6TH-7TH NOVEMBER 2020
MOTOR NEURON DISEASES
UNDERSTANDING THE PATHOGENETIC MECHANISMS
TO DEVELOP THERAPIES

VIRTUAL CONFERENCE

Organizers: Marina Boido & Serena Stanga

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SCIENTIFIC PROGRAM

DAY 1 (6th November 2020)

ZOOM ACCESS: https://zoom.us/j/93842867404

9.00-9.15: Opening ceremony

Welcome by the Organizers
Greeting address by Prof. A. Vercelli, Deputy Rector for Biomedical Research, Univ. Turin

9.15-9.45: Lecture D. Rossi, ICS Maugeri, Pavia - *Motor neuron diseases: an overview of ALS and SMA*

9.45-10.45: Session I - "ALS disease mechanisms"

9.45-10.00: V. Crippa, Univ. Milan - *Proteostasis in ALS*
10.00-10.15: G. Nardo, Mario Negri Institute Milan - *Immune response in the peripheral nervous system and skeletal muscles is pivotal to counteract ALS*
10.15-10.30: M. Milanese, Univ. Genoa - *Modulating the reactive phenotype of astrocytes as a therapeutic approach in amyotrophic lateral sclerosis*
10.30-10.45: C. Rouaux, Inserm, Strasbourg - *Evaluation of the corticofugal hypothesis of ALS*

10.45-11.15: Q&A Session I - Meet the speakers

11.15-11.30: Break

11.30-11.45: F. Biancardi (Zeiss) - *Beyond CLEM: Multiscale and Multidimensional Microscopy approaches in Neuroscience*

11.45-12.15: Session II - "ALS therapeutic approaches"

11.45-12.00: D. Ferrari, Univ. Milan Bicocca - *Human neural stem cells for experimental cell therapies approaches in ALS: a synopsis of the experience from preclinical and Phase I clinical trials*
12.00-12.15: A. Calvo, Univ. Turin - *New therapeutic strategies for ALS*

12.15-12.30: Q&A Session II - Meet the speakers

12.30-13.40: Poster Session I

SCIENTIFIC PROGRAM

DAY 2 (7th November 2020)

ZOOM ACCESS: https://zoom.us/j/94050671493

9.00-9.45: **Session III - "SMA disease mechanisms"**

9.00-9.15: G. Viero, CNR Trento - *Translational defects by positional sequencing: the case of Spinal Muscular Atrophy*
9.15-9.30: R. Soler, Univ. Lleida - *Using motor neurons and iPSCs cultures to understand intracellular mechanisms leading to motoneuron degeneration in Spinal Muscular Atrophy*
9.30-9.45: E. Di Schiavi, CNR Naples - *Use of C. elegans as experimental model to study SMA disease mechanisms*

9.45-10.15: **Session IV – "SMA therapeutic approaches"**

9.45-10.00: T. Mongini, Univ. Turin - *The new era of SMA therapy: results from real world experience and ongoing clinical trials*
10.00-10.15: P. Konieczny, Univ. Valencia - *From flies to mice: evaluation of combinatorial therapy for SMA*

10.15-10.45: Q&A Sessions III-IV - Meet the speakers

10.45-11.00: Break

11.00-12.30: **Poster Session II**

12.30-12.45: L. Clario (Media Lab System) - *Unlocking the mysteries of neurite growth in neurons: a quantitative approach to live cell imaging*

12:45-13:15 **Lecture** S. Lefebvre, Univ. Paris INSERM - *The SMN complex in motor neuron diseases*

13.15: **Awards ceremony** (Best Posters)

13.20: Closing remarks & Goodbye to the next edition!

POSTER SESSION I (presentations' order)

1. Torazza Carola "mGluR5 as a target to modulate the reactive phenotype of astrocytes in the SOD1G93A mouse model of amyotrophic lateral sclerosis"

2. Januel Camille "Using Human pluripotent stem cells derived motor neurons to address the pathogenesis of SMA"

3. Lauria Fabio "Survival motor neuron protein regulates ribosome fluxes along mRNAs relevant to Spinal Muscular Atrophy"

4. Maniscalco Federica "Transcripts associated with SMN-primed ribosomes share specific features and are translationally defective in cellulo and in vivo models of Spinal Muscular Atrophy"

5. Scarpetta Valentina "Characterization of peripheral alterations in a murine model of Spinal Muscular Atrophy: a focus on skeletal muscles and spleen"

6. Gras Artells Sílvia / Blasco Alba "Muscular alterations with aging are associated with motoneuron deafferentation and gliosis in the spinal cord of C57BL/6J mice"

7. Bombaci Alessandro "Use of plasmatic Neurofilaments and muscle biomarkers in differential diagnosis between Amyotrophic Lateral Sclerosis and Spinal Bulbar Muscle Atrophy"

8. Rosenstock Tatiana "Mitochondrial deregulation in Peripheral Blood Mononuclear Cells from ALS patients as a potential tool for biomarker research"

9. Baghdoyan Sandrine "Molecular screening of FDA-approved drugs in human pluripotent stem cells for the treatment of rare monogenic diseases."

10. Menduti Giovanna / Rasà Daniela Maria "Drug screening and drug repositioning: modern horizons in spinal muscular atrophy therapy"

11. Cavallina Ilaria "Motor function progression in Spinal Muscular Atrophy (SMA) patients treated with Nusinersen in Turin over the last 3 years. A longitudinal study"

POSTER SESSION II (presentations' order)

12. Gatius Alaó "Expression of the Y172-related antigen in cholinergic synapses (C-boutons) on motoneurons and its changes in association with pathology"

13. Paganin Martina "Multi-level translatome analysis reveals unexpected robust pre-symptomatic defects in Spinal Muscular Atrophy"

14. D'amico Agata Grazia "Pacap counteracts motorneurons degeneration by modulating autophagy process in an in vitro model of Amyotrophic Lateral Sclerosis"

15. Salvany Montserrat Sara "Identification of distinct pathological motor neuron phenotypes according with the expression of misfolded SOD1 and microgliosis during the progression of disease in the SOD1G93A ALS mice"

16. Fulceri Federica "Detailed spinal cord and muscle morphology in a mouse model of spinal muscle atrophy type III"

17. Bonifacino Tiziana "Metabotropic glutamate receptor 5 as a potential pharmacological target in ALS"

18. Wurtz Guillaume "Modulation of cholesterol metabolism as a new therapeutical approach for amyotrophic lateral sclerosis"

19. Bersani Margherita "Morpholino oligomers improve pathological phenotype in C9orf72 ALS iPSC-derived lines"

20. Kumar Mandeep "Effects of specific miRNAs shuttled by exosomes derived from Mesenchymal Stem Cells on late symptomatic SOD1G93A mouse astrocyte primary cultures"

21. Pagliari Elisa "Cell penetrating peptide-conjugated Morpholino rescues SMA in a symptomatic preclinical model"

22. Virla Federica "Exosomes isolated from adipose-derived stem cells ameliorate the disease progression in Smndelta7 murine model"

23. Caretto Anna "A new potential supportive role of MR409, a GHRH agonist, in an experimental mouse model of Spinal Muscular Atrophy"

24. Bankole Molakun "Resveratrol and Valproate improves motor function and induces neuroprotective effects in ALS murine model"

25. Bombaci Alessandro "Stapedial reflex: a novel biomarker of early bulbar involvement in ALS patients"

MGLUR5 AS A TARGET TO MODULATE THE REACTIVE PHENOTYPE OF ASTROCYTES IN THE SOD1^{G93A} MOUSE MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder due to motor neuron (MN) degeneration in the spinal cord, brainstem, and motor cortex. One major cause for MN degeneration in ALS is represented by glutamate-mediated excitotoxicity. Group I metabotropic glutamate receptors (mGluR1, mGluR5) play a role in ALS, since they are largely over-expressed during disease progression and are involved in the altered neuronal and glial cellular processes. We demonstrated that mGluR1 and mGluR5 produce abnormal glutamate release in the spinal cord of the SOD1^{G93A} mouse model of ALS and that halving their expression in SOD1^{G93A}mGluR5$^{+/-}$ mice has a positive impact on _in-vivo_ disease progression.

We here investigated the consequences of reduced mGluR5 expression in SOD1^{G93A} mice on the reactive phenotype of spinal cord astrocyte cell cultures from late symptomatic SOD1^{G93A}, age matched SOD1^{G93A}mGluR5$^{+/-}$and WT mice.

[Ca^{2+}]$_c$ was increased in SOD1^{G93A} astrocytes under basal and 3,5-DHPG-stimulated conditions. The mGluR5 down-regulation reduced the excessive [Ca^{2+}]$_c$. GFAP, Vimentin and S-100β, three astrogliosis markers, were increased in SOD1^{G93A} astrocytes and over-expression was reduced in SOD1^{G93A}mGluR5$^{+/-}$astrocytes. mGluR5 down-regulation resulted in a lower cellular presence of misfolded SOD1. The expression and secretion of pro-inflammatory cytokines was strongly reduced in SOD1^{G93A}mGluR5$^{+/-}$respect to SOD1^{G93A} astrocytes. Notably, the viability of spinal MNs co cultured with SOD1^{G93A}mGluR5$^{+/-}$astrocytes, instead of SOD1^{G93A} astrocytes, was significantly increased. The _in-vitro_ acute treatment with the mGluR5 antisense oligonucleotide, ASO, reduces mGluR5 expression and astrogliosis in SOD1^{G93A} adult spinal astrocytes. Moreover, the treatment with CTEP, a negative allosteric modulator of mGluR5, reduces the expression of S-100β and GFAP in SOD1^{G93A} astrocytes respect to controls.

Thus, mGluR5 ablation in SOD1^{G93A} mice has a positive impact on astrocytes. This supports the idea that mGluR5 may be a potential therapeutic target aimed at preserving MNs death, possibly by modulating the reactive astroglia phenotype in ALS.

USING HUMAN PLURIPOTENT STEM CELLS DERIVED MOTOR NEURONS TO ADDRESS THE PATHOGENESIS OF SMA

Januel C.[1], Tarhaoui J.[1], Come J.[2], Lesueur L.[2], Morizur L.[2], Peschanski M.[2], Martinat C.[1]

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Spinal muscular atrophy is the most common genetic cause of infant mortality characterized by the specific degeneration of lower motoneurons (MNs) in the spinal cord, leading to progressive paralysis and muscle atrophy. SMA etiology relates to an insufficient amount of SMN (survival motor neuron) protein, which results from mutations in the _SMN1_ gene. Despite the ubiquitous expression of SMN protein, it is still unclear why MNs are one of the most affected cell types. Understanding this specific cellular tropism is critical but requires access to the relevant cell type. In this present study, we demonstrated that the reduced expression of SMN lead to a decreased survival of hiPSC-derived MNs rather than a defect in their generation. We identified that this phenotype can be rescued by kenpaullone, an inhibitor of several CKDs as well as JNK, likely through a JNK dependent mechanism. By a transcriptomic approach, we identified SMA-specific changes in early MNs that include genes involved in synaptic plasticity. Interestingly, these genetic defects were rescued by kenpaullone treatment. These findings suggest that alteration in synaptic organization might be a new therapeutic target for SMA. Furthermore, several studies suggest that pathological changes of the neuromuscular junction (NMJ) precede the motor neuronal loss. Therefore, it is critical to evaluate the NMJ formed by SMA patients' MNs, and to identify drugs that can restore the normal condition. We thus developed an _in vitro_ co-culture strategy to study the interaction between MNs and its skeletal muscle target. Altogether, our results demonstrate the potential offered by hiPSC to shed light on the cellular and molecular bases of selective MN vulnerability in SMA.

SURVIVAL MOTOR NEURON PROTEIN REGULATES RIBOSOME FLUXES ALONG MRNAS RELEVANT TO SPINAL MUSCULAR ATROPHY

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Spinal muscular atrophy (SMA) is caused by low levels of the Survival Motor Neuron (SMN) protein and is characterized by progressive degeneration of lower alpha motor neurons. SMA results in hypotonia, respiratory failure and, in its most severe form, the death occurs within two years of age. Recent findings demonstrated that low levels of SMN lead to dysregulation of protein synthesis, suggesting a closely related connection between SMN and the translation machinery, but the mechanisms linking SMN to defective translation have yet to be elucidated. Ribosomes have been placed in the spotlight as putative direct influencers of translation by acting as mRNA regulatory elements. An intriguing hypothesis is that ribosome composition is heterogeneous in ribosomal protein composition, rRNA variants and ribosome-associated proteins. However, the contribution of ribosome heterogeneity and ribosome-associated proteins to the molecular control of translation and to disease development remains enigmatic. Here, we investigated the role of the SMN as ribosome-associated protein. Using multiple complementary approaches we demonstrate that SMN binds to ribosomes (SMN-primed ribosomes) _in vivo_ and _in vitro_. We observed a significant accumulation of SMN-primed ribosomes within the first five codons of the coding sequence of a specific population of mRNAs. These SMN-specific transcripts are characterized by enrichment of translational enhancer sequences in the 5'UTR and rare codons at the beginning of their coding sequence. Moreover, we showed that SMN-specific mRNAs are organized in seven functionally well-defined communities, associated with processes known to be defective in SMA such as translation, neurogenesis, lipid metabolism, ubiquitination and chromatin regulation. Loss of SMN induces translational defects and ribosome depletion from SMN-specific and mRNAs, especially at the beginning of the coding sequence.

TRANSCRIPTS ASSOCIATED WITH SMN-PRIMED RIBOSOMES SHARE SPECIFIC FEATURES AND ARE TRANSLATIONALLY DEFECTIVE *IN CELLULO* AND *IN VIVO* MODELS OF SPINAL MUSCULAR ATROPHY

Maniscalco F.[1,2], Lauria F.[1], Tebaldi T.[2,%], Groen E.J.N.[3,4], Rossi A.[2], Orri J.[1,5], Quattrone A.[2], Inga A.[2], Gillingwater T.H.[3], Viero G.[1]

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Spinal Muscular Atrophy (SMA) is a motor neuron disease caused by loss of SMN expression. We demonstrated that SMN associates with ribosomes which control the translation efficiency of a specific subset of mRNAs. Decrease in SMN levels in a mouse model of SMA leads to ribosome drop-off at the beginning of the coding sequence of SMN-specific mRNAs. Computational analyses identified specific features on the sequences of these transcripts: translational enhancer sequences in the 5' untranslated region (UTR) and rare codons at the beginning of the CDS.

We validated these features analyzing the translation efficiency of reporter constructs bearing *c-Myc* translational enhancer or rare arginine codons in *in cellulo* model of SMA. Results of luciferase assays indicated that the combination of these two features is required for SMN-specific mRNAs to be controlled translationally by SMN-primed ribosomes. To further validate the role of ribosome-associated defects in SMA pathogenesis, we investigated the effect of SMN loss on SMN-specific mRNAs which were downregulated in SMA. Co-sedimentation analysis of selected mRNAs along sucrose gradients of control and early symptomatic SMA brain confirmed that at early stage of the disease these transcripts are depleted from the polysomal fractions leading to possible changes at the protein level. Finally, we selected the neuron-specific gene Acetylcholinesterase (Ache) for further validations. Results showed that AChE protein was decreased in SMA and its expression was significantly impaired at the neuromuscular junction (NMJ). This identified AchE as molecular marker of impairment at early stages of the SMA.

CHARACTERIZATION OF PERIPHERAL ALTERATIONS IN A MURINE MODEL OF SPINAL MUSCULAR ATROPHY: A FOCUS ON SKELETAL MUSCLES AND SPLEEN

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Spinal Muscular Atrophy (SMA) is a genetic neurodegenerative disease that affects children: the lack of the SMN (Survival Motor Neuron) protein results in the loss of MNs, leading to progressive muscle atrophy, respiratory failure and premature death. Although historically considered a "MN disorder", mounting evidence suggests that peripheral districts are also strongly affected by SMN lack.

In this work we investigated some peripheral alterations, in particular focusing on skeletal muscles and spleen. We performed the analyses by comparing SMNΔ7 (the most used SMA murine model) and WT mice at different stages of the disease.

Degenerative processes in skeletal muscle are evident, but little is known about a potential glial involvement. For this reason, we analysed gastrocnemius and quadriceps muscles: we observed a higher number of GFAP-positive cells, selectively located close to NMJs and around clusters of dense nuclei, and a diffused S100β signal, probably correlated with denervated and damaged muscular fibers, in SMA mice. Increased muscular fibrosis - with a higher number of cell nuclei in SMA samples' connective tissue - was detected, too.

Furthermore we studied the immunological dysregulation in SMA mice, analysing morphological and functional alterations of the spleen and peripheral white blood cells (WBCs): we showed loss of white/red pulp organisation and a significant increased number of CD68+ and CD206+ splenic macrophages, suggesting an ongoing inflammatory process or an altered immune system response. Total CD3+ lymphocytes, CD4+ (T-helper) and CD8+ (T-cytotoxic) sub-populations were significantly augmented in SMA spleens, too. On the contrary, we observed a decreased number of circulating WBCs.

Such preliminary data further confirm that the pathogenesis of SMA is more complex than expected: a deeper knowledge of peripheral alterations will be also important for developing more specific therapeutic strategies.

MUSCULAR ALTERATIONS WITH AGING ARE ASSOCIATED WITH MOTONEURON DEAFFERENTATION AND GLIOSIS IN THE SPINAL CORD OF C57BL/6J MICE

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Aging is associated with sarcopenia, a decline in skeletal muscle mass, strength and function. The causative factors of aging sarcopenia are controversial and poorly understood, hampering the development of effective therapeutic interventions. Here, we performed a detailed characterization of age-associated pathophysiological changes occurring simultaneously in distinct components of the neuromuscular system of young, adult, middle-aged and old C57BL/6J mice, including: motoneurons (MNs), glia, motor nerves, neuromuscular junctions (NMJs) and different types of skeletal muscles. We found that aging was not accompanied by a significant loss of spinal MNs, although a proportion of them in old mice exhibited an abnormally dark appearance. Morphological alterations in motor axons were already observed in adulthood but substantially increased with age. Old MNs were depleted of cholinergic and glutamatergic inputs. Prominent microgliosis and astrogliosis were found in old spinal cords, with increased density of pro-inflammatory phenotypes. Aging resulted in significant reductions in the nerve conduction velocity and the compound muscle action potential amplitude in old distal plantar muscles. Compared with adult muscles, old muscles exhibited significantly higher numbers of both denervated and polyinnervated NMJs, changes in fiber type composition, higher proportion of fibers showing central nuclei and lipofuscin aggregates, depletion of satellite cells and augmented expression of different molecules related to development, plasticity, and maintenance of NMJs, including: calcitonin gene-related peptide, growth associated protein 43, agrin, fibroblast growth factor binding protein 1 and transforming growth factor-β1. Overall, these alterations occurred at varying degrees in all the muscles analyzed, with no correlation between the age-related changes observed and myofiber type composition or muscle topography. These data provide a global view of age-associated changes in the mouse neuromuscular system, which can help to a better understanding of mechanisms leading to aging sarcopenia.

USE OF PLASMATIC NEUROFILAMENTS AND MUSCLE BIOMARKERS IN DIFFERENTIAL DIAGNOSIS BETWEEN AMYOTROPHIC LATERAL SCLEROSIS AND SPINAL BULBAR MUSCLE ATROPHY

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Background and aim: Kennedy disease (KD), also known as spinal-bulbar-muscular-atrophy (SBMA), is a progressive, adult-onset X-linked neuromuscular disease[1]. Although traditionally considered a motor neuron disorder (MND), recent advances have highlighted a primary myopathic component[4,5]. In order to identify novel biomarkers for KD, we evaluated levels of Neurofilament-Light-chain (NfL) and phosphorylated-Neurofilament-Heavy-chain (pNfH) as indicators of neuronal damage, and CK and creatinine levels as markers of muscle damage in KD patients and a mouse model of disease.

Materials and methods: We collected plasma and serum from 93 KD, 50 ALS and 50 healthy control cases, alongside with plasma from a mouse model of KD (AR100) and littermate controls. We measured NfL and pNfH plasma levels using Single-Molecule-Array (Simoa)[3] and assessed CK and creatinine levels using standard laboratory testing. We analysed data using Kruskal-Wallis test and Cox regression analysis.

Results: Both NfL and pNfH were elevated in ALS, as previously reported[2], but, intriguingly, there was no change in KD. This finding was confirmed in the KD mice. ROC curves support the use of NfL and pNfH as diagnostic biomarkers to differentiate between these two disorders. Importantly, both CK and creatinine were significantly changed in KD vs controls, and creatinine changes correlated with disease severity.

Discussion and conclusions: This study finds, unexpectedly, that levels of neurofilaments are normal in KD, differently from MNDs[2], whilst CK and creatinine are altered. These findings
support the hypothesis of primary muscle damage in KD. In summary, neurofilaments could be used as biomarkers to differentiate KD from ALS and creatinine as a biomarker to evaluate disease progression in KD.

MITOCHONDRIAL DEREGULATION IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM ALS PATIENTS AS A POTENTIAL TOOL FOR BIOMARKER RESEARCH

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Amyotrophic lateral sclerosis (ALS) is a multifactorial and progressive neurodegenerative disease of unknown etiology. Due to ALS's unpredictable onset and progression rate, the search for biomarkers that allow the detection and tracking of its development and therapeutic efficacy would be of significant medical value. Considering that alterations of energy supply are one of ALS's main hallmarks and that a correlation has been established between gene expression in human brain tissue and peripheral blood mononuclear cells (PBMCs), the present work investigates whether changes in mitochondrial function could be used to monitor ALS. To achieve this goal, PBMCs from ALS patients and control subjects were used; blood sampling is a quite noninvasive method and is cost-effective. Different parameters were evaluated, namely cytosolic calcium levels, mitochondrial membrane potential, oxidative stress, and metabolic compounds levels, as well as mitochondrial dynamics and degradation. Altogether, we observed lower mitochondrial calcium uptake/retention, mitochondria depolarization, and redox homeostasis deregulation, in addition to a decrease in critical metabolic genes, a diminishment in mitochondrial biogenesis, and an augmentation in mitochondrial fission and autophagy-related gene expression. All of these changes can contribute to the decreased ATP and pyruvate levels observed in ALS PBMCs. Our data indicate that PBMCs from ALS patients show a significant mitochondrial dysfunction, resembling several findings from ALS' neural cells/models, which could be exploited as a powerful tool in ALS research. Our findings can also guide future studies on new pharmacological interventions for ALS since assessments of brain samples are challenging and represent a relevant limited strategy.

MOLECULAR SCREENING OF FDA-APPROVED DRUGS IN HUMAN PLURIPOTENT STEM CELLS FOR THE TREATMENT OF RARE MONOGENIC DISEASES

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Given the high attrition rates, substantial costs and slow pace of new drug discovery and development, repurposing of 'old' drugs to treat both common and rare diseases is increasingly becoming an attractive proposition. In this study, we evaluated the potential of using human pluripotent stem cells to identify repurposable drug candidates. For this purpose, we treated derivatives from human embryonic stem cells with 50 marketed drugs and we annotated the induced molecular changes by RNA deep sequencing. Focusing on genes previously involved in monogenic diseases, we identified drugs capable to modulate the expression of p62/SQSTM1, a gene known to be involved in Amyotrophic lateral sclerosis (ALS). Most ALS cases are sporadic but 5-10% of cases are familial, and involve a mutation in the SOD1, TARDBP, C9orf72, OPN or p62/SQSTM1 gene. We treated motor neurons differentiated from human embryonic stem cells and we showed that one of the drugs identified was capable to upregulate p62/SQSTM1, to promote its aggregation in puncta, appearing during autophagosme formation. To further explore this therapeutic potential, we have generated p62+/- and p62-/- human pluripotent stem cells by using CRISPR-Cas9 technology. Thanks to these cellular tools, we highlighted the impairment of autophagy in p62+/- and p62-/- motor neurons. Treatment of the cells with the drug was able to restore autophagy. We next tested the potential of the drug in a zebrafish model of ALS caused by p62 knockdown. In vivo treatment improved the swimming of fishes without the induction of toxicity. Altogether, these results identify a FDA-approved molecule capable to modulate the autophagy pathway in human motor neurons through the induction of p62/SQSTM1. These data raise the question of the therapeutic potential of this molecule both for ALS but also for other neuromuscular disease associated with abnormal protein aggregation.

DRUG SCREENING AND DRUG REPOSITIONING: MODERN HORIZONS IN SPINAL MUSCULAR ATROPHY THERAPY

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease affecting children and young adults, due to the mutation/deletion of the Survival Motor Neuron 1 (SMN1) gene. The lack of functional SMN protein determines MN impairment, skeletal muscle atrophy and premature death. Even if the genetic cause of SMA is well known, many aspects of its pathogenesis remain unclear. To date, there are only three FDA-approved drugs (Spinraza; Zolgensma; Evrysdi), which show some important limits including high costs, side effects and still unknown long-term effects. Moreover, the approved drugs are all SMN-dependent therapies disregarding other disease-related mechanisms and targets. Therefore, the research of new therapeutic strategies is still a hot topic in the SMA field and, to provide rapidly novel efficient therapies to patients, many efforts are spent in modern drug discovery approaches. We describe two promising strategies that could successfully and rapidly lead to the identification of new molecules for the SMA treatment: drug screening (DS) and drug repositioning (DR). We also show the most used SMA experimental models that can be exploited for DS/DR approaches. By using libraries of chemical/natural compounds and/or FDA-approved substances, DS aims at identifying new potentially effective compounds, whereas DR at testing drugs originally designed for the treatment of other pathologies. The drastic reduction in risks, costs and time expenditure assured by these strategies make them particularly interesting compared to the long and expensive canonical drug discovery process. Moreover, DS/DR approaches already allowed both an in depth understanding of SMA pathological mechanisms and the identification of modulators of SMN2 transcription. Finally, we highlighted a convergence of some DS/DR-targeted molecular cascades contributing to SMA pathology, including cell death related-pathways, mitochondria and cytoskeleton dynamics, neurotransmitter and hormone modulation.

MOTOR FUNCTION PROGRESSION IN SPINAL MUSCULAR ATROPHY (SMA) PATIENTS TREATED WITH NUSINERSEN IN TURIN OVER THE LAST 3 YEARS. A LONGITUDINAL STUDY

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Background: SMA is an autosomal recessive neuromuscular disorder with an incidence of 4-10 cases per 100,000 live births. Recently, the natural history of the disease has radically changed thanks to the implementation of the standards of care and the approval of innovative pharmacological treatments, especially Nusinersen, the first drug for all types of SMA.

Aim: to describe motor function progression in SMA patients treated with Nusinersen at the A.O.U. Città della Salute e della Scienza, Turin (Italy) over the last 3 years.

Study design: observational retrospective study.

Population: 38 subjects aged < 14 years (n=14) or ≥ 14 years (n=24) with SMA type 1, 2 and 3 treated with Nusinersen between January 2018 and August 2020.

Motor function scales: CHOP INTEND, HFSME, RULM and 6MWT.

Descriptive analysis: medians and IQRs. Statistical analysis: Generalized Least Square analysis (longitudinal data).

Results: the median age at first administration of Nusinersen was 2.03 years (range 0.19-20.66 years) in 9 SMA 1 patients, 13.7 (1.7-47.1) in 15 SMA 2 patients, 40.9 (6.4-63.9) in 14 SMA 3 patients. Patients aged <14 years: increase of 1.06 points (CI 0.39-1.73, p=0.004) at every additional Nusinersen infusion at CHOP INTEND (SMA 1), 1.50 points (CI 0.95-2.05, p<0.001) at every additional Nusinersen infusion at HFSME (SMA 1, 2, 3). Patients aged ≥14 years: increase of 0.15 points (CI -0.13-0.42, p=0.295) at every additional Nusinersen infusion at HFSME (SMA 1, 2, 3), 0.27 points (CI 0.006-0.48, p=0.014) at RULM (SMA 1, 2, 3), 1.55 meters (CI -8.39-11.49, p=0.762) at 6MWT (SMA 3).

Conclusions: despite the limited sample size of this study, the motor function scales showed motor function improvement, especially in the paediatric population compared to the adult one. Further function scales are needed to detect minimal motor function variations and to overcome the plateau effect of the current ones.

ABSTRACT - POSTER SESSION II

EXPRESSION OF THE Y172-RELATED ANTIGEN IN CHOLINERGIC SYNAPSES (C-BOUTONS) ON MOTONEURONS AND ITS CHANGES IN ASSOCIATION WITH PATHOLOGY

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C-boutons are cholinergic inputs to motoneurons (MNs) that modulate their excitation state, which is essential to drive motor behavior. Alterations in C-boutons appear to play an important role in MN pathology, particularly in amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). During an immunocytochemical study on the role of c-Jun in MNs with a monoclonal (clone Y172) antibody against phospho-c-Jun (serine 63), unexpected labeling was identified in cytoplasmic structures closely associated with C-boutons, but not with other nerve afferent types contacting MNs. By ultrastructural analysis, cytoplasmic Y172 immunostaining was selectively located at the subsurface cistern of C-boutons. The analysis of Y172 immunoreactivity in injured MNs after peripheral nerve transection, and in ALS and SMA mouse models, revealed a significant depletion of cytoplasmic immunostaining at advanced stages, which preceded the C-bouton loss occurring in these paradigms. RNA interference experiments to knock down c-Jun *in vitro* by using different shRNA constructs resulted in a dramatic decrease in nuclear Y172 immunostaining in MNs without any reduction in the density of cytoplasmic Y172-positive profiles. Studies in skeletal muscles revealed that Y172-immunoreactivity was also present in neuromuscular junctions (NMJs), suggesting that this protein might be axonally transported between MN soma and muscle. Our results lay the foundation for further studies aimed at identifying the Y172-related protein and determining its role in the context of the development, maintenance, plasticity and pathology of both C-boutons and NMJs.

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MULTI-LEVEL TRANSLATOME ANALYSIS REVEALS UNEXPECTED ROBUST PRE-SYMPTOMATIC DEFECTS IN SMA

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease representing the most common genetic cause of infant mortality. SMA is caused by deletions or mutations in the Survival Motor Neuron gene (*Smn1*) which induces reduced levels of the SMN protein.

Even though SMA has been mainly associated with motor neuron defects, structural and functional impairments have been observed in numerous tissues, suggesting that SMA is a multi-systemic disorder.

It has been recently observed that loss of SMN expression leads to a decrease of the translation efficiency with the disease progression in brain and spinal cord in a Taiwanese mouse model suggesting that dysfunction of translation is likely an early hallmark of the disease. However, the impact of SMN loss in diverse tissues is still understudied and the mechanisms linking SMN to the existence of tissue- and stage-specific translational defects have not yet been clarified.

To understand if SMN loss induces precocious and tissue-specific translational impairments underlying the primary cause of SMA, we performed a multi-level analysis of translational defects in different tissues at multiple stages of disease progression. We obtained and integrated preliminary translational maps by ribosome profiling (RiboSeq and RiboLace) from three tissues at pre- and early-symptomatic stages of disease in the Taiwanese mouse model.

Preliminary results allowed us to identify a set of genes with altered ribosome occupancy. To our surprise, we observed stronger perturbations of translation at pre- than at early- stage of disease. Additionally, we observed that brain, rather than spinal cord, is a primary source of robust and remarkable translational defects. These impairments were also confirmed by co-sedimentation qPCRs.

Together, our results demonstrate that very strong and pre-symptomatic variations in translation occur in SMA, suggesting a new scenario in SMA pathogenesis.

PACAP COUNTERACTS MOTORNEURONS DEGENERATION BY MODULATING AUTOPHAGY PROCESS IN AN IN VITRO MODEL OF AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease of complex etiology leading to motoneurons degeneration. Mutation in Cu, Zn superoxide dismutase (mSOD1) are the most frequent mutation characterizing familial form of ALS. Even though the pathogenetic mechanism underlying ALS due to mSOD1 is not yet well understood, it is largely demonstrated that
this gene mutation affects many biological processes including autophagy. A recent study of genomic profiling of motor cortex samples of ALS patients have identified many deregulated genes in ALS, including pituitary adenylate cyclase-activating polypeptide (PACAP), suggesting their use as potential drug targets for the disease treatment. Its involvement in counteracting motorneurons degeneration it is recently demonstrated. However, the potential ability of PACAP to interfere with dysregulated-autophagy process in ALS disease has not been studied, yet. In the present work, we have analyzed the autophagy-modulating properties of PACAP by using an *in vitro* model of ALS, consisting in neuroblastoma-spinal cord-34 (NSC-34) cells line stably expressing human wild type or mutant G93A SOD1. Since the hypoxic stress is strictly linked to the disease progression and induces uncontrolled autophagy leading to enhance cell death, we exposed the cells to 100-µM desferroxamine for 12h, mimicking the hypoxic stress affecting motorneurons during ALS. Data showed that PACAP treatment significantly increases cell survival rate in hypoxic condition with concomitant reduction of hSOD1 levels, significantly upregulated following hypoxia. PACAP modulates autophagy process by decreasing LC3II and increasing p62/SQSTM1 levels, two autophagy indicators. Moreover, PACAP reduces the autopathic vacuoles formation, as detected by evaluating LC3II immunolocalization through immunofluorescence analysis. Finally, we demonstrated that PACAP exerts a neuroprotective effect on mSOD1 motorneurons by inhibiting DFX-induced autophagy through MAPK/ERK induction. Overall, our investigation elucidate a possible molecular mechanism underlying PACAP neuroprotection in mSOD1 motor neurons.

IDENTIFICATION OF DISTINCT PATHOLOGICAL MOTOR NEURON PHENOTYPES ACCORDING WITH THE EXPRESSION OF MISFOLDED SOD1 AND MICROGLIOSIS DURING THE PROGRESSION OF DISEASE IN THE SOD1G93A ALS MICE

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Misfolded SOD1 (mfSOD1) accumulation, microgliosis and motor neuron (MN) loss are hallmark events in familial ALS. An important variability in the vulnerability of distinct MN subtypes in ALS was also seen. This fact appears to be correlated with different characteristics of their excitability. C-boutons are cholinergic inputs to α-MNs which are important regulators of its excitability. However, the contribution of C-boutons to ALS pathology is controversial. To understand the relation between the mentioned pathological aspects, we performed a quantitative correlative analysis of the neuroinflammatory reaction, mfSOD1 accumulation and C-bouton alteration in the SOD1G93A ALS mice. The effects of a supplementary stress on ALS MNs induced by peripheral nerve injury were also assessed. Focusing on the accumulation of mfSOD1 we defined three MN phenotypes. In phenotype 1, there was no evidence of mfSOD1 accumulation, but the postsynaptic organization of C-boutons was impaired. In phenotype 2, mfSOD1 accumulation was observed in MN neuropile together with important microgliosis; however, no changes in C-boutons were observed. In phenotype 3, mfSOD1 accumulation was extended to MN somata; microglia was active and both pre- and post-synaptic C-bouton compartments were reduced. After sciatic nerve axotomy performed in a presymptomatic stage, MNs worsen their phenotype, but no effect was observed when performed in early symptomatic stages. By classifying MNs according to mfSOD1 amount, we dissociate the alterations in MNs from the clinical time-course points of disease. Compensatory mechanisms acting in early disease stages contribute to hide the pathological changes early seen in the vulnerable MNs. Any added stress worsens the MN pathology phenotype in early but not in late stages of ALS.

DETAILED SPINAL CORD AND MUSCLE MORPHOLOGY IN A MOUSE MODEL OF SPINAL MUSCLE ATROPHY TYPE III

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Spinal muscular atrophy (SMA) is a neurodegenerative autosomal recessive disorder caused by mutation of the survival motor neuron gene (*SMN1*). Symptoms vary depending on the disease genotype/phenotype, ranging from early onset and death during infancy (SMA I and SMA II, the most severe forms), to later disease onset and a slighter severity (SMA III and SMA IV). SMA disease severity relates with the copy number of *SMN1* homologous gene, namely *SMN2* gene and the levels of SMN protein.

Over the last two decades, several SMA mouse models have been developed. Here we focus on a model of SMA III. This is featured by long survival and slow disease progression. Mice are knock-out for mouse *SMN* (*Smn−/−*) gene and are double transgenic featuring a human mutation of the *SMN1* gene (*SMN1A2G*), along with human wild type *SMN2* gene.

In the present study we characterize spinal cord pathology and motor deficit at prolonged survival times (18 months). To our knowledge, such a long time interval was never analysed so far in this model.

Delayed disease progression was characterized by fair motor activity despite a dramatic loss of large motor neurons and a significant reduction of SMN protein levels in the spinal cord. At this stage, spared motor neurons feature cell body enlargement and may occur heterotopically within the ventral white matter. Remarkably, altered pathology was evident also at muscle level.

The present study validates over a long time period a SMA III mouse model, which shows neuropathology reminiscent of what observed in human patients. This strengthens its potential role as a useful tool to test novel therapeutic strategies.

METABOTROPIC GLUTAMATE RECEPTOR 5 AS A POTENTIAL PHARMACOLOGICAL TARGET IN ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to motor neuron (MN) death. Among different pathological mechanisms, glutamate (Glu)-mediated excitotoxicity plays a major role in MN degeneration. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in Glu-mediated excitotoxicity. We previously reported that mGluR1 and mGluR5 produce abnormal Glu release and that halving mGluR1 or mGluR5 or dampening mGluR5 expression in SOD1G93A mice significantly prolongs survival, ameliorating disease progression as well as several biochemical, cellular and functional parameters. Moreover, we have demonstrated that the down-regulation of mGluR1 or the ablation of mGluR5 in SOD1G93A mice positively affects motor skills in both males and females, while the down-regulation of mGluR5 ameliorates motor performances in males only. On this basis, we investigated the translational clinical potential of the mGluR5 in SOD1G93A mice. We treated SOD1G93A mice with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy) phenyl)-1H-imidazol-4-yl)ethynyl)-pyridine (CTEP), an orally available mGluR5 negative allosteric modulator, at the doses of 2 mg/kg/48h or 4 mg/kg/24h from 90 day of life, corresponding to the onset of the disease. CTEP dose-dependently ameliorates clinical features in SOD1G93A mice. The lower dose increases survival and improves motor skills in female mice, while it barely produces positive effects in male mice. The higher dose significantly ameliorates disease symptoms and survival in both males and females, being females always more responsive. CTEP treatment also reduces motor neurons death, astrocyte and microglia activation and normalizes abnormal Glu release in the spinal cord. Our previous and present results suggest that mGluR5 represents a promising target to counteract ALS and highlights mGluR5 inhibitors as CTEP or structural analogues as favourable new pharmacological tools with a possible translational perspective.

MODULATION OF CHOLESTEROL METABOLISM AS A NEW THERAPEUTICAL APPROACH FOR AMYOTROPHIC LATERAL SCLEROSIS

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Amyotrophic Lateral Sclerosis (ALS) is the most common motor neuron disease and is characterized by the progressive loss of upper and lower motor neurons, leading to paralysis and death. Accumulation of cholesterol in the central nervous system (CNS) has been reported to actively contribute to the disease progression in Alzheimer's disease, Huntington's disease, Spinocerebellar ataxia and more recently ALS. Cholesterol is essential for myelin compartment, but also for its functional and structural role in plasmatic membrane. However, in the CNS, cholesterol is synthetized in situ and is not able to freely cross the blood brain barrier (BBB). Cholesterol-24-hydroxylase (CYP46A1) allows the conversion of cholesterol to 24-hydroxycholesterol, able to cross the BBB, thus regulating cholesterol homeostasis. Furthermore, this enzyme is a key neuronal stress response such as oxidative stress or protein aggregation. Therefore, we hypothesized that CYP46A1 could be relevant for a therapy in ALS to target both familial and sporadic forms of ALS independently from their genetic origin. In the severe SOD1^{G93A} model, we overexpressed CYP46A1 using an AAVPHP.eB able to cross the BBB after intravenous injection. As a first step, we confirmed that the AAVPHP.eB viral vector has a specific tropism for the CNS and especially motoneurons. Secondary, we demonstrated a significant and prolonged motor rescue of animals treated pre or post-symptomatically, but also a preventive effect on myelin loss, compared to untreated animals. Evaluation of this therapeutic strategy is ongoing in another model of ALS.

EVALUATION OF MORPHOLINO OLIGOMERS THERAPEUTIC EFFICACY IN C9ORF72 ALS IPSC-DERIVED LINES

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GGGGCC repeat expansions in C9ORF72 gene are the most common identified genetic cause of amyotrophic lateral sclerosis (ALS), a fatal disorder characterized by progressive degeneration of motor neurons (MNs). Many possible pathogenic mechanisms have been proposed, including loss of function of the C9Orf72 protein, gain of function from accumulation of RNA foci, and toxicity caused by dipeptide repeats proteins, however processes underlying C9-ALS are still largely unknown. Patient-specific induced pluripotent stem cells (iPSCs) and iPSC-derived MNs can provide fundamental insights to better understand C9-ALS pathogenesis. Antisense oligonucleotides (ASOs) are single-stranded nucleic acids designed to bind complementary mRNA and interfere with specific biological processes. We designed two different ASOs with Morpholino chemistry to target C9ORF72 expansion and C9ORF72 gene promoter. We aimed to characterize the pathological phenotype of the C9-ALS iPSC-derived lines and evaluate the therapeutic effect of ASOs administration on specific pathological markers. We reprogrammed iPSCs from C9-ALS patients and controls and differentiated them in motor neurons (MNs). We investigated the phenotype of the C9-ALS iPSC-derived lines compared to controls, evaluating cells survival, pluripotency and motor neuronal markers and identifying pathological features such as increase of DNA damage, R-loops accumulation, impairment of nucleolar size, reduced axonal elongation and impairment of expression of genes involved in axonal growth as Nfh, Stmn1, Stmn2 and Sept7. We transfected C9-ALS cells with different Morpholinos and we evaluated modification of the pathological markers: Morpholinos treatments could partially rescue the pathological phenotype observed in in vitro models. Our results suggest that patient specific iPSCs and iPSC-derived MNs are a valuable tool to deepen the knowledge of C9ORF72 pathogenic mechanisms, and that Morpholino-mediated approaches represent a promising therapeutic strategy that needs to be further validated.

EFFECTS OF SPECIFIC miRNAs SHUTTLED BY EXOSOMES DERIVED FROM MESENCHYMAL STEM CELLS ON LATE SYMPTOMATIC SOD1 G93A MOUSE ASTROCYTE PRIMARY CULTURES

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Amyotrophic lateral sclerosis (ALS) is a motor neuron-involving neurodegenerative disease affecting about 4.5 per 100,000 people per year. Despite the significant progress in genetic studies, managed to explain many cases of ALS through mutations in several genes, the cause of a majority of sporadic cases remains unknown, even if the clinical and biomolecular features of genetic and sporadic ALS are very similar. Currently, epigenetics, involving miRNA studies, shows some promising aspects. We previously reported that intravenous administration of mesenchymal stem cells (MSCs) in the SOD1 G93A mouse model of ALS produced positive effects on survival and disease progression, also modulating astrocytes and microglia reactive phenotypes. We proposed that MSC effects were paracrine, possibly involving exosome-mediated cell communication. Indeed, unpublished results substantiate the positive impact of MSC-derived exosomes on SOD1 G93A mouse-derived astrocytes. Here, we investigated the activity of nine miRNA, which were found up-regulated in IFNɣ-primed MSCs and shuttled by MSC-derived exosomes, on spinal cord astrocyte primary cell cultures from late symptomatic 120 day-old SOD1 G93A mice. At this purpose, we transfected SOD1 G93A astrocytes with the single synthetic miRNAs and analyzed their effect on the astrocyte phenotype and the involved pathways. Seven out of nine miRNA mimics were able to affect the reactive phenotype of SOD1 G93A astrocytes by significantly decreasing the over expression of GFAP, IL1β, and TNFα, detected by confocal microscopy. Four miRNAs (466q, 467f, 466m5p, 466i3p), over expressed in MSCs, were overexpressed also in exosomes. We selected in-silico their relevant pathways (p38, TNFα and NFKB) and validated them by determining the miRNA effects on MAP3K8, MAPK-APK2, MAPK11 and TRAF6 by qPCR. Two of them (466q, 467f) strongly reduced MAPK11 mRNA expression, thus inhibiting TNFα formation. Our results suggest that the amelioration of the reactive phenotype of spinal cord SOD1 G93A astrocytes, brought about by in-vivo MSC treatment, operates through exosome-shuttled specific miRNAs.

CELL PENETRATING PEPTIDE-CONJUGATED MORPHOLINO RESCUES SMA IN A SYMPTOMATIC PRECLINICAL MODEL

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Spinal muscular atrophy (SMA) is a motor neuron disease and the leading genetic cause of infant mortality. Recently approved SMA therapies have transformed a deadly disease into a survivable one, but these compounds show a wide spectrum of clinical response and effective rescue only in the early stages of the disease. Therefore, safe, symptomatic-suitable, non-invasive treatments with high clinical impact across different phenotypes are urgently needed. We used morpholino (MO) chemistry to conjugate antisense oligonucleotides that increase SMN protein levels to cell-penetrating peptides (CPPs) for better cellular distribution. Systemically administered MOs linked to r6 and (RXRRBR)₂XB peptides crossed the blood-brain barrier and increased SMN protein levels remarkably, causing striking improvement of survival, neuromuscular function, and neuropathology, even in symptomatic SMA animals. Our study demonstrates that MO-CPP conjugates can significantly expand the therapeutic window through minimally invasive systemic administration, opening the path for clinical applications of this strategy.

EXOSOMES ISOLATED FROM ADIPOSE-DERIVED STEM CELLS AMELIORATE THE DISEASE PROGRESSION IN SMNΔ7 MURINE MODEL

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Spinal Muscular Atrophy (SMA) is an autosomal-recessive neuromuscular disease that occurs in early childhood. It is caused by the mutation or deletion of the survival motor neuron 1 (_smn1_) gene resulting in spinal motor neurons (MNs) degeneration followed by motor impairment, progressive skeletal muscle paralysis and respiratory failure. In addition to already existing or emerging therapies, a possible and alternative role could be played by the use of mesenchymal stem cells; in particular, we focused on adipose-derived stem cells (ASCs) because they can be obtained easily and in large amount from adipose tissue. Their efficacy seems to be correlated to their paracrine activity and the production of soluble factors released through extracellular vesicles, like exosomes. Exosomes are main mediators of intercellular communication with a diameter between 30 and 100 nm; thanks to their dimensions, they can easily cross the blood brain barrier. Their use in other neurodegenerative disorders, like Amyotrophic Lateral Sclerosis, showed a neuroprotective effect thanks to the release of their content, especially proteins, miRNA and mRNA. In this study, we wanted to evaluate the effect of exosomes isolated from ASCs (ASC-exosomes) in the SMNΔ7 mouse model of SMA. With this purpose, we performed intracerebroventricular (i.c.v.) administrations of ASC-exosomes in P3 and P6 SMA pups and assessed different motor behavioural tests. The results showed a delay in the disease progression with improved motor performance and a significant increase of the density of MNs in the ventral horns of lumbar spinal cord of treated animals. ASC-exosomes could also reduce the apoptotic activation (cleaved Caspase-3) and modulate the neuroinflammation with an observed decreased astrocytes activation in lumbar spinal cord. Thus, our results could encourage the use of ASC-exosomes as a therapeutic treatment for SMA, bypassing the controversial use of stem cells.

A NEW POTENTIAL SUPPORTIVE ROLE OF MR409, A GHRH AGONIST, IN AN EXPERIMENTAL MOUSE MODEL OF SPINAL MUSCULAR ATROPHY

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Spinal Muscular Atrophy (SMA) is a pediatric neurodegenerative pathology caused by the deletion/mutation of the survival motor neuron gene 1 (*SMN1*), resulting in the loss of motor neurons in the brainstem and in the spinal cord. Patients show a progressive skeletal muscular atrophy and neuromuscular junction (NMJ) defects, often leading to premature death. Thus, focusing on this peripheral district, we have investigated the role of MR409, a growth hormone-releasing hormone (GHRH) agonist that has already shown a remarkable activity in preventing apoptosis and proteolysis in an *in vitro* model of muscle atrophy. To this aim, from postnatal day 2 (P2) to P12, we daily injected SMNdelta7 mice (the most used SMA murine model) with vehicle or MR409 (1mg/Kg and 2mg/Kg). During this period, we observed a progressive weight gain, more striking in the mice treated with the highest dose, accompanied by a significant improvement in motor behavior. These results positively correlated with preliminary histological and molecular analyses on quadriceps and gastrocnemius, respectively a proximal and a distal hindlimb skeletal muscle, sequentially affected in the pathology: these promising outcomes were proportional to the administered dose. Indeed, H/E staining has revealed a significant increase in the size of the muscular fibers of MR409-treated mice. Moreover, immunofluorescence analyses on NMJs have shown a higher monoinnervation (indicating an increased NMJ maturation) and a reduced denervation of the endplates. Finally, molecular analyses revealed a significant enhancement in the expression of different isoforms of myosin heavy chains (MYH1, MYH2, MYH7 and MYH8) and of markers of myogenesis and muscular damage repairing (respectively, Myogenin and MyoD1), and a remarkable downregulation of MuRF1 and Atrogin-1 (whose increased expression seems correlated with muscular atrophy). Thus, our results suggest MR409 as a promising therapeutic approach for the treatment of SMA, possibly in combination with SMN-dependent therapies.

RESVERATROL AND VALPROATE IMPROVES MOTOR FUNCTION AND INDUCES NEUROPROTECTIVE EFFECTS IN ALS MURINE MODEL

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ALS is a neurodegenerative disease that affects motor neurons (MNs). Transcriptional dysfunction which involves a defect in histone homeostasis has recently been implicated in MN degeneration. Histone homeostasis strongly depends on the activity of histone deacetylases (HDACs). These enzymes, which includes an important group known as sirtuins have been implicated in cellular processes such as cell death. Studies have demonstrated that the combination of two epigenetic drugs, MS-275 which inhibits HDACs, and Resveratrol, an activator of the sirtuin 1 pathway, provided neuroprotective effects and improved motor performance in ALS mice. However, MS-275 is currently not approved for clinical trials. Several studies have indicated that Valproate, another pharmacological inhibitor of HDACs, improves cell survival by promoting histone acetylation and gene transcription in ischemic stroke, and is currently been used in clinical trials. The overall aim of this study is to investigate the efficacy of MS-275 replacement with Valproate, and explore the effect of these two drugs, Valproate and Resveratrol, in modulating histone homeostasis and protecting MNs. Experiments were performed using SOD1(G93A) mice separated into treated and control groups. Animals in the treated group were administered Valproate and Resveratrol in combination every day from post-natal day 50 until death. Behavioural tests were carried out to test motor function. Stereological count of MNs in the lumbar tract was performed to determine MNs survival and the acetylation state of histone 3 (H3) was examined by immunofluorescence staining. Western blot was carried out to detect the acetylation of RelA protein in the lumbar tract. Overall results showed that the drugs significantly delayed the loss of motor function. Stereological count showed a significant increase in the MNs number of treated animals. Immunofluorescence revealed a restoration of H3 acetylation after drug treatment, while western blot analysis also showed a restoration of RelA protein acetylation state.

STAPEDIAL REFLEX: A POSSIBLE NOVEL BIOMARKER OF EARLY BULBAR INVOLVEMENT IN AMYOTROPHIC LATERAL SCLEROSIS PATIENTS

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Background and aim: Amyotrophic lateral sclerosis (ALS) is a neuromuscular progressive disorder, characterized by limb and bulbar muscle wasting and weakness. 30% of patients present a bulbar onset, while 70% a spinal outbreak, although most of them develop bulbar impairment later on. Due to the lack of an early biomarker of bulbar involvement, we chose to evaluate the role of stapedial reflex (SR) in order to predict pre-clinical bulbar impairment in ALS.

Materials and methods: We enrolled 36 ALS patients. We assessed revised-ALS functional-rating-scale and SR for a total of 4 visits. We established the presence of SR, Acoustic Reflex Latency Test (ARLT) and SR's Decay. Patients who had not develop bulbar signs at 4th visit continued follow-up up to fifteen months. Data were analyzed by using Mann-Whitney U test, Friedman test, and Cox regression analysis.

Results: We observed that SR's Decay at 500 and 1000 Hz is the first parameter of SR to get altered in all ALS patients before the development of bulbar impairment. 28 patients developed bulbar impairment during the study. We highlighted a correlation between the progression rate of disease and both time of SR's Decay alteration and time of bulbar impairment from disease onset. 4 patients who did not develop bulbar impairment had a progression rate lower than the other ones ($p < 0.05$).

Discussion and conclusions: This study shows that stapedial reflex Decay test could be a sensitive measure for detecting pre-symptomatic bulbar involvement in ALS and could represent a simple, non-invasive and useful biomarker of disease progression.

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