Assessing the role of citrullination and lipid interaction with Myelin Basic Protein in Multiple Sclerosis etiology

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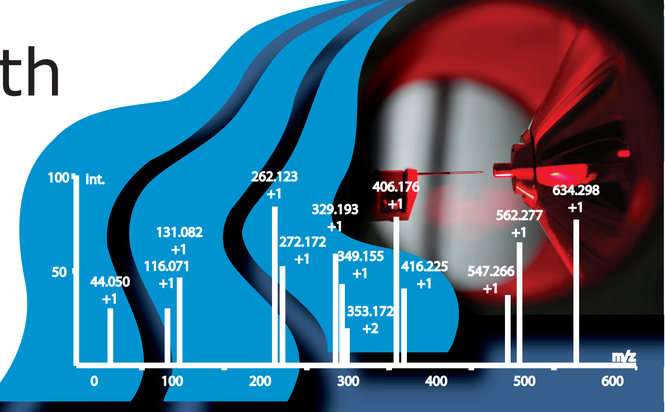


AIM

Assign covalently bond membrane lipids to Myelin Basic Protein from the Myelin Sheet of Rattus norvegicus.

Assign novel modifications on Myelin Baisc protein from healthy Rattus norvegicus which could be relevant to the disease etiology of Multiple Sclerosis and the general function of Myelin Basic Protein.

Conclusion

By hand annotations of denovo sequenced Peptide-Spectrums with high similarities to Myelin Basic Protein derived peptides, The lipidation Myristoylation was found and verified, together with the post translational modifications; Biotinylations as well as Levugladin and Malondialdehydes adducts.

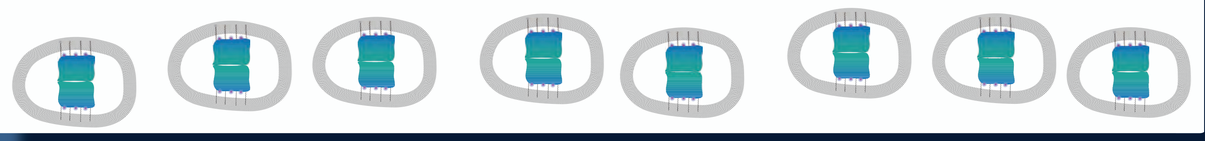
Perspectivation

- How is the lipidation regulated in Multiple Sclerosis?
- Enzyme catalyzed lipidation?
- How dependent is lipidation on the surrounding lipid level?

- How do citrullinations and peroxidation adduct modification of Myelin Basic protein correlate to disease in animals model?
- How is it comparable to humans?

Novel Etiology Question

How is lipid shielding of citrulline Myelin Basic Protein occurring in the Myelin Sheet?
Are peptides containing Levugladin and Malondialdehydes adducts or citrullination the initial immunogenic epitopes in Multiple Sclerosis?
Is dysfunction of lipid Metabolism the ignition point for generation of the autoimmune response.



Introduction

Multiple sclerosis (MS) is thought to be an autoimmune disease and initiated by demyelinating of the Myelin Sheet. Studies of MS have suggested that the role of Citrullination of Myelin Basic Protein (MBP) and fatty acid metabolism is essential for the Myelin sheet and the understanding the initiation and progression of the disease.
MBP is known to form lipid complexes Figure 1. The complex structure has show to partially be determined by Citrullination of Arginine residues.
The lipid complexes are believed to be generated by electrostatic interactions.
In this study, we seek to prove the new theory that MBP is covalently bond to membrane lipids, thus forming the lipidcomplexes. The study is performed on white matter from healthy Rattus norvegicus. We also seek to discover and map modification on MBP, which is potential epitopes, igniting the autoimmune response, thus key players in the Multiple Sclerosis etiology.

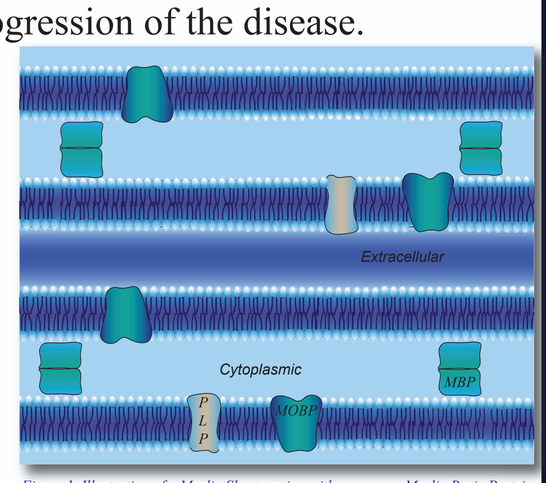


Figure 1. Illustration of a Myelin Sheet section with a zoom on Myelin Basic Protein membrane lipid complex. The Myelin sheet consist of lipid bilayers and is rich of Myelin Basic Protein (MBP), Proteolipid protein (PLP) and Myelin-Associated Oligodendrocyte Basic Protein (MOBP).

Results and Discussion

We identified the isoforms of Myelin Basic Protein with sequence coverage of ≥ 98% with 1% FDR on at peptide spectrum matches.

Protein	Mass (Da)	Coverage (%)	Description
sp|P02688|MBP_RAT	21502	99	Myelin Basic Protein
sp|P02688-2|MBP_RAT	18488	99	Isoform 2 of Myelin Basic Protein
sp|P02688-3|MBP_RAT	17225	98	Isoform 3 of Myelin Basic Protein
sp|P02688-4|MBP_RAT	14211	98	Isoform 4 of Myelin Basic Protein
sp|P02688-5|MBP_RAT	22940	99	Isoform 5 of Myelin Basic Protein

- Citrullination
- Biotinylation
- Myristoylation
- Levugladin adducts
- Malondialdehyde adducts

Figure 2. Modification of interest found on Myelin Basic Protein.

Five modifications were found, figure 2, which could be highly relevant for Myelin Basic Proteins' function in both healthy and diseased Myelin Sheets.

Citrullination is a known modification, suspected of being a key epitope in numerous autoimmune diseases. Biotinylation, Myristoylation, Levugladin- and Malondialdehyde adducts are all connected to lipid metabolism. Levugladin- and Malondialdehyde adducts are similar to citrullination, which is considered potential key epitopes in autoimmmune responses. Raising the question how is these three modifications connected to lipid metabolism? MBP was found to be lipidated by Myristoylation. In healthy myelin sheets this lipidation could facilitate lipid shielding of normal occurring citrullinated residues, which, when not shielded, would activate the immune system. This connection could explain, how the lipid metabolism is essential for understanding the etiology of MS, as dysfunctional lipid metabolism would lead to delipidation and thereby deshielding of the citrulline epitopes. Additional peroxidation would lead to further epitope generation doe to Levugladin- Malondialdehydes modification, Figure 3,.

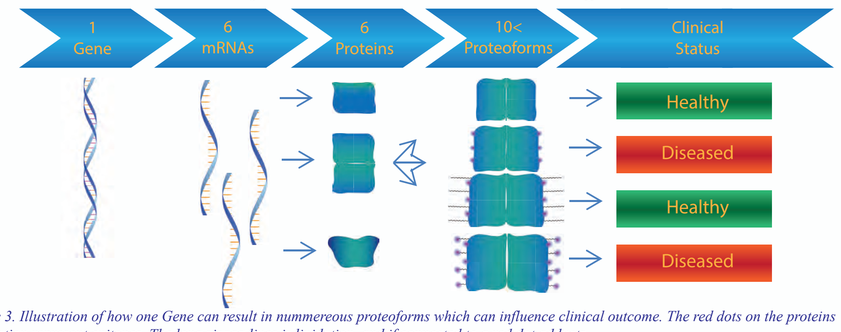


Figure 3. Illustration of how one Gene can result in nummereous proteoforms which can influence clinical outcome. The red dots on the proteins illustration represent epitopes. The long zigzag lines is lipidation, and if connected to a red dot adducts.

Methods

White matter was extracted from Rattus norvegicus brain biopsies. The white matter was homogenized, the protein content was extracted with methanol and chloroform precipitation. SDS-PAGE was performed as well as In-Gel digestions of bands suspected to contain Myelin Basic Protein.

The Protein fractions were analyzed by LC-MS/MS on a Q Exactive HF MS using a UPLC-nanoESI with a 75 cm C18 column and a 30 min gradient. Post Translational Modifications analysis and denovo sequencing was performed in PEAKS.

Poster

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