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NOVEL MARKERS FOR NEURODEGENERATION

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Abstract — Prion diseases are incurable and fatal neurodegenerative disorders that affect both humans and animals. The causative agent is an infectious protein called prion (PrP^{Sc}), which is the pathological form of a normal protein (PrP^{C}) present on the cell membrane. The molecular mechanisms underlying prion replication and subsequent degeneration of the Central Nervous System (CNS) are still poorly understood and therefore innovative approaches are needed to build diagnostic, therapeutic, taxonomic, and disease surveillance tools. We adopted an unbiased genomic approach and conducted whole transcriptome analyses using microarray and RT-qPCR gene expression methods in brain of infected macaques versus healthy controls. We identified a set of genes that could become novel biomarkers for early diagnosis and/or therapeutic strategies for prion diseases and other neurodegenerative disorders.

Index Terms — TRANS2CARE, prion, prion protein, neurodegeneration, gene expression, genomics

1 BACKGROUND

Prion diseases are fatal neurodegenerative disorders that affect humans and animals. Their pathogenesis mechanisms are not fully understood and there is no diagnostic tool, nor a cure for them [1]. Among these disorders, the best known to the general public is the Bovine Spongiform Encephalopathy (BSE) or "mad cow disease" with the related human form called variant Creutzfeldt-Jakob Disease (vCJD). In this context, the analysis of gene expression alterations occurring in prion-infected animal models represents a powerful tool that may contribute to unravel the molecular basis of prion diseases and therefore discover novel potential targets for diagnosis and therapeutics.

2 OBJECTIVES

The goal of our project is identifying differentially expressed genes in infected animals versus controls that can become potential targets for diagnostic and/or therapeutic approaches.
Recent findings support the idea that neurodegenerative diseases may all share a common mechanism that implies a prion-like behavior. Therefore, even though prion diseases are rare disorders, basic research on their mechanisms may be useful to explain all the neurodegenerative maladies, like Alzheimer's and Parkinson's diseases, that affect large portions of the world population.

3 APPROACH & METHODS

General approach

We performed for the first time a large-scale gene expression profiling of brains from BSE-infected macaques, an excellent model for human prion disorders.

Methods

We performed an mRNA expression analysis using microarrays with subsequent validation of selected targets by RT-qPCR. mRNA was extracted from brain frontal cortex (superior frontal gyrus) of 6 intracranially BSE-infected animals (A1 to A6) and 5 non infected controls (CovA, CovB, CovC, CovDI, CovDII). All samples were analyzed using the GeneChip® Rhesus Macaque Genome Array (Affymetrix®) that contains 52,024 rhesus probe sets to enable gene expression studies of Macaca mulatta transcriptome interrogating more than 47,000 transcripts. RT-qPCR was performed using SYBR® Green-based assays (Bio-Rad Laboratories, Inc.) and then TaqMan® MGB probes (Life Technologies) at a later stage for results confirmation.

4 RESULTS

The microarray analysis revealed about 300 transcripts with expression changes around twofold. Among these, the Gene Ontology analysis identified 86 genes with known functions, most of which are involved in cellular development, cell death and survival, lipid homeostasis, and acute phase response signaling. 12 of these genes were analyzed by RT-qPCR and the regulation trend was confirmed. In particular, a 5 gene signature was identified in infected vs. control animals with statistical significance. These genes are involved in oxygen or lipid transport and in innate immunity as well as inflammatory response. These genes are known to be deregulated in other neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Their protein products may become potential targets for both general diagnosis and therapeutic purposes of many neurodegenerative diseases.

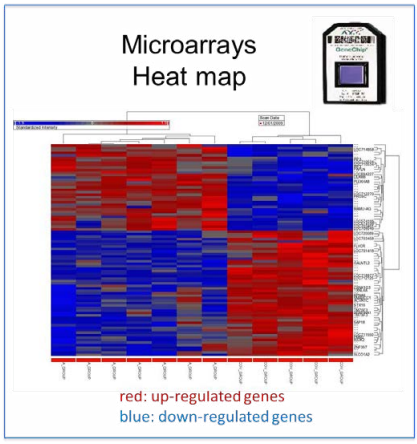

Figure 1: Condition trees of the clustering analysis

The hierarchical cluster analysis (Euclidean distance clustering algorithm) was performed using "R" and Partek® Software (Partek Inc.): 86 clones/genes showed a differential expression. Each colored bar represents a gene. The color represents the level of expression (red: up-regulation, blue: down-regulation) and the sample information is listed across the bottom. The names of the known genes are indicated on the right handside. A picture of a representative Affymetrix chip is captured on the upper right corner.

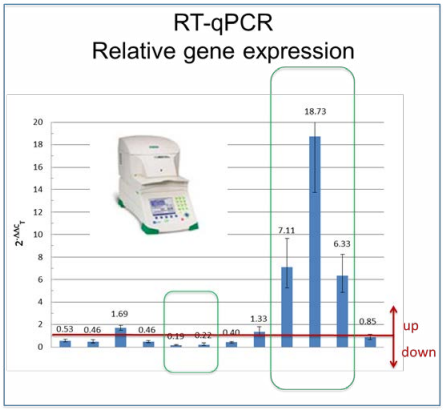

Figure 2: SYBR® Green-based RT-qPCR validation of microarray results

Validation by RT-qPCR was performed using gene-specific primer pairs and cDNA synthesis was accomplished using 100 ng RNA per sample. The relative expression ratio was calculated using the $\Delta\Delta C_T$ method and significance was calculated with the unpaired student t-test ($p < 0.05$). Among the 12 genes analyzed, 5 were found to be differentially regulated: 2 were down-regulated (HBA2 and HBB) and 3 were up-regulated (TTR, APOC1,SERPINA3) (green squares) with statistical significance [2].

5 POTENTIAL NEW PRODUCTS & SERVICES

Product 1: A qPCR-based diagnostic test for neurodegenerative diseases. We can conceive a microtiter plate containing lyophylized probes for the identified markers; patient samples will be added (brain biopsies, liquor, blood) and qPCR will be performed .

Product 2: An antibody-based diagnostic test for neurodegenerative diseases: major work is needed to validate the differential expression of these targets at protein level and in additional tissues more easily accessible than brain (e.g., liquor, blood). The antibodies will be absorbed on the microtiter plate and a colorimetric reaction will happen after adding the patient sample.

6 CURRENT COLLABORATIONS

6.1 With other researchers

Dirk Motzkus, Unit of Infection Models, German Primate Center, Göttingen, Germany;
Uroš Rajčević and Vladka Čurin Šerbec, BTCS, Ljubljana, Slovenia (PP10)

6.2 With hospitals

Policlinico and University of Verona, Italy; Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

7 CONTACT OR COLLABORATIONS NEEDED

Hospitals: access to diseased human samples and controls
Proteomics research centers: validation at protein level
Diagnostic companies: development of IVD/GMP/FDA-approved reagents and kits

8 COMMUNICATION TOOLS

Scientific publications
Barbisin M, Vanni S, Schmädicke AC , Motzkus D, Opitz L, Salinas-Riester G, Legname G. Gene expression profiling of brains from bovine spongiform encephalopathy (BSE)-infected cynomolgus macaques. BMC Genomics, 15:434, 2014.

Conference poster
Schmädicke AC , Barbisin M, Motzkus D, Opitz L, Gasperini L., Salinas-Riester G, Vanni S, Legname G. Whole transcriptome analysis in brains from BSE-infected macaques. Prion 2012, Amsterdam, May 9-12 2012.

Dissemination workshop
Barbisin M, Legname G. Gene expression profiling of BSE-infected macaques. Molecular tools to study neurodegeneration, Trieste, Sep 19 2013.

Media
http://it.linkedin.com/pub/maura-barbisin/2/6a3/598
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9 FUNDS NEEDED

9.1 For basic research (investigation of biological mechanisms): € 100,000.00

9.2 For applied research (solutions for real-world problems): € 300,000.00

9.2 For pilot & demonstrator activities (to develop a prototype): € 500,000.00

10 CONCLUSION

To our knowledge, this is the first genome-wide expression study in frontal cortex of macaques inoculated with BSE. Using microarray and RT-qPCR technologies we identified a gene signature able to distinguish infected macaques from control animals. These results could be extremely helpful in understanding the progression of the disease, allowing for the identification of some key players which, if not being the cause of the onset, could be some of the target genes affected by the disease. Our animal model is the closest available model for human vCJD and these results, obtained with an unbiased methodology as the gene expression microarray technology, are contributing to shed some light on the molecular basis of prion diseases as well as neurodegeneration as a whole.

ACKNOWLEDGEMENT

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[2] Barbisin M, VanniS, et al.: Gene expression profiling of brains from bovine spongiform encephalopathy (BSE)-infected cynomolgus macaques. BMC Genomics 2014; 15:434.