Introductory Considerations

In the present project, we have outlined an ambitious, although feasible looking collaborative effort to identify key gene products regulating de- and remyelination in order to provide novel therapeutic targets for causal treatment of multiple sclerosis and other sclerotic diseases. Feasibility of the proposal was based upon the success of a previous collaboration with the present international principal investigator, Prof. Zsolt Illes, an acknowledged authority on multiple sclerosis. This previous collaboration determined that demyelination in multiple sclerosis and in its degenerative experimental model; the cuprizone model, involves parallel mitochondrial mechanisms. Also, it resulted in four best presentation awards, a research paper published in *Brain* (impact factor 9.5), and a registered patent. Feasibility of the proposal was also strengthened by a separate preliminary study that identified genes overexpressed or suppressed in de- and remyelination by DNA chip technique.

Consequently, the present project aimed at confirming and validating the DNA chip results at the protein level, and proposing key mechanisms in de- and remyelination. To this end, we proposed five specific aims: (i) comparing proteomics profile of normal, demyelinating, early-, and late remyelinating white matter in brains of cuprizone treated mice, (ii) transfecting mixed glia cultures with constructs expressing different abundances of µRNA-s identified in the preliminary study, and investigating their effect on proteomic pattern of the cultures, (iii) comparing proteomics profile of normal and affected white matter in brains of cuprizone treated mice with those of post-mortem multiple sclerosis patients, (iv) determining post-translational modifications and (v) performing proteomic imaging of the proteins selected from the experiments i-iii.

Achievements

We have experienced a number of drawbacks from the beginning. Administrative problems delaying purchase of important reagents, early quitting of a key technician set the cell culturing experiments back by a year, detector desensitisation rendered our own MALDI-TOF MS unsuitable for proteomic imaging, and maternity leave of a key investigator. Fortunately, we have received two 6-month extensions to the project that allowed completion of all experiments outlined in the research plan but (v) proteomic imaging of the selected proteins.

(i) Corpus callosum of mice (control, 4-week cuprizone treated, 4-week cuprizone treated followed by 2-day remyelination and 4-week cuprizone treated followed by 2-week remyelination) were homogenised, proteins were precipitated, solubilised, alkylated by iodoacetamide and digested by lysyl endopeptidase and trypsin. Peptides were purified on a C8 then a C18 reverse solid phase extraction columns before isobaric tags for relative and absolute quantitation labeling was performed on them. The peptides were analysed with liquid chromatography coupled with a nano electron spray ionisation mass spectrometry instrument. The scanning range was set to 100–3000 m/z. Each intensive peptide was fragmented and the completed data was processed with Thermo Proteome Discoverer software. Protein idetification was carried out by searching for mus musculus taxonomically restricted in the databases of the NCBI and

the Swiss-Prot using Mascot V2.4.1. Significance limit was set to >200 MASCOT score. Limma 0.1 significant proteins were analysed for canonical pathway, disease and function, and upstream regulator comparisons by using QIAGEN's Ingenuity® Pathway Analysis (IPA) tool.
Out of the identified 4282 proteins, 1980 were present in 4/5 experiments. We selected 275 proteins that were Limma 0.1 significant, and differed from the control in any of the treatment groups. After 4 weeks of demyelination, 39 and 36 were significantly down- and upregulated, respectively. Similarly, there were 13 and 26, and 32 and 31 down- and upregulated early and late remyelination specific genes. However, we could not find overlap with Mir-146a regulated genes. Genes involved in energy production were downregulated in demyelination, and upregulated in remyelination, and some other patterns consistent with general metabolism could be observed. IPA analyses suggested that amyloid precursor protein may regulate demyelination while prolactin and brain derived neurotrophic factor may promote remyelination.
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Proteomic analysis of gene products that regulate cuprizone-indiced de- and remyelination

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Background: Cuprizone (CPZ) induces reversible, inflammation-free and well localised demyelination and oligodendrocyte loss, which shares molecular pathways with those observed in brain lesions of patients with multiple sclerosis (MS).
Methods: Corpus callosum of mice (control, 4-week CPZ treated, 4-week CPZ treated followed by 2-day remyelination and 4-week CPZ treated followed by 2-week remyelination) were homogenised, proteins were precipitated, solubilised, alkylated by iodoacetamide and digested by lysyl endopeptidase and trypsin. Peptides were purified on a C8 then a C18 reverse solid phase extraction columns before isobaric tags for relative and absolute quantitation labeling was performed on them. The peptides were analysed with liquid chromatography coupled with a nano electron spray ionisation mass spectrometry instrument. Limma 0.1 significant proteins were analysed for canonical pathway, disease and function, and upstream regulator comparisons by using QIAGEN's Ingenuity® Pathway Analysis tool.
Results: Out of the identified 4282 proteins, 1980 were present in 4/5 experiments. We selected 275 proteins that were Limma 0.1 significant, and differed from the control in any of the treatment groups. After 4 weeks of demyelination, 39 and 36 were significantly down- and upregulated, respectively. Similarly, there were 13 and 26, and 32 and 31 down- and upregulated early and late remyelination specific genes. However, we could not find overlap with Mir-146a regulated genes. Genes involved in

energy production were downregulated in demyelination, and upregulated in remyelination, and some other patterns consistent with general metabolism could be observed. IPA analyses suggested that amyloid precursor protein may regulate demyelination while prolactin and brain derived neurotrophic factor may promote remyelination.

Interpretation: Shifts in the equilibrium of signalling networks rather than expressional changes of a few specific genes seems to be important among the mechanisms of de- and remyelination.

(ii) Cells on confluent 10 cm culture plates were harvested by a rubber policeman, washed in chilled phosphate buffered saline containing protease and phosphatase inhibitor cocktail, taken up in 300 uL of the washing solution and homogenised by a Teflon-glass homogeniser. The proteins were precipitated, solubilised, alkylated by iodoacetamide and digested by lysyl endopeptidase and trypsin. Peptides were purified on a C8 then a C18 reverse solid phase extraction columns before isobaric tags for relative and absolute quantitation labelling was performed on them. The peptides were analysed with liquid chromatography coupled with a nano electron spray ionisation mass spectrometry instrument exactly as for (i).

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MicroRNA-146a deficiency protects against cuprizone-induced demyelination

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Background: Cuprizone (CPZ)-induced demyelination and oligodendrocyte loss share molecular pathways with those observed in brain lesions of patients with multiple sclerosis (MS), and differential expression of microRNAs in MS lesions has been described.

Methods: MicroRNA expression was examined by Agilent Mouse miRNA Microarray in the corpus callosum during demyelination and remyelination induced by CPZ. MiR-146a expression was investigated by quantitative PCR during postnatal myelination. Systemic effects of CPZ on weight of the body, thymus and spleen were examined in miR-146a deficient (KO) and wild type (WT) mice. Demyelination, axonal loss, changes in number of oligodendrocytes, oligodendrocyte precursors, and macrophages/microglia was compared by histology/immunohistochemistry between KO and WT mice. Differential expression of target genes and proteins of miR-146a was analyzed in the transcriptome (4x44K Agilent Whole

Mouse Genome Microarray**)** and proteome (liquid chromatography tandem mass spectrometry) of CPZ-induced de- and remyelination in WT mice. Down-regulated proteins were examined by ELISA in WT and KO mice. Levels of proinflammatory molecules in the corpus callosum were compared between WT and KO mice by Meso Scale Discovery multiplex protein analysis.
Results: We found differential expression of miR-146a, miR-181b and miR-193a during CPZ-induced de/remyelinaton. The absence of miR-146a in KO mice protected against demyelination, axonal loss, body weight loss and atrophy of thymus and spleen. The number of CNP^+ oligodendrocytes was increased during demyelination in the miR-146a KO mice. MiR-146a target genes, SNAP25 and SMAD4 were downregulated in the proteome of demyelinating corpus callosum in WT mice. Higher levels of SNAP25 were measured by ELISA in the corpus callosum of miR-146a KO mice, but there was no difference between KO and WT mice during demyelination. Multiplex protein analysis of the corpus callosum lysate revealed upregulated TNF-RI, TNF-RII and CCL2 in the WT mice in contrast to KO mice, and the number of $Mac3^+$ macrophages/microglia was reduced in the demyelinating corpus callosum of the KO mice.
Conclusions: Absence of miR-146a reduces inflammatory responses, decreases the number of infiltrating macrophages, increases the number of myelinating oligodendrocytes in the demyelinating corpus callosum, and protects against demyelination and axonal loss.

(iii) Tissue samples were punched out from multiple sclerosis lesions and not-affected white mater from archived pathological brain samples of patients and non-multiple sclerosis controls by an experienced neuropathologist blind to the experiment. Homogenisation and quantitative proteomic analyses of the tissue samples were performed exactly as described for (i). Changes among the proteome of the human brain samples were compared to those found in the cuprizone model.
Additionally, we screened the cerebrospinal fluid (CSF) proteome of 97 CSF samples of relapsing and progressive multiple sclerosis patients for proteins that were similarly regulated in brains of the multiple sclerosis patients and cuprizone treated mice.
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De- and remyelination-related proteins in the CSF of multiple sclerosis subtypes

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Objective: To identify key molecules in multiple sclerosis (MS) pathogenesis and biomarkers to differentiate relapsing from progressive MS.
Methods: In order to relate differentially expressed genes in MS lesions to de- and remyelination, transcriptomes of cuprizone-induced de- and remyelination were compared to MS lesion transcriptomes. Protein products of overlapping orthologous genes were screened in the cerebrospinal fluid (CSF) proteome, and 129 peptides were compared by targeted quantitative proteomics in 97 CSF of relapsing and progressive MS. Differentially regulated proteins were correlated with 37 biomarkers of inflammation and axonal degeneration in the CSF by Meso Scale. Expression of orthologous genes/proteins were related to de- and remyelination in the cuprizone model by immunohistochemistry.
Results: Agilent Whole Mouse Genom Expression Microarray detected 98 MS gene orthologs connected to experimental de- and remyelination. Pathway analysis suggested the importance of the TYROBP (DAP12)/TREM2 pathway, TNF-receptor 1, CYBA and the proteasome subunit PSMB9. We designed 129 peptides representing 51 orthologous proteins, and measured their levels in 97 CSF of MS by targeted proteomics (parallel reaction monitoring). Four proteins were differentially regulated among relapsing and progressive MS: tyrosine protein kinase receptor UFO (UFO), TIMP-1, apolipoprotein C-II (APOC2), and beta-2-microglobulin (B2M). In the mouse brain, their orthologous genes/proteins were up-regulated during acute remyelination in different resident cells. Levels of UFO, TIMP-1 and B2M negatively correlated with inflammation in the CSF (IL-6, MCP-1/CCL2, TARC/CCL17). APOC2 showed positive correlation with IL-2, IL-16 and eotaxin-3/CCL26.
Interpretation: Pathology-based multi-omics identified molecules/pathways important in MS pathogenesis and four potential biomarkers in multiple sclerosis.

(iv) We have proposed to determine post-translational modifications of the selected proteins by immunoprecipitating the proteins in various samples by using post-translational modification-specific primary antibody, then performing proteomic identification of the precipitated proteins. However, this method has limitations in the specificity and avidity of the primary antibody, and it entirely lacks in quantitativity. Therefore, we used a TiO_2-Sequential elution from Immobilized Metal Affinity Chromatography (SIMAC)-Hydrophilic Interaction Liquid Chromatography (HILIC) procedure for enrichment of phosphopeptides and sialylated glycopeptides. It indeed limited the post-translational modifications to phosphorylation and glycosylation, but had the advantage of quantitativity, and allowed studying all detectable proteins, not just the selected ones. For these experiments, we used the

aforementioned protocol until lysyl endopeptidase and trypsin digestion. Then we performed TiO_2-SIMAC-HILIC enrichment. For the different fractions (glycosylated, and single- and multiphosphorylated peptides) we used the aforementioned liquid chromatography - nano electron spray ionisation mass spectrometry measurement. It enabled us to determine expressional changes of proteins together with their phosphorylation and glycosylation. Therefore, we could identify a given protein as a key regulator of de- and/or remyelination even if it's steady state expression level remained the same when e.g. its phosphorylation state (indicating activation or inactivation) changed. We intend to determine post-translational modifications by immunoprecipitation only for those few selected key proteins, in which the position of the post-translational modification is important (e.g. phosphorylation of one site causes activation while the other results in inhibition). In these cases we use post-translational modification and protein specific primary antibody.
We have identified 1370 mono-, 615 multiphosphorylated, and 1197 gycosylated proteins. Out of these, expression level of 184 phospho- and 4 glycoproteins has changed significantly during de- and remyelination. These results have not yet considered for publication because the data are not conclusive.

In the meantime, we could publish several related parallel studies. These studies are related to the present project either because they involve the cuprizone model, inhibitors of poly(ADP-ribose) polymerase, the substance that was found to inhibit cuprizone-induced demyelination in our previous collaborative study, or investigate the same signalling mechanisms that are participating in the pathomechanism of demyelination.

1/ We examined whether PARP-inhibitor (L-2286) treatment could prevent the development of hypertensive cardiopathy in spontaneously hypertensive rats (SHR). 6-week-old SHR animals were treated with L-2286 (SHR-L group) or placebo (SHR-C group) for 24 weeks. Wistar-Kyoto rats were used as aged-matched, normotensive controls (WKY group). Echocardiography was performed, brain-derived natriuretic peptide (BNP) activity and blood pressure was determined at the end of the study. We detected the extent of fibrotic areas. The amount of heat-shock proteins (Hsps) and the phosphorylation state of Akt-1(Ser473), glycogen synthase kinase (GSK)-3β(Ser9), forkhead transcription factor (FKHR)(Ser256), mitogen activated protein kinases (MAPKs), and protein kinase C (PKC) isoenzymes were monitored. The elevated blood pressure in SHRs was not influenced by PARP-inhibitor treatment. Systolic left ventricular function and BNP activity did not differ among the three groups. L-2286 treatment decreased the marked left ventricular (LV) hypertrophy which was developed in SHRs. Interstitial collagen deposition was also decreased by L-2286 treatment. The phosphorylation of extracellular signal-regulated kinase (ERK)1/2(Thr183-Tyr185), Akt-1(Ser473), GSK-3β(Ser9), FKHR(Ser256), and PKC ε(Ser729) and the level of Hsp90 were increased, while the activity of PKC α/βII(Thr638/641), ζ/λ(410/403) were mitigated by L-2286 administration. We could detect signs of LV hypertrophy without congestive heart failure in SHR groups. This alteration was prevented by PARP inhibition. Our results suggest that PARP-inhibitor treatment has protective effect already in the early stage of hypertensive myocardial remodelling.
Published in PLOS One

2/ We used a well-characterized monocrotaline (MCT)-induced rat pulmonary arterial hypertension (PH) model, and analysed lung morphology, expression of cytokines, mitogen-activated protein kinase (MAPK) phosphorylation, and phosphatidylinositol 3-kinase-Akt (PI-3k-Akt) pathway and nuclear factor (NF)-κB activation in order to elucidate the mechanisms by which sildenafil's protective effect in PH is exerted. We found that besides its protective effect on lung morphology, sildenafil suppressed multiple cytokines involved in neutrophil and mononuclear cells recruitment including cytokine-induced neutrophil chemoattractant (CINC)-1, CINC-2α/β, tissue inhibitor of metalloproteinase (TIMP)-1, interleukin (IL)-1α, lipopolysaccharide induced CXC chemokine (LIX), monokine induced by gamma interferon (MIG), macrophage inflammatory protein (MIP)-1α, and MIP-3α. NF-κB activation and phosphorylation were also attenuated by sildenafil. Furthermore, sildenafil reduced extracellular signal-regulated kinase (ERK)1/2 and p38 MAPK activation while enhanced activation of the cytoprotective Akt pathway in PH. These data suggest a beneficial effect of sildenafil on inflammatory and kinase signalling mechanisms that substantially contribute to its protective effects, and may have potential implications in designing future therapeutic strategies in the treatment of pulmonary hypertension.
Published in PLOS One.

3/ We studied whether mitochondrial permeability transition (mPT) can actively regulate the immune response. To this end, we compared bacterial lipopolysaccharide (LPS)-induced inflammatory response in cyclophilin D (CypD) knock-out and wild-type mouse resident peritoneal macrophages. CypD is a regulator of mPT; therefore, mPT is damaged in CypD(-/-) cells. We chose this genetic modification-based model because the mPT inhibitor cyclosporine A regulates inflammatory processes by several pathways unrelated to the mitochondria. The LPS increased mitochondrial depolarisation, cellular and mitochondrial reactive oxygen species production, nuclear factor-κB activation, and nitrite- and tumour necrosis factor α accumulation in wild-type cells, but these changes were diminished or absent in the CypD-deficient macrophages. Additionally, LPS enhanced Akt phosphorylation/activation as well as FOXO1 and FOXO3a phosphorylation/inactivation both in wild-type and CypD(-/-) cells. However, Akt and FOXO phosphorylation was significantly more pronounced in CypD-deficient compared to wild-type macrophages. These results provide the first pieces of experimental evidence for the functional regulatory role of mPT in the LPS-induced early inflammatory response of macrophages.
Published in Biochem Cell Biol.

4/ We have concluded our study on the possible suppression of T-cell functions by cuprizone. Peripheral T-cell function in the cuprizone model has already been explored; therefore, in the present study, we investigated, for the first time, how cuprizone feeding affects the thymus, the organ of T-cell maturation and selection. We found that even one week of cuprizone treatment induced significant thymic atrophy, affecting the cortex over the medulla. Fluorescent microscopy and flow-cytometric analyses of thymi from cuprizone- and vehicle-treated mice indicated that eradication of the cluster of the differentiation-4 (CD4)-CD8 double-positive T-cell subset was behind the substantial cell loss. This result was confirmed with CD3-CD4-CD8 triple-staining experiments. Ultrastructurally, we observed degraded as well as enlarged mitochondria, myelin-bodies, large lipid droplets, and large lysosomes in the thymi of cuprizone-treated mice. Some of these features were similar to those in physiological and steroid-induced accelerated aging. According to our results, apoptosis was mainly of mitochondrial origin

mediated by both caspase-3- and apoptosis inducing factor-mediated mechanisms. Additionally, mitogen activated protein kinase activation and increased pro-apoptotic B cell lymphoma-2 family protein expression were the major underlying processes. Our results do not indicate a functional relationship between cuprizone-induced thymus involution and the absence of inflammatory responses or the selective demyelination observed in the cuprizone model. On the other hand, our results suggest that cuprizone affects the most vulnerable differentiating cells preferably; a characteristics shared by T-cells and oligodendrocytes.
These results were published in PLoS One.

5/ We investigated how cytoplasmic abundance of HSPB11 small human heat-shock protein augments tumour malignancy. We up- and downregulated the cytoplasmic level of HSPB11 before paclitaxel exposure in NIH3T3 and HeLa cells, which normally express low and high levels, respectively, of the HSPB11 protein. We examined the paclitaxel-mediated induction of cell death, mitochondrial fission, HSPB11 mitochondrial translocation and inhibitory phosphorylation of dynamin-like protein-1 (DLP1). We found that increasing cytoplasmic abundance of HSPB11 in NIH3T3 cells protected against paclitaxel-induced apoptosis, while suppressing HSPB11 in HeLa cells sensitised the cells toward paclitaxel. Also, paclitaxel enhanced mitochondrial translocation of HSPB11 in wild type HeLa but not in NIH3T3 cells. More importantly, increased cytoplasmic level of HSPB11 in NIH3T3 cells enhanced the inhibitory phosphorylation of DLP1 and attenuated paclitaxel-induced mitochondrial fission. All these results suggest that increased cytoplasmic abundance of HSPB11 augments inhibitory phosphorylation of DLP1 thereby reduces mitochondrial fission that eventually leads to decreased apoptosis. This novel mechanism may explain the resistance to apoptosis and increased malignancy of HSPB11-overexpressing tumours. The clinical significance of this mechanism has already been highlighted by the finding that the kinase inhibitor tyrphostin A9 induces cancer cell death by DLP1-mediated mitochondrial fragmentation.
Published in J Cancer.

6/ Oxidative stress induces DNA breaks and PARP-1 activation which initiates mitochondrial reactive oxygen species (ROS) production and cell death through pathways not yet identified. Here, we show the mechanism by which PARP-1 influences these processes via PARylation of activating transcription factor-4 (ATF4) responsible for MAP kinase phosphatase-1 (MKP-1) expression and thereby regulates MAP kinases. PARP inhibitor, or silencing, of PARP induced MKP-1 expression by ATF4-dependent way, and inactivated JNK and p38 MAP kinases. Additionally, it induced ATF4 expression and binding to cAMP-response element (CRE) leading to MKP-1 expression and the inactivation of MAP kinases. In contrast, PARP-1 activation induced the PARylation of ATF4 and reduced its binding to CRE sequence in vitro. CHIP-qPCR analysis showed that PARP inhibitor increased the ATF4 occupancy at the initiation site of MKP-1. In oxidative stress, PARP inhibition reduced ROS-induced cell death, suppressed mitochondrial ROS production and protected mitochondrial membrane potential on an ATF4 and MKP-1 dependent way. Basically identical results were obtained in WRL-68, A-549 and T24/83 human cell lines indicating that the aforementioned mechanism can be universal. Here, we provide the first description of PARP-1-ATF4-MKP-1-JNK/p38 MAPK retrograde pathway, which is responsible for the regulation of mitochondrial integrity, ROS production and cell death in oxidative stress, and may represent a new

mechanism of PARP in cancer therapy since cancer stem cells development is JNK-dependent. Published in Free Radic Biol.

7/ Vascular remodeling during chronic hypertension may impair the supply of tissues with oxygen, glucose and other compounds, potentially unleashing deleterious effects. In this study, we used Spontaneously Hypertensive Rats and normotensive Wistar-Kyoto rats with or without pharmacological inhibition of poly(ADP-ribose)polymerase-1 by an experimental compound L-2286, to evaluate carotid artery remodeling and consequent damage of neuronal tissue during hypertension. We observed elevated oxidative stress and profound thickening of the vascular wall with fibrotic tissue accumulation induced by elevated blood pressure. 32 weeks of L-2286 treatment attenuated these processes by modulating mitogen activated protein kinase phosphatase-1 cellular levels in carotid arteries. In hypertensive animals, vascular inflammation and endothelial dysfunction was observed by NF-κB nuclear accumulation and impaired vasodilation to acetylcholine, respectively. Pharmacological poly(ADP-ribose)polymerase-1 inhibition interfered in these processes and mitigated Apoptosis Inducing Factor dependent cell death events, thus improved structural and functional alterations of carotid arteries, without affecting blood pressure. Chronic poly(ADP-ribose)polymerase-1 inhibition protected neuronal tissue against oxidative damage, assessed by nitrotyrosine, 4-hydroxinonenal and 8-oxoguanosine immunohistochemistry in the area of Cornu ammonis 1 of the dorsal hippocampus in hypertensive rats. In this area, extensive pyramidal cell loss was also attenuated by treatment with lowered poly(ADP-ribose)polymer formation. It also preserved the structure of fissural arteries and attenuated perivascular white matter lesions and reactive astrogliosis in hypertensive rats. These data support the premise in which chronic poly(ADP-ribose)polymerase-1 inhibition has beneficial effects on hypertension related tissue damage both in vascular tissue and in the hippocampus by altering signaling events, reducing oxidative/nitrosative stress and inflammatory status, without lowering blood pressure. Published in PLOS One.

8/ Reactive oxygen species (ROS) play a critical role in the progression of mitochondria-related diseases. A novel insulin sensitizer drug candidate, BGP-15, has been shown to have protective effects in several oxidative stress-related diseases in animal and human studies. In this study, we investigated whether the protective effects of BGP-15 are predominantly via preserving mitochondrial integrity and reducing mitochondrial ROS production. BGP-15 was found to accumulate in the mitochondria, protect against ROS-induced mitochondrial depolarization and attenuate ROS-induced mitochondrial ROS production in a cell culture model, and also reduced ROS production predominantly at the complex I-III system in isolated mitochondria. At physiologically relevant concentrations, BGP-15 protected against hydrogen peroxide-induced cell death by reducing both apoptosis and necrosis. Additionally, it attenuated bacterial lipopolysaccharide (LPS)-induced collapse of mitochondrial membrane potential and ROS production in LPS-sensitive U-251 glioma cells, suggesting that BGP-15 may have a protective role in inflammatory diseases. However, BGP-15 did not have any antioxidant effects as shown by in vitro chemical and cell culture systems. These data suggest that BGP-15 could be a novel mitochondrial drug candidate for the prevention of ROS-related and inflammatory disease progression.
Published in PLOS One.

Concluding Considerations

In the project proposal we have hypothesised that results of the total RNA microarray experiments of the international collaborator could suggest several proteins as potential mediators and/or regulators of de- and remyelination. Unfortunately, it did not prove to be so. All in all, we have identified 4282 proteins, as well as 1370 mono- and 615 multiphosphorylated, and 1197 gycosylated proteins. Out of these, expression level of 431 proteins, and 184 phospho- and 4 glycoproteins has changed significantly during de- and remyelination. Comparing the hits with those provided by the total RNA microarray experiments did not give useful hints for selecting candidates. Because of the too high number and dubious significance of the hits co-emerging from the cuprizone model and the human patients, there was no point to perform the planned immunohistochemical analyses on affected areas and normal-appearing white matter in cuprizone treated mice and postmortem multiple sclerosis brains (see v) at this stage. We postponed these experiments until we could settle on <10 hits.

Despite of the fact that the proteome data on brain samples of multiple sclerosis patients and cuprizone treated mice are not self-revealing, we think that they could be of great value for data miners. On the other hand, most journals request data sharing when an omics paper is accepted. That is why we have delayed publishing our results, and tried submitting simultaneously various aspects of our collaborative research. They are at different stage of publication (1 resubmitted, 1 submitted and 1 in preparation).