CHAPTER 4

CONCLUSIONS AND SUGGESTIONS

FOR FUTURE WORK
4.1 CONCLUSION:

The far western proteomic approach was employed in order to find out novel protein interactions that could be further exploited for biomarker discovery and thereby early diagnosis of MS. It is imperative to introduce evidence based biochemical markers in MS since MRI and CSF OCB analysis stands as the only two confirmative methods of diagnosis. Importantly, diseases that affect the brain demand considerable importance since it is an immunologically privileged site and thus provides minimal access to the immune molecules and therapeutic agents. In many instances, MS is initiated as a single neurological episode attributed to the development of single demyelinating lesion in the white matter of the brain. Moreover, OCB analysis may or may not be positive for CIS that makes it challenging for the differential diagnosis of MS.

Here in our study, using far western clinical proteomic method with 2DE as the basic platform, we were able to identify candidate potential biomarkers for early diagnosis of MS by predicting the conversion of CIS to clinically definite MS.

In the first part of our study, we standardized the myelin sample preparation for 2DE. Here we exploited the thermodynamics behind solubilization of highly lipid enriched myelin directly into membrane solubilizing detergent, ASB-14-4. We found that addition of myelin into ASB-14-4 could effectively solubilize myelin and thus resulted in better profiling of myelin proteins on a 2D gel compared to any other methods. The other methods included direct solubilization of myelin in ASB-14-4 where ASB was added to myelin, and precipitation methods using organic solvents like ethanol, acetone and ammonium acetate in methanol. We found that order of addition of detergent plays crucial role in the solubilization of material in a solvent.
We concluded that maximum solubilization of myelin proteins for 2DE could be attained by gently adding myelin into ASB-14-4 and not by adding ASB-14-4 into myelin. Thus, this strategy of adding myelin into ASB-14-4 was employed for further downstream experiments.

In the second part of the study, far western analysis using biotinylated CSF samples from patient and controls were conducted where we showed that a set of proteins in myelin, myelin axolemmal complexes and nodal proteome were engaged in differential protein interactions specific to CIS. Some of these reactivity’s were carried to RRMS as well. The identity of some of these proteins was revealed by LC-MS-MS and found to be 2’3’CNPase, DHPR, PEBP, ApoliproteinA1, Fructose bis phosphate Aldolase C and Synaptosomal Associated protein 25. Among these, DHPR was found to be promising based on literature survey. DHPR is a BH4 regenerating enzyme that maintains the pools of BH4 in the brain. The significance of this enzyme has been documented in other autoimmune diseases like vitiligo and diabetic nephropathy as well. But here for the first time we show that this enzyme suggestive to have an immense role along with other factors in the early pathogenic mechanisms in MS. Moreover, the specific reactivity of CSF of CIS patients towards DHPR also is an immense leap towards the possibility of exploitation of DHPR as potential candidate biomarker of MS in the CIS stage of MS. Also, analysis using CSF obtained from NMO patients would be important in order to decipher whether DHPR reacts with CSF of NMO patients to further prove the CIS reactivity to DHPR is specific to MS.

In the third part of the study, the validation of DHPR was performed in the animal model of MS, the EAE, which was generated in female C57BL6 mice. Here the levels of DHPR expression in the EAE and control brains were analyzed using immunoblot and quantified using imageJ software. We found that the expression levels of DHPR were considerably elevated in the brains of EAE mice and found associated with the increasing clinical symptoms than the controls.
Thus, the increased expression of DHPR in animal model as well as the presence specific reactive proteins in the CSF of CIS patients suggests the importance of DHPR in the early pathogenesis of MS and may serve as an early potential candidate diagnostic marker in MS. Further validation of DHPR reactivity to CIS CSF samples using increased numbers of patient samples is required. In addition, we speculate a pathway involving DHPR’s role in the pathogenesis of MS where the increased expression of DHPR results in subsequent increase in BH4 levels thereby increasing the function of NOS and decrease in the levels of TGF beta. This would eventually augment autoimmune response in the brain.

4.2 SUGGESTIONS FOR FUTURE WORK

Identification of other proteins involved in differential interaction to myelin proteome in CIS by mass spectrometry is suggested. This helps to explore the possibility of role of additional proteins in MS pathogenesis.

It would be important to confirm the significance of this enzyme in a larger patient population. In addition, analysis of the DHPR expression levels in MS brain would help in better understanding of the role of DHPR in pathogenesis of MS.

Moreover, analysis of CSF from NMO confirmed patients based on aquaporin reactivity will have to be performed for DHPR reactivity to confirm the specificity of CIS CSF reactivity to DHPR.

In the context of early diagnosis of MS, ELISA based methods using the identified protein molecules could be helpful in order to predict the conversion of CIS to MS. Thus, incorporation of evidence based biochemical markers along with existing diagnostic methods can help the clinicians for differential diagnosis of MS at the CIS stage itself. This would subsequently augment better treatment modalities to patients.