Relazione Scientifica dell'attività svolta (Programma STM 2016)

Il Fruitore:

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Istituto di afferenza:

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con qualifica:

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Istituzione ospitante:

Centre for Misfolding Diseases (CMD), Department of Chemistry - University of Cambridge, Lensfield Road, Cambridge CB2 1EW

Titolo del programma:

MudPIT proteomics of C. elegans models for neurodegenerative diseases

Descrizione del progetto ed attività svolta:

Neurodegenerative diseases, such as Alzheimer's disease (AD), are incurable conditions that consist in the progressive degeneration and death of specific neurons in the brains of the victims. These pathologies are a major cause of disability and premature death particularly among older people. They are not only fatal illnesses, but that they also represent a devastating social justice issue; such pathologies dramatically affect the life of the victims, their caretakers, and relatives. Early diagnosis and effective treatment for these pathologic conditions remain elusive. This fact results, at least to some extent, from our limited understanding of the fundamental nature and mechanisms of such diseases. A common early feature of these disorders, however, is that specific peptides and proteins may give rise to toxic aggregates in the brain matter. In the case of AD, the conversion of the Aβ-amyloid peptides into pathogenic aggregates is linked to its onset and progression. The presence of these aberrant aggregates can generate a cascade of pathological events, leading to the failure of protein

homeostasis and the loss of normal biological function. One promising avenue for progress in the development of therapeutic approaches for neurodegenerative disorders is to improve our understanding of the mechanisms by which cellular dysfunction arises from the initial protein aggregation events.

In light of this, we proposed to develop an integrated platform based on *C.elegans* models and shotgun proteomics for the identification of novel therapeutic targets, correlating proteomic data with phenotypic changes in aβ42-amyloid worm models in the absence/presence of specific therapeutic treatments against Alzheimer's disease.

The nematode *Caenorhabditis elegans* (*C. elegans*, Fig.1) is an excellent animal model for the study of pathologic conditions and particularly neurodegenerative disorders. It is a small (1 mm in length), transparent roundworm, and easy to manipulate. It has a short maturation period of 3 days from egg to adult at 25°C and its life span is between 2 and 3 weeks, which facilitates the study of its biology. For all these reasons, *C. elegans* has developed into an important model for biomedical research, particularly in the functional characterization of novel drug targets that have been identified using genomics technologies. The Centre for Misfolding Diseases (CMD) has access to a library of pathology models of *C. elegans* and it is equipped with the state of art instrumentation for protein aggregation studies and in vivo toxicity analysis in *C. elegans*. At the same time, ITB-CNR Proteomics Unit, employed a strategy based on application of gel-free proteomic methodology named MudPIT (Multidimensional Protein Identification Technology) in order to evaluate the differential protein expression profiles in Alzheimer-related *C.elegans* models. Proteomic methodologies are of primary importance in clinical investigations because mass spectrometry (MS) has the ability to monitor tryptic peptides in complex biological mixtures with high sensitivity and specificity. In particular, mass spectrometry-based proteomics represents a great promise as a discovery tool for biomarker candidates in the early detection of human disease.

In details, the activities of this project involved the application of our proteomic expertise to investigate potential therapeutic antibodies, available at CMD, against

Alzheimer's disease using aβ42-amyloid *C.elegans* models towards the identification of a sub-proteome (Fig.2).

During the visiting period at Cambridge University, it was prepared the experimental design with the Vendruscolo's Team and performed the optimization of *C.elegans* models of Alzheimer's disease to evaluate a specific antibody's treatment, tested the efficacy of specific single-domain antibodies (10KDa), rationally designs by CMD, in order to inhibit the aβ42-protein aggregation in worms. Specifically, aβ-neuronal (CL2355) and aβ-muscular (GMC) *C.elegans* models were submitted to the therapy and morphological changes were registered (Motility Assay).

In detail, for each biological replicates were cultured:

- wild type *C. elegans* worms (N2);
- aβ-neuronal model (CL2355);
- aβ-muscular model (GMC);
- CL2355 treated with the antibody;
- GMS treated with the antibody.

All experiments were carried out in triplicate and the worms pellets from each condition were freezing and sent to ITB-CNR Proteomics Unit for proteomics analysis. Currently, we proceeding with the development of experimental protein extraction protocol for *C. elegans* and the subsequent MudPIT analysis of protein extracts. The next step will be to obtain the protein profiling of *C. elegans* models of AD and evaluate, at the proteomic level, the effect of antibody treatment. In particular, the differentially expressed proteins between different growth/treatment conditions will be identified by means of MAProMa software and the metabolic pathway changes extracted by means of PCA and Cytoscape analysis will be investigated.
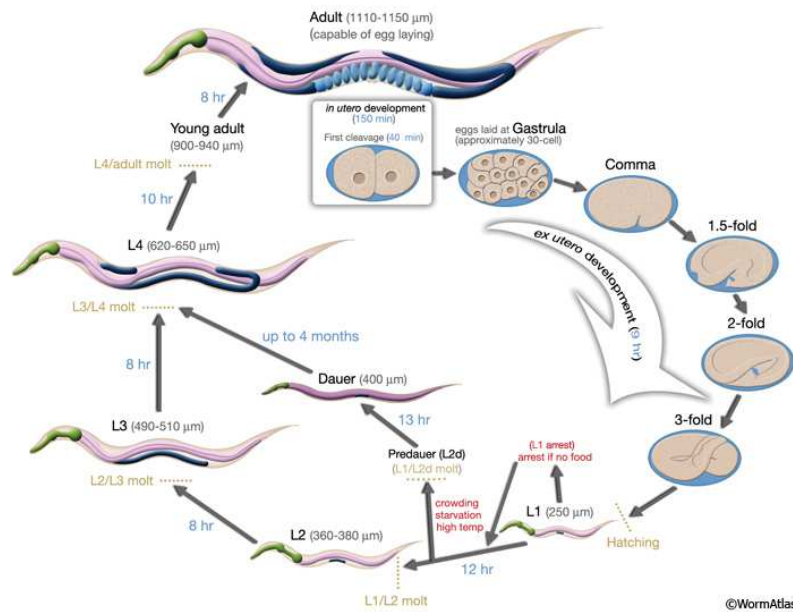
Fig. 1. Life cycle of *C. elegans*. The life cycle of *C. elegans* is comprised of the embryonic stage, four larval stages (L1-L4) and adulthood.
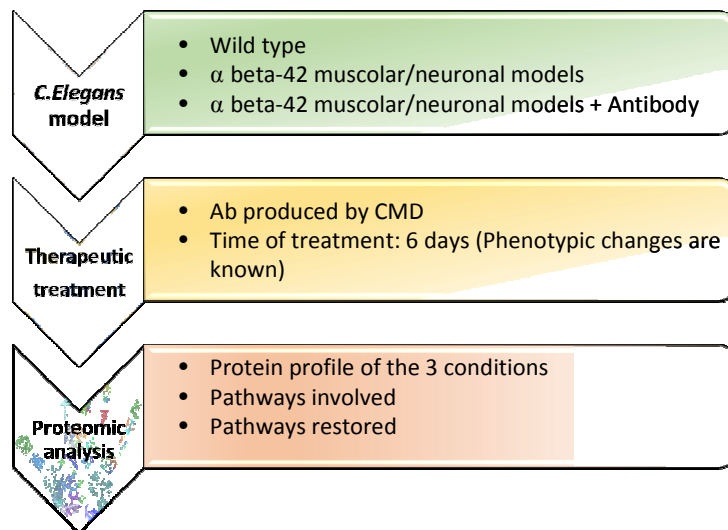

Fig. 2. Experimental Design. The project involved the application of proteomic approaches to investigate potential therapeutic antibodies designed against Alzheimer's disease using aβ42-amyloid *C.elegans* models.