GiC XXXIX WEB CONFERENCE 14 - 18 GIUGNO 2021
CONFERENZA NAZIONALE DI CITOMETRIA
AGGIORNAMENTI E INNOVAZIONI DELLA CITOMETRIA NELLE APPLICAZIONI CLINICHE E DI RICERCA

KNOCKING DOWN THE METABOTROPIC GLUTAMATE RECEPTOR 5 IN A MOUSE MODEL OF AMIOTROPHIC LATERAL SCLEROSIS REDUCES THE REACTIVE PHENOTYPE OF EX-VIVO CULTURED SPINAL CORD ASTROCYTES

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disease characterized by a selective death of upper and lower motor neurons (MNs). ALS is known to be a multifactorial and non-cell autonomous disease, where the non-neuronal supporting cells are directly involved in MNs degeneration. Glutamate-(Glu)-mediated excitotoxicity has been proposed as one major cause that trigger MNs degeneration. Although the etiopathogenesis is not completely understood, *in-vitro* and *in-vivo* studies demonstrated that damage within MNs is sustained by the degeneration of non-neuronal cells such as microglia and astrocytes. Group I metabotropic glutamate receptors (mGluR1, mGluR5) likely play a role in ALS, since they are over-expressed and functionally altered in different experimental model of ALS. In a previous work, we demonstrated that knocking-down mGluR1 or mGluR5 significantly prolongs survival and ameliorates the clinical progression in the $SOD1^{G93A}$ mouse model of ALS.

The aim of this work is to investigate the effects of mGluR5 down-regulation on the reactive phenotype of astrocytes in ALS. We used here spinal cord astrocyte cell cultures from adult B6SJL-TgN (SOD1-G93A)1Gur mice expressing high copy number of mutant human SOD1 with a Gly93Ala substitution ($SOD1^{G93A}$) and mGluR5+/- mice expressing half dose of mGluR5. The two strains were appropriately crossed to obtain mice carrying the $SOD1^{G93A}$ transgene and lacking one allele of the mGluR5 encoding genes.

Experiments with the FURA-2 dye showed a significantly higher cytosolic calcium concentration ($[Ca^{2+}]_i$) in $SOD1^{G93A}$ than in WT mice, both under basal condition and after exposure to a 30 µM concentration of the Group I mGluRs agonist 3,5-DHPG (3,5-dihydroxyphenylglycine). mGluR5 knocking-down significantly reduced the excessive $[Ca^{2+}]_i$.

Confocal microscopy revealed that the astrogliosis markers GFAP (Glial fibrillary acidic protein), vimentin and S100β (S100 calcium binding protein B) were more expressed in $SOD1^{G93A}$ respect to WT mice and decreased in $SOD1^{G93A}mGluR5^{+/-}$ mice. The same was true for the expression of the autophagy activation marker LC3-II (Microtubule-associated protein light chain 3). Of note, mGluR5 knocking-down also translates in a significant lower presence of misfolded-SOD1 protein when comparing $SOD1^{G93A}mGluR5^{+/-}$ and $SOD1^{G93A}$ mice.

To conclude, a lower constitutive level of mGluR5 had a positive impact in $SOD1^{G93A}$ mouse astrocytes, supporting the idea that mGluR5 may be a potential pharmacological target for cell specific therapeutic approaches in ALS, aimed at preserving MNs by acting at the neighboring astroglial cell.