

Supplementary

Flow cytometric analysis

Viability of cell cultures was determined by flow cytometric analysis using Fixable Viability Dye eFluor® 780 (eBioscience, San Diego, CA, USA). Flow cytometric analysis of stained cells was performed using a FACSCanto Flow Cytometer equipped with FACSDiva software (BD Biosciences) and data analysis was performed using FlowJo 7.6 (FlowJo LLC, Ashland, OR, USA).

Western blot analysis

Frozen surgical hippocampal specimens or cells in culture were homogenized in lysis buffer containing 10 mM Tris (pH 8.0), 150 mM NaCl, 10% glycerol, 1% NP-40, 0.4 mg/ml Na-orthovanadate, 5 mM EDTA (pH 8.0), 5 mM NaF and protease inhibitors (cocktail tablets, Roche Diagnostics, Mannheim, Germany). Protein content was determined using the bicinchoninic acid method. For electrophoresis, equal amount of proteins (50 µg/lane) were separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE, 12% acrylamide). Separated proteins were transferred to nitrocellulose paper by electroblotting for 1 h and 30 min (BioRad, Transblot SD, Hercules, CA). After blocking for 1 h in TBST (20 mM Tris, 150 mM NaCl, 0.1% Tween, pH 7.5)/5% non-fat dry milk, blots were incubated overnight at 4°C with the primary antibodies (Table 2; proteasome β 1, 1:1000; proteasome β 1i, 1:1000; proteasome β 5, 1:2500; proteasome β 5i, 1:1000; β -actin, mouse monoclonal, Sigma, St. Louis, MO; 1: 10,000; β -tubulin, mouse monoclonal, Sigma, St. Louis, MO; 1: 2000). After several washes in TBST, the membranes were incubated in TBST / 5% non-fat dry milk, containing the goat anti-rabbit or rabbit anti-mouse antibodies coupled to horse radish peroxidase (1:2500; Dako, Denmark) for 1 h. After

washes in TBST, immunoreactivity was visualized using ECL PLUS western blotting detection reagent (GE Healthcare Europe, Diegen, Belgium).

Quantification

The images were captured with an Olympus microscope (BX41, Tokyo, Japan) equipped with a digital camera (DFC500, Leica Microsystems-Switzerland Ltd., Heerbrugg, Switzerland). A total set of 6 images from 6 different cases were collected per pathology. Fiji (ImageJ2) was used for image processing. In a first step colour deconvolution (RGB colour space) was performed in order to separate positive cells from background according to the following channel parameters: red: 0.21408768, green: 0.8171735, blue: 0.4782719. Then a threshold (= 233) was applied and subsequently the images were converted to 8 bit gray-scale. The positive pixels/total assessed pixels, indicated as staining percentage area and intensity was calculated.

In situ hybridization

In situ hybridization (ISH) for $\beta 1i$ and $\beta 5i$ was performed using double digoxigenin (DIG) -labeled custom LNA oligonucleotides (Exiqon A/S, Denmark; see supplementary Table 1). The hybridizations were done on 5 μm sections of paraffin embedded materials as previously described [1]. The probes were hybridized at 56°C for 1 hour and the hybridization was detected with alkaline phosphatase (AP) labeled anti-DIG (Roche Applied Science, Basel, Switzerland). NBT (nitro-blue tetrazolium chloride)/BCIP (5-bromo-4-chloro-3'-indolylphosphate p-toluidine salt) was used as chromogenic substrate for AP. Negative control assays were performed without probes (sections were blank).

[1] Prabowo AS, van Scheppingen J, Iyer AM, Anink JJ, Spliet WG, van Rijen PC, et al. Differential expression and clinical significance of three inflammation-related microRNAs in gangliogliomas. *J Neuroinflammation* 12: 97.(2015).

Legends supplementary figures

Figure 1S. Representative immunoblot analysis of total homogenates from (n=3) surgical hippocampal specimens; β -subunits ($\beta 1$, ~ 25 kDa; $\beta 1i$, ~ 22 kDa; $\beta 5$, ~ 25 kDa; $\beta 5i$, ~ 25 kDa; β -actin ~ 42 kDa).

Figure 2S. $\beta 1i$ and $\beta 5i$ intensity signal in control, mMCD, FCDII and TSC.

FCD: Focal Cortical Dysplasia; TSC: Tuberous Sclerosis Complex; mMCD: mild malformations of cortical development.

Figure 3S. Effect of the different treatments on fetal astrocyte cell cultures. **A:** scatterplots of eFluor viability dye staining as analyzed by flow cytometry after different treatments. **B:** quantification of viable cells based on eFluor viability staining. Neither treatment with IL-1 β nor rapamycin negatively influenced viability of cell cultures. **C:** Western blot analysis showed effective reduction of phosphorylated S6 after 24 hours of 100 nM rapamycin treatment. FSC: forward scatter.

Figure 4S. Proteasome subunit immunoreactivity ($\beta 1$, $\beta 1i$ $\beta 5$ and $\beta 5i$) in mild MCD (mMCD) and in Alzheimer' s disease (Alz)

Panels A,C,E,G: mMCD. **A:** low $\beta 1$ expression (insert: high magnification of a neuron, with light nuclear expression). **C:** low $\beta 1i$ expression (insert: high magnification of a neuron). **E:** nuclear expression of $\beta 5$ (arrows; neuron in insert). **G:** low $\beta 5i$ expression (neuron in insert).

Panels B, D, F, H (**Alz**; hippocampus). **B**: $\beta 1$ expression in neurons (CA1; arrows, cytoplasmic expression) and around amyloid plaques (arrow-heads); **D**: $\beta 1i$ expression in glial cells (arrows, cytoplasmic expression). **F**: low $\beta 5$ expression in neuronal cells (arrows). **H**: $\beta 5i$ expression in glial cells (arrows, cytoplasmic expression). Scale bar in B: A,C, F,G: 80 μm ; B,D,H: 40 μm .

Figure 5S. *In situ* hybridization of $\beta 1i$ and $\beta 5i$, proteasome subunit immunoreactivity in control, focal cortical dysplasia (FCD) type IIb and Tuberos Sclerosis Complex (TSC).

Panels A-D: control cortex (A-C) and with matter (B-D); $\beta 1i$ (A-B) and $\beta 5i$ (C-D)
Panels E-F (FCD IIb) and panels C-G (TSC) shows strong expression within the dysplastic region with several positive dysmorphic neurons [arrows and inserts in E(a) and F], giant cells [inserts in G and H(a)] and glial cells [inserts (b) in E and H) Scale bar in H: A-H: 80 μm .

Supplementary table 1

| mRNA | Sequence | Hybridization temperature |
|--------------------|---|---------------------------|
| β 1i (PSMB9) | 5'DIG- +TmUmC+CmUmC+CmAmG+TmUmC+TmAmU+CmCmC+ A -3'DIG | 56°C |
| β 5i (PSMB8) | 5'DIG- +AmAmU+CmUmC+AmAmU+CmAmC+CmUmU+GmUmU+C -3'DIG | 56°C |

+ = LNA modification; m = 2'-O-methyl RNA base; DIG = digoxigenin label