Al collegio docenti del Dottorato in Medicina Molecolare

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"Study of the molecular characteristic of spastic paraplegia type 11, its impact on oxidative and gangliosides metabolism"

INTRODUCTION

Spastic paraplegia type 11 (SPG11) is a common form of autosomal recessive hereditary spastic paraplegia (HSP) accounting for about 10% of all HSP patients. The *SPG11* gene encodes spatacsin, a protein of unknown function potentially associated with neurite plasticity, autophagosome recycling and ganglioside metabolism. Our goal is to better characterize the role of spatacsin in HSP and omic technologies and to perform attempts to treatments in cell systems.

METHODS

Proteins and RNA from patient's cultured skin fibroblasts were extracted and spatacsin and mRNA levels were evaluated through Western blotting and Real time PCR, respectively. The mitochondrial oxygen consumption rates (OCR) and basal ATP levels were measured with a micro-oxymetry using the Seahorse XF analyzer. We treated SPG11 and control cells with and without 50 μM gentamicin (positive control) and 40 μM ataluren (PTC-124) to test potential effects on ability to rescue mRNA expression. Cells with ataluren were sampled 24h after the initial compound addition and Real time PCR of SPG11 mRNA was performed. Miglustat (100 μM) was tested to assess effects on oxidative stress and lipid profiling. Cells treated with miglustat were collected after 72h and stored for lipidomic analysis. The intracellular ROS levels in cultured skin fibroblasts were measured, with/without miglustat, using H2DCF-DA. To establish neuronal-like models of the disease, we designed single guide RNA (sgRNA) targeting SPG11 (and in its associated SPG15 gene), and cloned it in pSpCas9(BB)-2A-Puro V2.0 plasmid. Plasmids are ready for transfection for further omic studies.

RESULTS

In cultured skin fibroblasts from selected SPG11 patients we observed the following results:

- ✓ mRNA of SPG11 appeared reduced;
- ✓ spatacsin was absent/nearly absent in all the patients whereas its interacting proteins (spastizin and SPG48) as well as NRF2, the master regulator of endogenous antioxidant response, showed variable expression levels;
- ✓ micro-oxygraphy showed a decreased spare respiratory capacity (SRC) in almost all the patients with an impaired ATP production;
- ✓ high basal production of ROS in cells did not appear influenced by miglustat treatment;
- ✓ in a single patient, ataluren appeared to modify mRNA expression. Additional experiments will combine ataluren with caffeine, able to mitigate the Nonsense-Mediated mRNA Decay (NMD) activity.

ABSTRACTS AND COGRESSES

- Mero S, Doccini S, Nesti C, Santorelli FM. Study of the molecular characteristic of spastic paraplegia type 11: its impact on oxidative metabolism. IRPES – PISA, 27 novembre 2019. (Poster)
- "ZF-Med and Pisa Zebrafish Day 2.0". Pisa – 27,28 gennaio 2020.
- "La percezione pubblica della scienza: i giovani ricercatori di fronte a temi scientifici di forte interesse pubblico, politico e mediatico". Dipartimento di Bioscienze dell'Università degli Studi di Milano - 27,28 aprile 2020 in modalità telematica.

EDUCATIONAL ACTIVITIES

- Corso di formazione "Protezione degli animali impiegati nella ricerca: aspetti scientifici, etici e applicativi". Pisa, 8 - 15 - 22 ottobre 2019.
- Esperienze di Scienza "Quando la Biologia e la Comunità di incontrano". Pisa, 24 gennaio 2020.

PUBBLICATIONS

- Naef V, Mero S, Fichi G, D'Amore A, Ogi A, Gemignani F, Santorelli FM, Marchese M. Swimming in Deep Water: Zebrafish Modeling of Complicated Forms of Hereditary Spastic Paraplegia and Spastic Ataxia. Frontiers Neuroscience. 2019 Dec 10;13:1311.
- Pellacani S, Dosi C, Valvo G, Moro F, Mero S, Sicca F, Santorelli FM. Customized multigene panels in epilepsy: the best things come in small packages. Neurogenetics. 2020 Jan; 21(1):1-18.
- D'Amore A, Tessa A, Naef V, Bassi MT, Citterio A, Romaniello R, Fichi G, Galatolo D, Mero S, Battini R, Bertocci G, Baldacci J, Sicca F, Gemignani F, Ricca I, Rubegni A, Hirst J, Marchese M, Sahin M, Ebrahimi-Fakhari D, Santorelli FM. Loss of ap4s1 in zebrafish leads to neurodevelopmental defects resembling spastic paraplegia 52. Annals Clinical and Translational Neurology. 2020 Apr; 7(4):584-589.