Neuroproteomic profiling of human body fluids

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Abstract

This thesis provides results from affinity based studies where human body fluids were profiled to find markers for neurological diseases. Both proteins and autoantibodies were analysed using microarray technologies that can profile hundreds of analytes and hundreds of samples in parallel using small sample volumes. A central element in this work was to develop and apply new methods to study cerebrospinal fluid (CSF), which is the fluid in direct contact with the brain. CSF contains proteins reflecting the physiological state of the central nervous system and therefore offers a unique insight into proteins associated to neurological disorders. As a complement to CSF, blood-derived samples such as serum and plasma, were also investigated as these represent potential sources of disease related proteins. The work presented here summarises the development of assay protocols to study protein and autoantibodies in CSF and blood using planar and bead-based microarrays.

In Paper I, an antibody-based protocol was developed to enable multiplexed protein profiling in CSF. The protocol was then applied for a first analysis within multiple sclerosis (MS) patients. In Paper II, the results were further evaluated in additional CSF as well as plasma samples. Based on the CSF analysis we found two proteins associated to MS; GAP43, a protein related to disease progression and SERPINA3, a protein involved in inflammation. In addition, four other proteins; IRF8, METTL14, IL7 and SLC30A7, were found to have altered plasma levels between the patient groups. The expression of these proteins were further investigated by immunofluorescent staining of human brain tissue, revealing differential localisation of proteins in diseased and healthy brain. In Paper III, a study on extensive protein profiling of plasma in the context of another neurodegenerative disorder, amyotrophic lateral sclerosis (ALS), is described. The levels of three proteins, namely NEFM, RGS18 and SCL25A20, were found to be elevated in ALS patients compared to controls. Among these, NEFM also indicated association to disease subtype as the levels were elevated in patients with definite compared to suspected diagnosis.

In addition to antibodies, we also utilised antigens on microarrays to screen for the presence of autoantibodies in body fluids. In Paper IV, a strategy for this analysis was developed using protein fragments and two types of microarrays. This strategy was then applied for profiling of the autoantibody repertoire of MS patients, revealing 51 protein fragments with potential disease relevance. Interestingly, comparison of plasma and CSF samples obtained from the same patients indicated high concordance of antibodies between the two body fluids. In Paper V, a similar strategy was applied to narcolepsy, another neurological disorder. Our investigation of antibodies in serum revealed higher reactivity towards METTL22, NT5C1A and TMEM134 compared to controls in two independent sample materials.
In conclusion, the presented work constitutes a framework of proteomic assays for enhanced exploration of proteins and autoantibodies in neuroscience. Moreover, we have reported identification of several potential disease markers to be further investigated within neurological disorders.