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Review article

Proteomics technologies for biomarker discovery in multiple sclerosis

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ABSTRACT

Multiple sclerosis is a disabling inflammatory and neurodegenerative disorder that predominantly affects young adults. There is a great need for biomarkers, which could elucidate pathology as well as provide prognosis of disease progression and therapy response in multiple sclerosis. Rapidly evolving, technology driven applications such as mass spectrometry based proteomics are currently being developed for this purpose. In this review, we will outline the current status of the field and detail a number of the bottlenecks as well as future prospects of this type of biomarker research.

Contents

Abbreviations: 2-DGE, 2-dimensional gel electrophoresis; BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; ICAT, isotope coded affinity tags; ITRAQ, isobaric tags for relative abundance and quantitation; LC, liquid chromatography; LC–MS, liquid chromatography–mass spectrometry; MS, mass spectrometry; MS/MS, tandem mass spectrometry; SELDI, surface enhanced laser desorption/ionization; SRM, selective reaction monitoring.

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1. Introduction

Multiple sclerosis is a disease of the central nervous system (CNS), of which the precise pathogenesis and aetiology still remain a mystery. The disease appears to evolve/occur in individuals that are genetically susceptible to the disease, but only those who are exposed to certain unknown environmental triggers develop the disease. It is believed that the disease process has an early phase with increased migration of auto-reactive lymphocytes across the blood–brain barrier (BBB) into the CNS (Compston and Coles, 2008). The primary adverse effect that can be observed in multiple sclerosis patients is damage to the myelin sheath that enwraps axons, and is physiologically needed to increase the speed of signal transmission. This process culminates in the hallmark of the disease; the formation of the sclerotic lesions from which the disease gets its name. Other cells affected besides the myelin-forming oligodendrocytes are neurons with their axons. One of the main issues in multiple sclerosis is the heterogeneity of the disease, as observed by the different patterns of plaque formation in patients (Lucchinetti et al., 2000) and the variability of clinical symptoms in patients. The majority of patients (80–85%) have the relapsing remitting subtype of the disease, in which relapses are followed by periods of remission. Around two thirds of these patients eventually enter a secondary, progressive phase of the disease. In 15–20% of multiple sclerosis patients the disease is progressive from onset (Confavreux and Vukusic, 2006).

In recent years new techniques have become available for analysis of biological samples in search of potential biomarkers for diseases such as cancer, Alzheimer's disease and also multiple sclerosis. These biomarkers can be useful in a number of different ways, like for example prognosis, monitoring of disease progression and early detection of diseases, which are of specific importance in multiple sclerosis as early detection and subsequent early treatment retard disease development (Tintore, 2007). Additional purposes include detection of new possible therapy targets, monitoring of response to certain therapies as well as increasing our understanding of disease pathology (Bielekova and Martin, 2004). In this review we will discuss available mass spectrometry-based proteomics technologies, sample selection considerations and future prospects for multiple sclerosis research.

2. Biological samples

2.1. Blood

Blood is a promising body fluid for biomarker discovery because of its easy collection and the presence of brain specific proteins and peptides produced in the CNS. These may reflect pathological changes occurring within the brain and spinal cord (Schaarschmidt et al., 1994). In blood TNF-α and CCL2 have been suggested to be indicators of inflammatory responses in primary progressive multiple sclerosis (Hagman et al., 2011), whereas kallikreins have been shown to be a secondary progressive stage indicator of multiple sclerosis (Scarisbrick et al., 2008). Blood is a rich source of disease related proteins due to its large dynamic range ($>10^{15}$) (Thadikkaran et al., 2005). Since it is located distantly from CNS, the detection of disease related proteins may be complicated by dilution and fast clearance of proteins by the liver and kidneys. Additionally metabolic changes may occur and there is also the possibility that highly abundant proteins like albumin in blood may mask detection of low abundant proteins, therefore making detection difficult (Zolg and Langen, 2004; Davidsson and Sjogren, 2005). In addition, proteases may be effective during the travel from the CNS to the blood stream that may truncate proteins of interest and even more make the exercise to find reliable and clinical relevant markers a challenge.

2.2. Urine

Urine is another interesting body fluid that attracts attention because of its non-invasive collection method. Past studies have reported neopterin and nitric oxide metabolites as marker of disease activity in multiple sclerosis (Rejdak et al., 2010). The possibility of detecting interesting protein biomarkers in urine is limited by metabolic variations observed in urine, relatively low protein content overwhelmed by a few high abundant proteins (for example albumin and Tamm–Horsfall protein (Parsons et al., 2011)), high salt concentration and presence of relatively high levels of low molecular proteins. Based on these difficulties Thongboonkerd and co-workers concluded that urine may not be the best choice specially for proteomics based biomarker research for CNS disorders (Thongboonkerd, 2007). However, the proteolytic cleavage into low molecular weight products can also be related to protease activity that could be partly disease specific (Mischak et al., 2010b).

2.3. Cerebrospinal fluid

While blood and urine are easily obtainable, the key endorsement of cerebrospinal fluid (CSF) as a body fluid for biomarker detection of CNS disorders is its direct connection and close proximity to the CNS. However, its collection is obviously more invasive and elaborate than the collection of blood.

As a consequence of fluid transports in the CNS, most of CNS-disease related proteins are likely to diffuse into the CSF. Therefore CSF is able to reflect directly the ongoing pathological conditions in the brain (Spitzer et al., 2010). This fluid is partly produced in choroid plexus of the brain at a rate of 500 ml/day and very rich in brain specific proteins. Normally, the concentration of protein in CSF (0.2–0.5 g/l) is far lower than in serum (60–80 g/l) (Fishman, 1992), mainly because of the vastly lower concentrations of highly abundant blood proteins that could mask detection of low abundant proteins by sophisticated techniques. Together this makes CSF an interesting body fluid for biomarker research for CNS disorders.

2.4. CNS tissue

Post-mortem brain tissue, and in particular brain lesions, allow for the possibility to detect changes caused by the pathology of multiple sclerosis at possible sites of the disease activity. These lesions are a combination of demyelination and inflammation occurring in different parts of CNS. Histological patterns of active demyelinating lesions vary from patient to patient (Lee et al., 1999). Lesion tissue obtained post-mortem from multiple sclerosis patients has been shown to have differences in RNA expression compared to normal appearing white matter (Lindberg et al., 2004). Consequently, if differences exist on a transcriptomics level, then effector molecules like proteins and metabolites might also be differentially abundant at the site of disease activity. Active multiple sclerosis lesions could be of great interest to approach disease in a targeted manner using an efficient technique called laser capture microdissection. This technique does not alter or damage the morphology and chemistry of the tissue and the surrounding cells. Using this type of method Han et al. have reported increased protein C inhibitor protein and tissue factors in chronic active multiple sclerosis lesions (Han et al., 2008). Availability of this kind of material is limited, so it is difficult to obtain sufficient numbers of samples to perform meaningful statistics on detected biomarkers. Therefore biobanking of brain tissue material is, along a good description of the obtained material, essential. The Netherlands Brain Bank (www.nin.knaw.nl) is a nice example of brain tissue banking. Additionally, tissue is an interesting biological option for validation of biomarkers detected in other types of biological samples (Fig. 1).