RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis

Reporting

Project Information

ExItALS

Grant agreement ID: 752470

Project website

DOI
10.3030/752470

Project closed

EC signature date
2 March 2017

Start date
1 April 2017

End date
31 March 2019

Funded under
EXCELLENT SCIENCE - Marie Skłodowska-Curie Actions

Total cost
€ 180 277,20

EU contribution
€ 180 277,20

Coordinated by
UNIVERSITA DEGLI STUDI DI TRENTO
Italy

Periodic Reporting for period 1 - ExItALS (RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis)

Reporting period: 2017-04-01 to 2019-03-31

Summary of the context and overall objectives of the project

Amyotrophic Lateral Sclerosis, or ALS, is a rare neurodegenerative disorder the primarily targets upper and lower motor neurons – leading to the inability to control voluntary movements. Like other chronic neurodegenerative diseases, ALS starts in one focal point before spreading to the entire nervous system, including the brain and spinal cord.
As the disease spreads, motor neurons, that control specific muscle movements, begin to degenerate, causing the individual to initially experience difficulties swallowing or performing fine motor movements. Gradually all muscles under voluntary control are affected, causing the individual to lose the ability to speak, eat, move and even breath. Thus, most people with ALS die of respiratory failure, typically within three to ten years from when they first start experiencing symptoms.
There is no cure for ALS, so our focus is on trying to determine what causes the disease to spread. If we know this, then we can begin developing strategies for stopping it from spreading.
Motor neurons are supported by glial cells, accessory cells that have been shown to play an important role in the progression of ALS. One of the ways in which glia cells and neurons communicate is through nanoparticles called extracellular vesicles, or EVs. Extracellular vesicles are small pieces of cells that can be formed in various ways and that are released by the cells constitutively. EVs are loaded with proteins, RNA, and metabolites that reflect the content of the cells from which they are released. Identifying the EV content that breeds toxicity would allow us to better understand how ALS is spread.
In order to test whether glia-derived EVs propagate neuronal death, we took advantage of a mouse model that recapitulates ALS pathology. This mouse model expresses a mutant form of TDP-43, an RNA-binding protein that is the main constituent of protein inclusions present in the spinal cord and brain of sporadic and familial ALS patients. We produced glia cultures and purified EVs with a novel and efficient method, called NBI. We tested the effect of glia-derived EVs on wild type neurons and observed that ALS-derived EVs induced motor and cortical neuronal death. We searched for the EV components transmitting toxicity by using –omics techniques, associated with bioinformatics analysis. To validate our functional data, we also developed new protocols to ‘inactivate’ proteins or RNA cargoes of ALS-EVs. As a result, we have novel candidates for EV-mediated toxicity in ALS that we aim to validate in peripheral bio-fluids to accelerate ALS diagnosis. Furthermore, the outcome of this project will provide new pathways involved in disease progression that can be targeted for therapeutic intervention.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far

Taking advantage of genetically engineered TDP-43 Transgenic (Tg) mice expressing a mutation in glutamine (Q) to lysine (K) at amino acid 331 (TDP-43 Q331K), we tested the ability of EVs derived from mutant astrocytes to propagate toxicity to wild-type neurons and motor neurons. We further focused on which component of the EVs would be responsible for the propagation of toxicity. We set up a novel and not yet published methods to generate i) EVs unloaded of the majority of the protein cargos and ii) EVs containing ‘inactivated’ RNA. At the same time, we performed an unbiased characterization of the protein and RNA cargos through a iii) proteomic and iv) small RNA sequencing

analysis. Surprisingly, we did not detect the mutant RNA-binding proteins as the major constituents of the EVs protein cargos, suggesting that in ALS, RNA binding proteins do not propagate toxicity through EVs per se. Interestingly, protein cargos differ significantly between wild type and disease condition. Thus, we are currently testing the hypothesis that mutant TDP-43 modulates the proteins and the messages sent to the neighboring cells through EV release. We are investigating a new mechanism of TDP-43-mediated toxicity that will potentially unveil a new piece of relevant ALS biology, and, in turn, suggest novel venues of therapeutic intervention. Along with this mechanistic study, we correlated the data obtained in EVs derived from primary astrocytes to the plasma EVs from ALS patients and neurologic or healthy controls. We set up novel methods in which we show how EVs derived from the central nervous system (CNS) can be detected in the periphery.
In conclusion, our results support the notion that in ALS, the content of extracellular vesicles contributes to toxicity propagation and disease progression. In particular, our data suggest that the expression of mutant TDP-43 alters intracellular pathways that influence the protein loading in extracellular vesicles. This concept, presented at several international meetings, discussed at workshops and seminars, and part of upcoming scientific publications, is important to uncover new pathways contributing to neuronal degeneration in ALS and potentially applicable to other neurodegenerative conditions. On the other hand, our studies will possibly pave the way to new diagnostic markers to be analyzed as early as possible in ALS patients.

Progress beyond the state of the art and expected potential impact (including the socio-economic impact and the wider societal implications of the project so far)

We faced several technical challenges due to the fact that research on extracellular vesicles and the central nervous system is at its early stage. First, we had to find a purification method that could be used rapidly and efficiently, and that was also reproducible and pure. We benefited from collaboration, as another research group in the Host Institution happened to have such a method. We were able to compare their method to existing ones and then subsequently validate it. Then, we set up a pipeline of experiments that provided us with the opportunity to study the mechanism of intercellular communication using both in vitro and in vivo ALS models. We were then able to validate the data from these experiments against the samples derived from actual patients. From this, we could better understand how cells communicate with each other via nano-messages and how this communication influences cell performance and well-being. In order to decode the nature of the nano-messages, we established (part of this work is still in process) new methodologies to deplete EVs of their main components and tested their toxicity in our in vitro system. The future goal for ExItALS is to build on the results obtained during the MSCA fellowship to learn how to: 1.) modulate EV release to halt toxicity (Therapy) and 2.) use the phenotypic characterization of EVs to discriminate ALS patients from other neurodegenerative diseases at the early stages of the pathology (Early Diagnosis) and 3.) continue to develop new technologies to decipher the key players responsible for EV transmission from glia to motor neurons (Technology).

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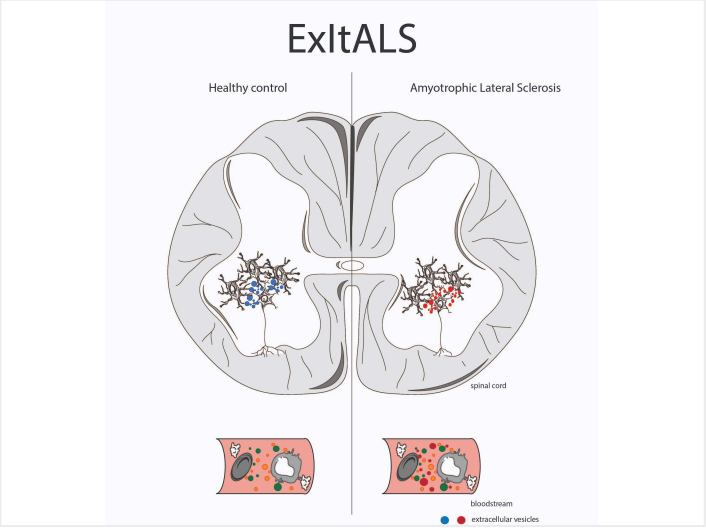

ExItALS- Glia-released EVs propagate toxicity to motor neurons in ALS and are detected in periphery

Last update: 10 February 2022

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