Abstract

Neurodegenerative diseases are incurable disorders associated with the loss of cells in the central nervous system. The neural cell loss severely affects brain functions which ultimately results in a patient's death. Cell-based therapy using neural stem cells represents a promising strategy. These neural stem cells are expected to stimulate the regeneration of damaged tissue either by protein secretion or through the direct replacement by cells that differentiate into neurons or glial cells upon transplantation. However, current protocols for neural stem cell differentiation yield heterogeneous cell populations so a better understanding of regulatory mechanisms is required. It is crucial to determine the proliferation and differentiation potential to fully characterise the cells intended for transplantation. One of the key factors driving the efficacy of cell therapy is the timing of its administration to the patient. Thus, a search for suitable biomarkers that would assess the development of the disease is essential not only for the precise timing of transplantation but also for monitoring cell therapy outcomes. This dissertation thesis provides a detailed study of human neural stem cells during *in vitro* differentiation, aiming to uncover the pathways that regulate this process. The first part of the thesis describes the development of a targeted mass spectrometry method based on selected reaction monitoring (SRM) that allows the sensitive quantification of protein markers of specific cell populations. Due to the precise quantification of several markers for each analysed cell type, it is possible to monitor the differentiation of the entire cell population. This method can be used for routine cell characterisation or an assessment of the differentiation protocol. The second part of the thesis presents the results of a proteome study focused on the differentiation of neural stem cells. Using the mass spectrometry-based method called sequential window acquisition of all theoretical mass spectra (SWATH-MS) together with a multiplex antibody method, a significant regulation of specific signalling pathways has been revealed during neural stem cell differentiation. In *in vitro* conditions, this regulation included the signalling pathways Hypoxia-inducible factor 1 (HIF-1), Wnt, Interleukin-6 and Vascular endothelial growth factor (VEGF). Such pathways are highly interconnected and positively regulate the expression of the gene encoding the VEGF-A protein. Further experiments demonstrated that the proliferation and survival of spontaneously differentiating neural stem cells is specifically stimulated by the VEGF121 isoform. The supplementation with VEGF121 should be tested to verify its effect on cell replacement therapy. The last part of this thesis reviews published proteomic data obtained from analyses of samples from patients and experimental models with Huntington's disease, providing a list of new potential biomarkers for monitoring the progression of this neurodegenerative disease.

Key words: neural differentiation, neural stem cell, Huntington disease, proteomics, vascular endothelial growth factor