evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název:
Sledování změn hladiny metalothioneinu-3 při vzniku prionových onemocnění

Školitel: Bc. Alžběta Cardová

Datum: 17. 1. 2014

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

Immunohistochemical determination of Metallothionein level in context of Prion diseases

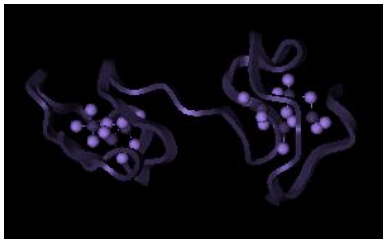
Alžběta Cardová

Laboratoř metalomiky a nanotechnologií
Centrum excelentní vědy

CAMBRIDGE NEUROSCIENCE

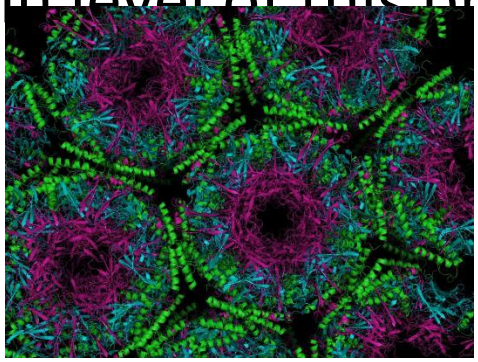
Metallothionein - III

- Brain specific subtype of MT is called MT-III and this protein seems to fulfil a unique biological role in the central nervous system.
- Observations that the down-regulation of MT-III could lead to formation of neurofibrillary tangles characteristic of the Alzheimer's disease provoked the intense scientific endeavour to discover a potential role of MT-III in context of neurodegenerative disorders.
- Prion diseases and other neurodegenerative disorders share many common attributes (familial as well as sporadic), aggregation of misfolded protein and neuronal forfeit.
- Each disorder is characterized by the misfolding of a specific protein: α-synuclein in Parkinson's disease, β-amyloid and tau in Alzheimer's disease, huntingtin in Huntington's disease and PrP in transmissible spongiform encephalopathies.



Aim of this experiment

- In this study we tried to determine and compare the metallothionein levels in non-infected and prion-infected brain tissues.
- The observation of these changes was performed by immunohistochemical method (western-blot) with anti – MT and anti-MT-III antibody applied to non-infected mice brains homogenates and Scrapie-infected mice brain homogenates.
- If metallothionein does play a role in PrP pathological change to PrP^{Sc}, there should be specific change in level of this protein.

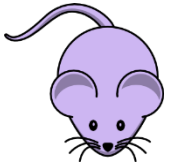


Mouse experiment

- Approximately 2 g of cerebral cortex brain material from confirmed positive cases of sheep with scrapie were homogenized and subsequently diluted in normal saline to produce a 10% (w/v) homogenate.
- Following confirmation of sterility by aerobic culture, each scrapie isolate was injected into the wild type C57BL/6 mice by combined intracranial (20-µl) and intraperitoneal (100-µl) routes.
- Mice were monitored for the development of clinical signs of mouse prion disease and euthanized at the time of appearance of terminal signs of disease.

Non-infected mice

Wild type mouse (genotypes C57BL/6, CD1, C57)



Mouse overexpressing PrP (genotype TGA 20)

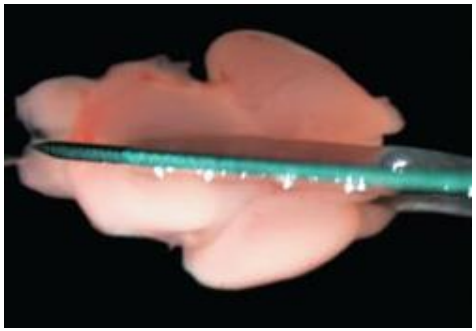
PrP knock-out mouse (genotype PrP -/-)

Prion-infected mice

Prion-infected mouse (genotypes RML, ME7, 470, 28, 37, 46, 475, 65, 634)

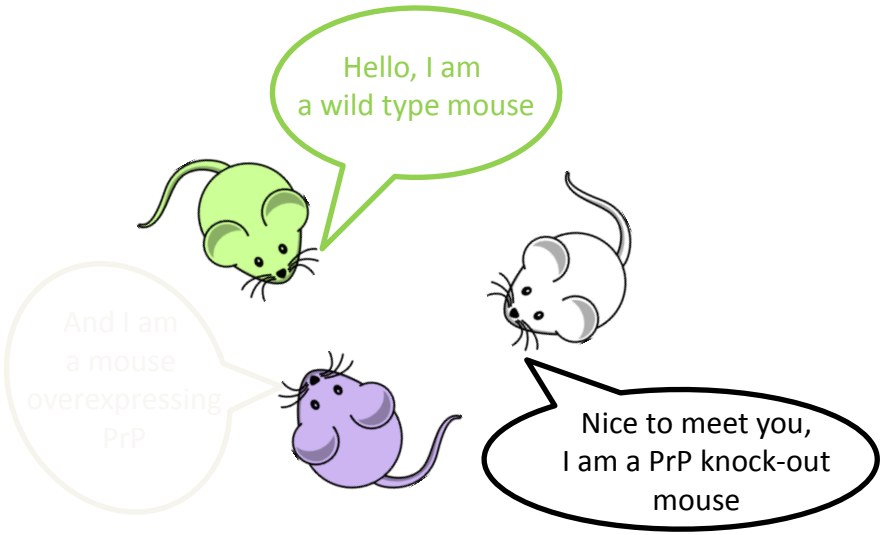
Methods

- A mouse brain was dissected in half by a saggital cut and homogenized at 10% (w/v) in lysis buffer (50mM Tris pH 7.5, 100mM NaCl, 0,5% Nonidet P-40, 1mM AEBSF) on Bio-Rad Homogenizer. After homogenization brain tissue was thoroughly resuspended with a pipet tip to achieve a homogeneous mixture and stored at -20°C.
- Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (180V/55min) and Western-blot (45mA/1hour) with various antibodies were performed.

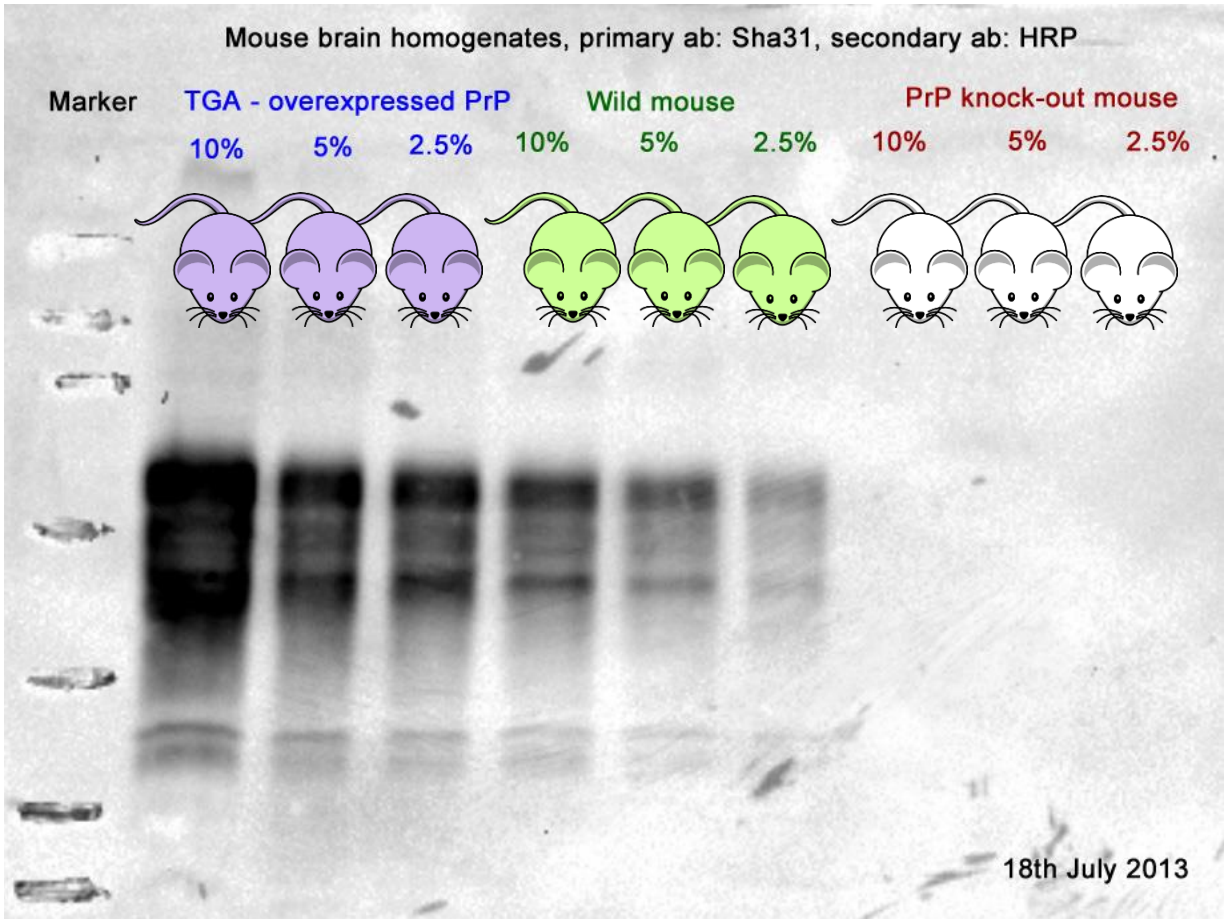


Results:

PrP and MT-III expression in non-infected mice brain tissues

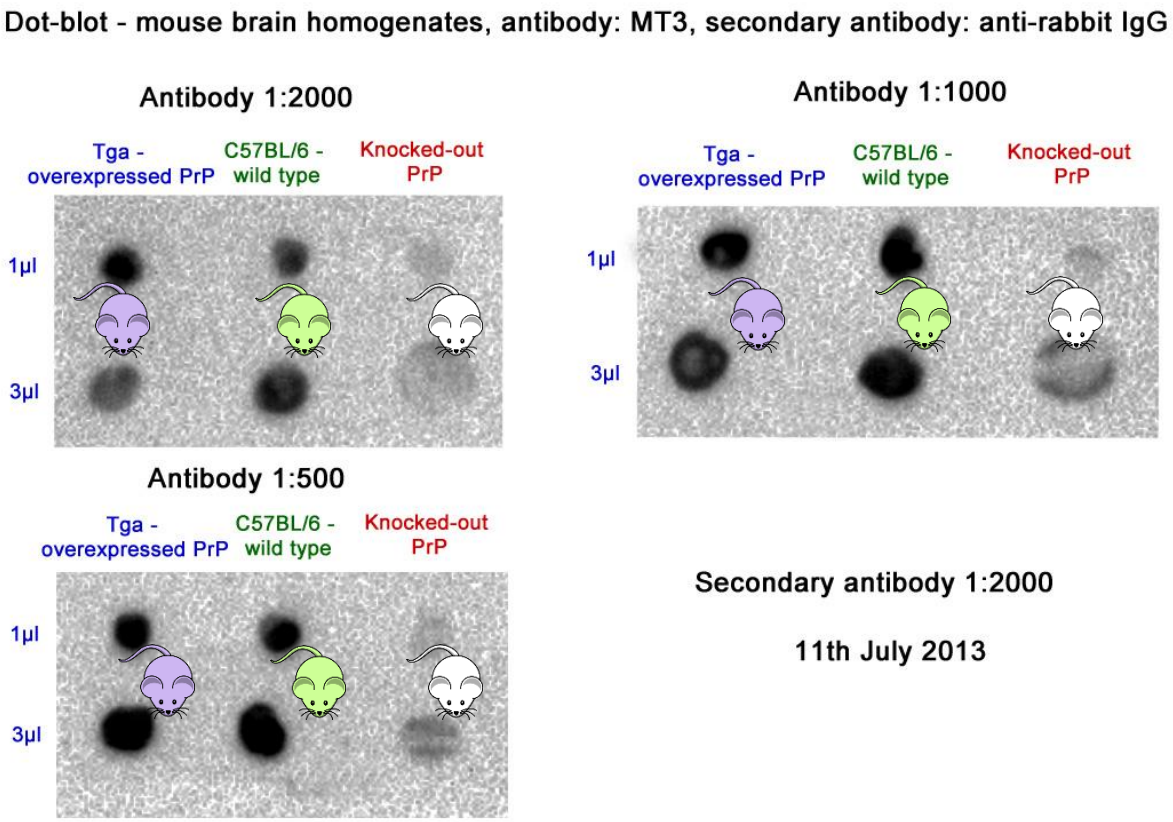


PrP expression in non-infected mice brain tissues (anti-PrP antibodies)



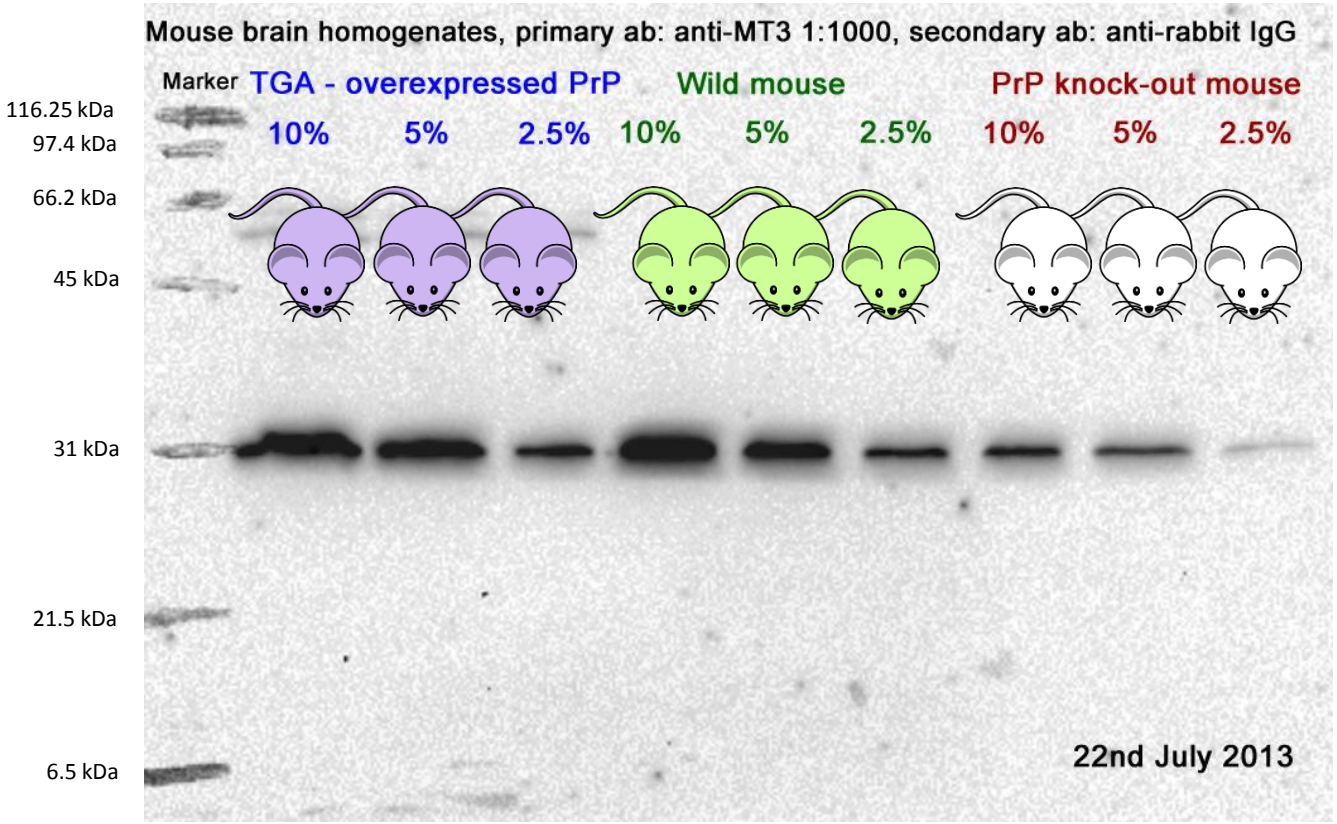
There is the highest level of PrP in case of mouse overexpressing PrP, lower level of PrP in case of wild type mouse and there is no PrP in case of PrP knock-out mouse. An identity of our samples was confirmed. Molecular weight of di-glykosylated PrP is 35 kDa, in case of mono-glykosylated PrP it is 27 kDa and non-glycosylated PrP is 20 kDa.

MT-III expression in non-infected mice brain tissues, dot-blot



MT-III level corresponds to the PrP level. The lowest level of MT-III protein expression can be seen in knock-out mouse brain tissue.

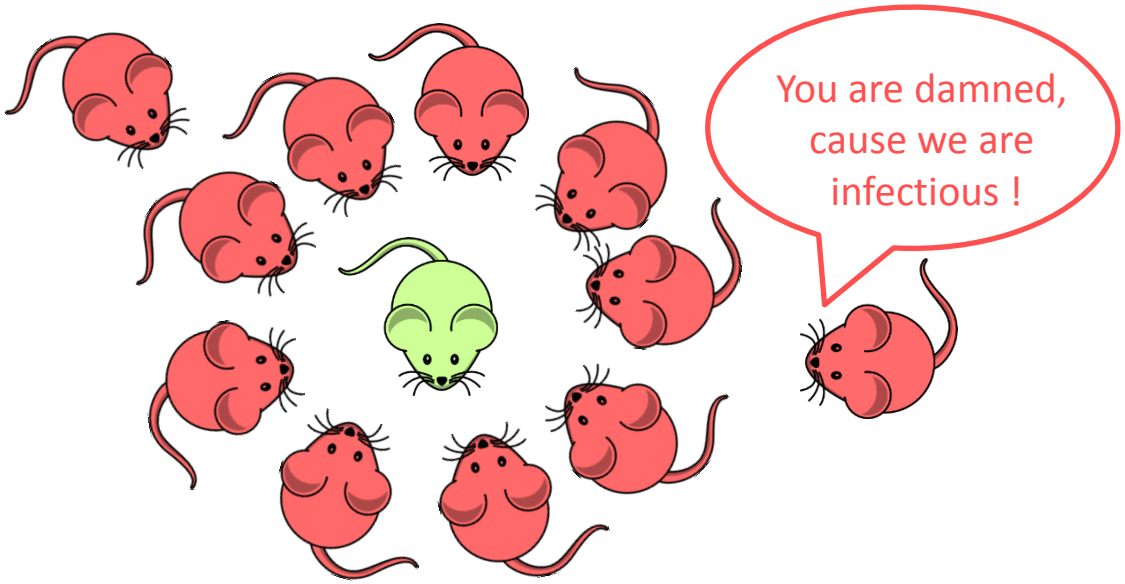
MT-III expression in non-infected mice brain tissues



The data in this figure show that MT-III level is decreased in case of PrP knock-out brain tissue but there is no important change of MT-III level between mouse overexpressing PrP and wild type mouse. **There is possible to see significantly lower level of MT-III in knock-out mouse brain homogenates.**

Results:

MT-III expression in prion-infected mice brain tissues



MT-III expression in prion-infected mice brain tissues

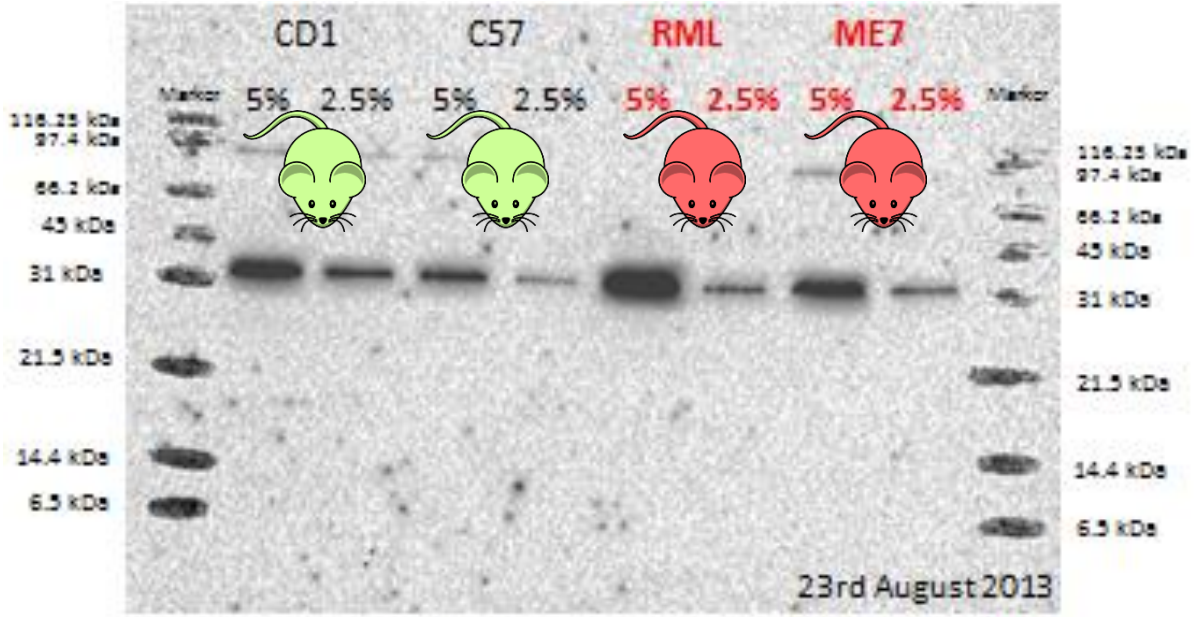
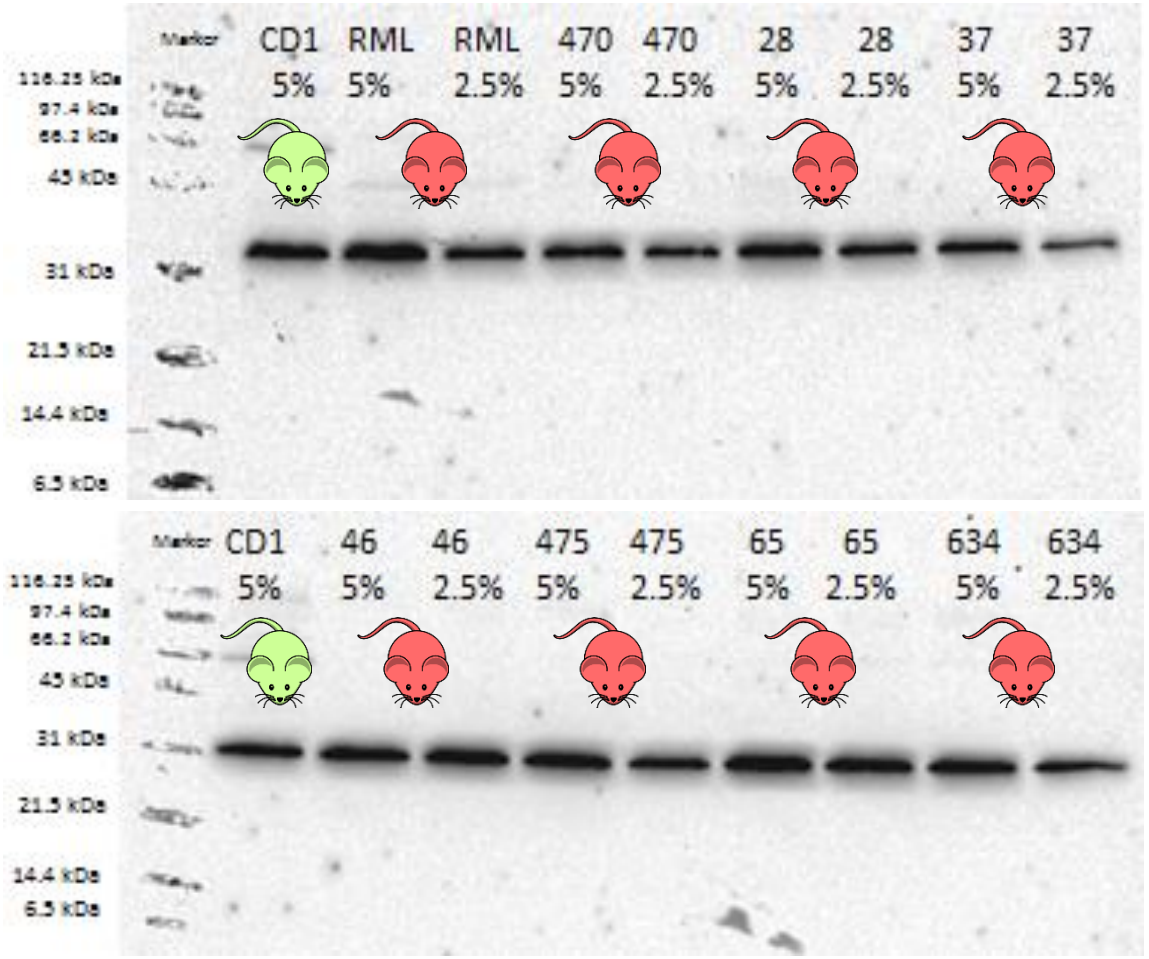


Fig. 14: Samples – CD1 and C57 – control mice brain homogenates
RML and ME7 – infectious (PrP^{Sc}) mice brain homogenates
Electrophoresis - 180V/57min, Western blot - 45mA/1hour

The data appear to show that MT-III level increases in case of RML prion-infected brain homogenate. In case of ME7 prion-infected brain homogenate there is no significant increase in MT-III level compared to CD1 or C57 non-infected mouse brain homogenates.
According to this figure we assumed that the MT-III level could be specific amongst various prion strains.

MT-III expression in prion-infected mice brain tissues – various prion strains



Prion-infected brain tissue homogenates have similar level of MT-III expression as non-infected tissues. Unfortunatelly, there is no significant difference between CD1 non-infected tissue and prion-infected tissues.

Conclusion

- There is a big difference in MT-III expression in wild type mouse, mouse over-expressing PrP and PrP knock-out mouse. There is dependence between the expression of PrP and MT-III amongst those tissues.
- **There is possible to see significantly lower level of MT-III in knock-out mouse brain homogenates.**
- The first experiment with prion-infected mice brain tissues showed the highest MT-III level in case of RML prion-infected brain homogenate whilst ME7 level is not significantly increased in comparison with non-infected tissues. This phenomenon appeared to be prion strain specific. However, further investigations of different mice adopted over the prion strains, showed this was not a case. The band intensity in case of prion infected brain homogenates is on similar level as non-infectious mouse brain homogenates.
- **There is no significant up or down regulation of MT-III in different prion strains in comparison with non-infectious mouse brain tissue.**
- In future we should perform an additional experiments focused on MT-III mRNA level in PrP knock-out tissues as well as in prion infected tissues.

- Doc. RNDr. Vojtech Adam, Ph.D.
- Dr. Raymond Bujdoso
- Dr. Alana Thackray
- Dr. Chang Zhang
- Ing. Pavlina Sobrova

First of all I would like to thank to the Laboratory of Metallomics and Nanotechnologies for the big opportunity I was given.

My other thanks belong to the whole team of Neuroscience and Molecular Immunology at the Department of Veterinary Medicine at University of Cambridge.

CAMBRIDGE INFECTIOUS DISEASES

Laboratoř metalomiky a nanotechnologií

Centrum excelentní vědy

evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Thank you for your attention

Reg.č.projektu: CZ.1.07/2.3.00/20.0148

Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik