UNIVERSITÀ DEGLI STUDI DI MILANO-BICOCCA

DOTTORATO DI RICERCA IN Tecnologie Convergenti per i Sistemi Biomolecolari – XL CICLO

Research Topic ID: XL – 1.21

Proponent: Prof. Maria Elena Regonesi

Project Title: Searching for natural compounds capable of counteracting the pathogenic aggregation of human ataxin-3

Scientific background and 'open issues'

Contrasting human neurodegenerative diseases and their extensive societal impact is becoming a global priority. Ageing is a major risk factor for neurodegenerative diseases, and with a growing elderly population, their prevalence is continuously increasing [1]. Despite their devastating effect on human quality of life and their huge economic impact on healthcare systems and families, many human neurodegenerative diseases remain incurable [1]. Belong these, the Spinocerebellar ataxia type 3 (SCA3) is the most common spinocerebellar ataxia worldwide, accounting for approximately 50–80% SCAs [2]. SCA3 belongs to the polyglutammine disease family, each caused by a different protein carrying a stretch of consecutive glutamines (polyQ) expanded beyond a critical threshold. The causative agent of SCA3 is ataxin-3 (ATX3) whose pathogenic variants have a polyQ tract of 54-89 residues [3,4]. This repetition leads to the expression of a pathological protein that is able to form intracellular amyloid aggregates, which lead to neuronal death by different cellular mechanisms [5]. To date, no effective treatment has been developed for SCA3. One possible therapeutic strategy is based on the treatment with compounds capable of contrasting and preventing the formation of such toxic aggregates. In this context, regular intake in diet of natural compounds capable of interfering with toxic oligomers could be very effective. Indeed, interest in nutraceuticals and functional foods has greatly increased in recent years, and the potential benefits of their consumption being related to the prevention not only of disorders of aging, but also of certain types of cancer, and cardiovascular diseases [6]. It has been reported that flavonoids belong to the well-known class of molecules capable of counteracting aggregation of a large variety of amyloid proteins [7-9]. In our previous works, we have demonstrated the capability of epigallocatechin-3-gallate (EGCG), the most represented tea catechin, to counteract pathogenic ATX3 aggregation *in vitro*, and substantially reduce toxicity *in vivo*, as shown in our ataxic *in vivo* model [10]. Recently we have published a work on the antiamyloidogenic properties of the *Lavado Cocoa* extract on ATX3, a natural matrix enriched of flavanols [11].

Objectives

In the present project will be pursued the following tasks:

- Identification of natural matrices extracts capable of affecting ATX3 aggregation process both *in vitro* and *in vivo* and reducing toxicity in our ataxic *C. elegans* model.

- Characterization and isolation of the extract components directly responsible of the anti-amyloidogenic properties.
- Uncover the pathways involved in the ataxic phenotypic rescue, in order to elucidate the mechanism of action of the compounds and their synergistic effect in the extract.

Methodologies

In the last years, we have developed a technological platform suitable for investigating structural changes and biological effects of pathogenic ATX3 variants. The available technology includes different biochemical assays useful to follow the ATX3 aggregation process and biophysical methods (FTIR spectroscopy) that detect *in vitro* the structural features of the aggregation intermediates. Moreover, our platform includes an animal model (*Caenorhabditis elegans*) which expresses ATX3 physiological or pathogenic variants in the nervous system, suitable to investigate protein toxicity. The extract will be provided by Tutor' laboratory of this project and this collaboration will further permit to characterize and isolate the bioactive compounds from natural extract combining advanced preparative and analytical techniques. Moreover, our *C. elegans* model will be employed to evaluate the benefic effect of the extract and of the isolate compounds on the ataxic phenotypes, i.e. lifespan and mobility, ROS level, amyloid plaques quantification, neural disfunctions. Confocal microscopy will be performed to determine the distribution and the size of the ATX3 aggregates, exploiting the GFP-TAG fluorescence. Finally, to deepen evaluate the mechanism at the basis of the phenotypic rescue observed will be relevant evaluate the changing in specific protein levels involved in mitochondrial functionality and oxidative stress pathways.

Collaboration / Co-tutoring opportunities

The FTIR analyses will be performed in collaboration with Prof. Antonino Natalello and Dr. Diletta Ami in the Biophysics laboratory of our department. The extract purification and characterization will be performed in collaboration with BioOrgNMR Laboratory led by Prof. Cristina Airoldi and Prof. Alessandro Palmioli.

Project's Sustainability & Mobility

The Proponent and the collaborators have knowledge of this disease pathology, master sophisticated technologies for structural studies, and have a clear know-how in biochemical/biophysical characterization of ATX-3 with a high-profile track record (the last published paper is: Sciandrone, B., Palmioli, A., Ciaramelli, C., Pensotti, R., Colombo, L., Regonesi, M.E., and Airoldi, C. (2024). Cell-Free and In Vivo Characterization of the Inhibitory Activity of Lavado Cocoa Flavanols on the Amyloid Protein Ataxin-3: Toward New Approaches against Spinocerebellar Ataxia Type 3. ACS Chemical Neuroscience, 15, 2, 278-289).

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