DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di assegnazione di una borsa di Dottorato

Titolo:

English:

Rescue strategies for reverting the phenotype caused by the autism-linked mutation R451C in neuroligin3: in vitro and in vivo approaches.

Italian:

Strategie per recuperare il fenotipo causato dalla mutazione R451C in neurolighina3: approcci *in vitro* ed *in vivo*.

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DESCRIZIONE DELLA RICERCA (max 2 pagine)

Objectives:

Autism Spectrum Disorders (ASDs) are neurodevelopmental syndromes, characterized by behavioral deficits and a strong genetic background.

Among the genetic risk factors, the R451C substitution in the synaptic protein Neuroligin3 (NLGN3) has been highly characterized. It is known from *in vitro* studies, that the mutation affects folding of the extracellular domain of the protein, causing its retention in the Endoplasmic Reticulum (ER) (Comoletti et al., 2004; De Jaco et al., 2010).

The ER plays a central role in the cell assisting synthesis, folding and glycosylation of one-third of the cellular proteome and allowing only proteins correctly folded to undertake the secretory pathway (Molinari, 2007). The accumulation of misfolded proteins and the activation of the Unfolded Protein Response (UPR) are hallmarks of several disorders, such as neurodegenerative diseases (Garcia-Huerta et al., 2016). Recently, increased levels of UPR markers have been correlated with Autism Spectrum Disorders (ASDs) both in a mouse model and in humans (Kawada and Mimori, 2017; Crider et al., 2017).

A growing interest is focused on small-molecule drugs with chaperone-like activity, able to restore protein folding, by reducing the accumulation of misfolded proteins and re-establish normal trafficking to the appropriate subcellular localization.

Scopo del presente progetto e (risultati preliminari)

With this project, we propose to develop a strategy to rescue NLGN3 folding allowing the exit of the mutant protein from the ER and restoring its physiological trafficking to the cell surface. This will represent a possible strategy to treat the monogenic form of ASDs, caused by the R451C mutation in NLGN3, but it is potentially useful for other protein misfolding diseases due to the retention of mutant proteins in the ER.

An *in vitro* cell-based model system to study NLGN3 folding and trafficking, has been generated in our lab, by using HEK293 cell lines stably expressing a truncated form of NLGN3, either WT or R451C, C-terminally fused to Venus, an analog of the Green Fluorescent Protein (Giepmans et al., 2006). These cell lines produce a fluorescent NLGN3 that is secreted by the cell, allowing to evaluate protein trafficking by measuring fluorescence levels in the cell culture medium. Preliminary results show that the fluorescence in the medium of R451C NLGN3-Venus cell lines

show a significative reduction of the mutant protein secretion (~50%) compared to the WT protein, that was also confirmed by western blot analysis.
From the screening of a library of 2662 compounds approved by the American Food and Drugs Administration (FDA), we selected 12 compounds, which showed a 2-fold increase in the fluorescence levels for the R451C NLGN3-Venus cell lines, in comparison to the untreated condition. It is noteworthy that most of these compounds belonged to the glucocorticoids family, which is known to increase the levels of ER-resident chaperones (Das et al., 2013).
We propose to further characterize the effects of these compounds on analyzing protein degradation through Endoplasmic associated degradation (ERAD), on restoring binding to Neurexin, the pre-synaptic partner protein and their effectiveness on improving trafficking of the full length form of the protein by co-localization staining with the ER marker calreticulin and confocal microscopy.
The effects of the selected compounds will be validated *in vivo* by using the knock-in (KI) mouse model with the R451C substitution in the endogenous gene (Tabuchi et al., 2007) available in the animal facility of our department. This mouse strain presents reduced NLGN3 levels in the brain, suggesting that the mutation causes protein instability *in vivo*, similarly to what observed *in vitro*. In addition, the R451C mice manifested autistic-like behaviors and a gain of function in the neuronal circuitry characterized by enhanced spatial learning and LTP in hippocampus (Etherton et al., 2011) and impaired LTD in the dorsal striatum (Martella et al., 2017).

Stato delle conoscenze

In the last years, the research field on diseases correlated to the accumulation of misfolded proteins has been growing quickly. Collectively called protein-misfolding diseases, they include common neurological and non-neurological disorders characterized by the accumulation of misfolded proteins resulting in a double effect: a loss-of-function due to the mis-localization of the protein, and protein accumulation, which in turn can lead to a toxic gain-of-function and in most cases to ER stress conditions. In this contest, the research for small molecules able to facilitate protein folding and their escape from the ER is of growing interest.
The focus of our work are the biological effects caused by the Arg451 to Cys substitution (R451C) in the extracellular domain of the post-synaptic protein Neuroligin3. This mutation, found in patients with ASDs, causes the retention of the protein in the ER and its loss at the synapses where it plays a crucial role as adhesion molecule (Baig et al., 2017). Our recent work established the first evidence between the retention of the R451C NLGN3 in the ER and the activation of UPR (Ulbrich et al., 2016). Moreover, mice carrying the R451C mutation in the endogenous NLGN3 gene show altered neurotransmission in several brain areas correlated to ASDs (Tabuchi et al., 2009; Etherton et al., 2001; Trobiani et al., 2018).
Our project aims at finding compounds able to restore the defective trafficking of R451C NLGN3 to the cell surface in order to rescue cellular phenotypes *in vitro* and *in vivo*. Helping the mutant NLGN3 to exit the ER would be beneficial in two ways: restoring proper protein localization for its function, and alleviating the overload of the ER, reducing ER stress and UPR activation.

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Lavori più rilevanti pubblicati negli ultimi 5 anni dal Docente Guida (2012-2017)

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Fondi attualmente disponibili per svolgere il programma di ricerca.

Progetti medi Ateneo **2017, 2018**
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