

**Mass spectrometry based proteomic strategies
applied in the study of central nervous system derived cells**

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av

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Avhandlingen baserad på följande delarbeten:

- I. Proteome analysis of conditioned medium from cultured adult hippocampal progenitors**
A. Dahl, P. Eriksson, A. Persson, G. Karlsson, P. Davidsson, R. Ekman,
A. Westman-Brinkmalm
Rapid Commun Mass Spectrom, (2003) 17: 2195-2202
- II. Proteome analysis of serum containing conditioned medium from primary astrocyte cultures**
A. Thorsell, J. Fajerson, F. Blomstrand, M. Nilsson, K. Blennow, P.
Eriksson and A. Westman-Brinkmalm
Submitted for publication
- III: Evaluation of sample fractionation using micro scale liquid phase iso-electric focusing on mass spectrometric identification and quantitation of proteins in a SILAC experiment**
A. Thorsell, E. Portelius, K. Blennow and A. Westman-Brinkmalm
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ABSTRACT

This thesis focuses on evaluation, improvement and development of mass spectrometry based proteomic strategies for identification and quantitation of proteins. Development of such strategies is important to achieve a better understanding of regulatory mechanisms of individual cell types, both during normal development and during the onset and progression of diseases. Cells derived from the central nervous system served as model systems. Proteins secreted by these cells can be predicted to be involved in a variety of biological processes, including protective/survival effects, promotion of brain plasticity or intercellular communication. Secreted proteins might also be potential biomarkers of clinical importance, a tool for early detection and diagnosis of diseases. A challenge in the analysis of conditioned media is that the secreted proteins are much less abundant than the media proteins. A strategy involving preparative two-dimensional gel electrophoresis based on liquid phase separation identified several secreted proteins in well-defined conditioned medium from neural stem/progenitor cells. Their identification demonstrates the potential of this approach in identifying lower abundance proteins in the medium. In a serum-containing astrocyte conditioned medium, secreted proteins were identified using metabolic labeling. The proteins released were distinguished from the components of the medium in the mass spectrometric analysis by the labeled amino acid that was incorporated into the cellular proteins during culturing. Both strategies employed on the conditioned media can be used as initial screening tools to identify released proteins. Furthermore, they can be extended to most cells lines for studying secreted proteins. Mass spectrometric methods based on metabolic labeling have also shown great promise for identification and quantitation of proteins in complex mixtures. A prefractionation step involving micro-scale iso-electric focusing in liquid phase of whole cell extracts was found to be useful in the analysis. The number of identified proteins was drastically increased and the quantitation of lower abundance proteins was improved as compared with direct analysis of the same sample.

Key words: Proteomics, mass spectrometry, protein identification, protein quantitation, fractionation