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trans2care
Transregional Network for Innovation and Technology Transfer to Improve Health Care

Transcriptome analysis of prion disease animal models

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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 are going to adopt an unbiased genomic approach and conduct whole transcriptome analyses using microarray gene expression methods in brain and/or blood of infected animals versus healthy controls. We hope to identify a set of genes that can be used for early diagnosis and/or as targets for therapeutic strategies. Within the Trans2Care project we intend to promote collaboration and exchange of knowledge to facilitate all partners' research objectives, and possibly find a common way to accelerate the process aimed at improving our healthcare system.

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

1 SISSA, INTERNATIONAL SCHOOL FOR ADVANCED STUDIES

SISSA, International School for Advanced Studies was established in Trieste in 1978, and it is one of the leading scientific institutions in Italy for postgraduate training in Mathematics, Neuroscience and Physics. There are thirteen Ph.D. courses available, covering the three main research areas, three professional master's courses and, thanks to an agreement with University of Trieste and University of Trento, three joint curricula for master's degrees (laurea magistrale) in Mathematics, Physics and Neuroscience. Besides being a school, SISSA is also an international research center

with financial support granted by private and public entities (Friuli Venezia Giulia Region, Italian Government and European Research Council). In 2010-2011 alone, Italian funding amounted to some € 5.5 million, while international grants provided over € 10 million. The Neuroscience Department (hereafter Department) is built on Cognitive Neuroscience and Neurobiology. The Cognitive Neuroscience group focuses on how the brain generates behavior (language, perception, action) using methods from artificial neural networks to human neuropsychology. The Neurobiology component of the Department is devoted to research on the nervous system, using a combination of molecular, cellular and integrative approaches. One specialized division of the Neurobiology area is devoted to the study of neurodegenerative diseases (such as Alzheimer's, Parkinson's, Huntington's and Prion diseases), focusing on Functional and Structural Genomics. The Prion Biology Laboratory is part of the Genomics branch of the Department.

2 THE PRION BIOLOGY LABORATORY

The Prion Biology Laboratory currently includes one associate professor, four post-doctoral fellows, nine Ph.D. students and two undergraduate students. We have a Biosafety Level 2 facility with a cell culture room as well as protein expression and purification equipment. In addition, we have access to core facilities and technologies available in the Department such as an animal facility carrying transgenic animals, histology room, DNA sequencing, confocal microscopy, Real-Time PCR and Affymetrix micro-array platforms.
The laboratory is supported by various grants and organizations such as IIT, PRIN, FIRB, FP7, and so on. Just to mention one example, recently € 5 million were granted to our laboratory and another group in the same Department to identify the changes in the human genome leading to a number of incurable neurodegenerative diseases (FIRB-Programme agreements 2011; project title: Functional Genomics of Neurodegenerative Diseases). Within this same line of research we became partners of the international strategic project Trans2Care, FESR 2007-2013 for the cooperation between Italy and Slovenia.

2.1 Research Activities

The focus of the Prion Biology Laboratory is studying prion diseases, rare and fatal neurodegenerative maladies that affect humans and animals, for which there is no diagnostic tool, nor a cure [1, 2]. In mammals, prions reproduce by recruiting the normal, cellular isoform of the prion protein (PrP^{C}) and stimulating its conversion into the disease-causing isoform (PrP^{Sc}). PrP^{C} and PrP^{Sc} have the same amino acid sequence, but distinct conformations: PrP^{C} is rich in α-helical content and has little β-sheet structure, whereas PrP^{Sc} has less α-helical content and is rich in β-sheet structure (Fig. 1). The conformational conversion of PrP^{C} to PrP^{Sc} is the fundamental event underlying prion diseases, and it is still poorly understood. The main research lines of the laboratory are: therapy of prion disease [3], physiology of the prion protein [4], syntethic prions and molecular determinant to infectivity [5], structural biology

and biophysics of the prion protein [6], transcriptomics and neurodegeneration [7], molecular mechanisms of neurodegeneration [8].
The project funded by the Trans2care grant is focused on the analysis of the whole transcriptome of animal models affected by prion disease. The goal is identifying genes that can become potential targets for diagnostic and/or therapeutic approaches. Our collaborators identified some candidate genes by microarray gene expression analyses in brain tissue of a primate model of prion disease and they appear to be very promising. Our objective is validating these candidates using a more sensitive and accurate technology such as qRT-PCR. A second model we are using is cattle infected with BSE (Bovine Spongiform Encephalopathy or mad cow disease). In this case the tissue is blood and we are performing some QC tests of the samples before embarking in the microarray gene expression analysis and subsequent validation with qRT-PCR.
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 disease, that affect large portions of the world population.

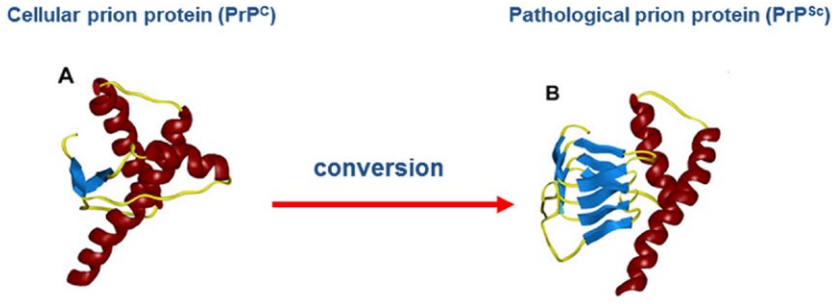

Fig.1. The conformational conversion of PrP^{C} (A) to PrP^{Sc} (B).

3 ROLES IN TRANS2CARE

3.1 What we can offer to T2C

We have focused our research projects on exploring the infection mechanisms of prions from various angles; therefore we have gained expertise in many fields, from protein expression and purification to animal models, as well as gene expression analyses. We can provide T2C partners with our knowledge to facilitate the exchange of expertise within the network, and at the same time we can take advantage of other partners' technological skills to further understand prion replication mechanisms and subsequent degeneration of the CNS. We have in fact an ongoing collaboration with ZTM - Blood Transfusion Centre of Slovenia (PP10) that we intend to exploit further.

So far PP10 has developed a panel of mAbs against different moieties of the prion protein and some of them can distinguish and differentiate between the wild type PrP^{C} and its pathogenic form PrP^{Sc}. They will be tested in our laboratory mainly for the detection of PrP in immunohistochemical procedures (i.e. Western Blot, ELISA and IF), but they may also become important diagnostic and/or therapeutic tools. We are trying to expand this collaboration in the direction of targeted proteomics using Multiple Reaction Monitoring assays (MRM Proteomics). In this way markers obtained by transcriptomic analyses could be directly screened at the protein level in samples of either animal models or patients.
We are going to seek additional opportunities to collaborate with other project partners.

4. CONCLUSIONS

The aim of the Prion Biology Laboratory is conducting high level research in the field of neurodegeneration and in particular unraveling aspects of prion diseases that are still poorly understood. Within the Trans2Care framework, we intend to employ gene expression profiling methods to identify gene candidates that may become potential diagnostic biomarkers and/or therapeutic targets. The participation in the Trans2Care initiative can be very helpful in opening new collaborations with the partners or expanding existing ones that may lead to joint discoveries and publications. In addition, having the opportunity of interacting with such diverse partners ranging from basic research institutes to technology centers and clinics, may foster exchange of ideas that can lead to potential technology transfer opportunities to improve our healthcare system.

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