Proceedings Book of the 10th Iberian Congress on Prions

19 - 20 May 2022, University of Trás os Montes e Alto Douro, Vila Real

Livro de Resumos do 10.º Congresso Ibérico de Priões

19 - 20 de Maio de 2022, Universidade de Trás os Montes e Alto Douro, Vila Real

in a group of humanized transgenic mice inoculated with certain CWD isolates. Such experimentally generated splenic humanized CWD prions (termed eHuCWD^{sp}) appear indistinguishable from prions in the brain sCJDMM1 patients on Western blot. Significantly, we found that eHuCWD^{sp} can efficiently infect not only humanized transgenic mice but also cervidized transgenic mice (Tg12). Tg12 mice infected by eHuCWD^{sp} produced prions and brain pathology that are practically identical to those of CWD-infected Tg12 mice. In contrast, prions from the spleen of humanized transgenic mice inoculated with sCJDMM1 (termed sCJD^{sp}), similar to prions from sCJDMM1 patient brains, is poorly transmissible in the Tg12 mice. These data demonstrate that high transmissibility of splenic prions in cervidized transgenic mice is a unique feature of acquired human CWD prions, and it may serve as a reliable marker to identify the first acquired human CWD cases.

O10 | Chronic wasting disease (CWD) risk assessment in Portugal - the genetic approach to study prion protein gene (PRNP) variability in Portuguese populations of three cervid species: Red deer, Fallow deer and Roe deer

Jorge C. Pereira[1], Nuno Gonçalves-Anjo[2], Estela Bastos[2], Leonor Orge[1,3], Ana C. Matos[4], Adelina Gama[1], Anabela Alves[1], Alexandra Esteves[1], Sara Rocha[2], Luís Figueira[4], Carla Lima[3], Filipe Silva[1], Fernanda Seixas[1], Isabel Pires[1], João Silva[3], Madalena Vieira-Pinto[1], Maria L. Pinto[1], Paula Mendonça[3], Paulo Carvalho[3], Paula Tavares[3], Roberto Sargo[1], Maria A. Pires[1]

1 Animal and Veterinary Research Centre (CECAV), University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal;
2 Centre for the Research and Technology of Agro-Environmental and Biological Sciences; (CITAB), UTAD, Vila Real, Portugal;
3 Pathology Laboratory, UEISPSA, National Institute for Agricultural and Veterinary Research, I.P.(INIAV) Oeiras and Vairão, Portugal;
4 Polytechnic Institute of Castelo Branco (IPCB), Castelo Branco, Portugal

Among the transmissible spongiform encephalopathies (TSEs), chronic wasting disease (CWD) in cervids is now the rising concern in wildlife within Europe, after the first case was detected in Norway in 2016, in a wild reindeer and until October 2021, a total of 34 cases were described in Norway, Sweden and Finland. The establishment of risk assessment projects, even in countries with no cases of CWD is very important to forecast possible contaminations.

The study of the genetics of the prion protein gene, *PRNP*, has been proved to be a valuable tool for determining the relative susceptibility to TSEs. In the present study we analyzed the exon 3 of *PRNP* gene in 235 samples from three species: red deer (*Cervus elaphus*), fallow deer (*Dama dama*) and Roe deer (*Capreolus capreolus*). Three single nucleotide polymorphisms (SNPs) were found in red deer – codon A136A, codon T98A, codon Q226E – and no sequence variation was found in fallow deer and roe deer. The low genetic diversity found in our samples are compatible with the ones found in previous studies in Europe. The comparison of our population with North American populations, suggest that the free-ranging deer from our study may present susceptibility to CWD, although lack of experimental data and the necessity of large survey are necessary to evaluate these populations.

Funding: This work was supported by the project WastingPrionRisk [POCI-01-0145-FEDER-029947 / PTDC/CVT-CVT/29947/2017] funded by the Portuguese Foundation for Science and Technology (FCT). FCT PhD grant [SFRH/BD/146961/2019] financed by FCT through FSE (Fundo Social Europeu). This work was also supported by national funds [UIDB/CVT/00772/2020], [LA/P/0059/2020] and [UIDB/04033/2020] by FCT.

O11 | Identification of biomarkers associated with endoplasmic reticulum stress in natural scrapie

Jenny Lozada[1], Marina Betancor[1], Sonia Pérez Lázaro[1], Rosa Bolea[1], Juan J. Badiola[1], Alicia Otero[1]

1 Centro de Encefalopatías y Enfermedades Transmisibles Emergentes, Universidad de Zaragoza-IA2, Zaragoza, Spain.

The accumulation of misfolded proteins such as PrP^{Sc} can alter endoplasmic reticulum homeostasis triggering the unfolded protein response (UPR). In this pathogenic event, the molecular chaperones play an important role. Several reports in humans and animals have suggested that neurodegeneration is related to endoplasmic reticulum stress in diseases caused by the accumulation of misfolded proteins. In this study,

we investigated the expression of three endoplasmic reticulum stress markers: PERK (endoplasmic reticulum kinase), BiP (binding immunoglobulin protein) and PDI (Protein Disulfide Isomerase) in sheep affected by natural scrapie in clinical and preclinical stages of the disease by immunohistochemical and western blot analyses. We observed a significant overexpression of BiP, PERK and PDI in clinical sheep compared to healthy controls in various areas of the brain. Our results suggest that the neuropathological and neuroinflammatory phenomena that develop in prion diseases cause endoplasmic reticulum stress in brain cells, leading to the accumulation of these proteins in different areas of the brain and triggering an UPR, which at the beginning could be a neuroprotective event, however, prolonged UPR activation could initiate neurodegeneration.

O12 | Ovine atypical scrapie failed to transmit to cattle

Timm Konold[1*], Janet Hills[1], Jemma Thorne[1], John Spiropoulos[1]

1 Pathology and Animal Sciences Department, Animal & Plant Health Agency Weybridge, United Kingdom.
** Timm.Konold@apha.gov.uk*

Classical bovine spongiform encephalopathy (BSE) in cattle was caused by the recycling and feeding of meat and bone meal contaminated with a transmissible spongiform encephalopathy (TSE) agent but it still remains unknown whether the agent originated from sheep or cattle. This study aimed to determine whether atypical scrapie could cause a BSE-like disease when transmitted to cattle.

Two groups of calves (five and two) were intracerebrally inoculated with atypical scrapie brain homogenate from two sheep with atypical scrapie. Controls were five calves intracerebrally inoculated with saline solution (group 1, culled as age-matched control for test group cull) and one non-inoculated animal (group 2). Cattle were clinically monitored until clinical end-stage or at least 96 months post inoculation (mpi). After euthanasia, the brain was collected for potential transgenic mouse bioassay and examined for TSE by Western immunoblot and immunohistochemistry.

Three of the five animals from test group 1 were lost due to intercurrent diseases at 23, 46 and 91 mpi, one animal was culled with BSE-like clinical signs at 48 mpi and one clinically unremarkable animal was culled at 106 mpi. Both test group 2 animals were culled with no obvious TSE signs at 106 mpi. One of the control animals in each group was also culled because of intercurrent diseases. None of the animals tested positive for TSEs. Bioassay in tg338 and tg110 mice using the brain of the clinical suspect was negative. The results do not provide any evidence that cattle are susceptible to atypical scrapie.

Oral Communications: Prion structure and biology

O13 | Cryo-EM Structure of the Amyloid Exon of a Human Prion-like Protein

Javier García-Pardo[1], Antonio Chaves-Sanjuan[2], Andrea Bartolomé-Nafría[1], Marcos Gil-Garcia[1], Cristina Visentin[2], Martino Bolognesi[2], Stefano Ricagno[2], Salvador Ventura[1*]

1 Institut de Biotecnologia i de Biomedicina (IBB) and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain.
2 Dipartimento di Bioscienze, Università degli Studi di Milano, Milano 20133.
**Salvador.Ventura@uab.cat*

The Heterogeneous ribonucleoprotein D-like (hnRNPDL) is a ubiquitously expressed prion-like RNA-binding protein required for RNA-processing. hnRNPDL has three alternative splicing (AS) isoforms differing in the number of low complexity domains (LCD): hnRNPDL1, hnRNPDL2 and hnRNPDL3. Mutations of the conserved Asp259 at the C-terminal Tyr/Gly-rich LCD of hnRNPDL cause autosomal dominant limb-girdle muscular dystrophy-3 (LGMDD3). Thus, elucidation of the molecular mechanisms underlying amyloid transitions in this protein is of biomedical relevance. We report the cryo-electron microscopy (cryoEM) maps at 2.5 Å resolution and the corresponding atomic model of hnRNPDL amyloid filaments reconstituted in vitro. The high-resolution structure revealed that fibril core is built up by the cross-β packing of a highly hydrophilic LCD region into a single filament. Importantly, this region includes Asp259, and precisely matches the sequence