site analysis on these enhancers using a de novo search and binding motifs in available databases. We found 15 overrepresented motifs, including known (MEF2, AP1, MEIS1) and new ones. Extensive mutagenesis on two enhancers shows that several of these motifs participate to the Six+Myod transcriptional synergy. Corresponding nuclear proteins were as well found enriched in cells under the control of Six and Myod. Altogether, these results provide an unprecedented regulatory dissection of genetic MEF reprogramming to a myogenic fate relying on synergistic gene activation by Six and Myod including EBF, MEF2 and new transcription factors, in a feedforward manner.

Myod Six reprogramming

Skeletal muscle development- #3253

P23- 357- Slow muscle precursors lay down a matrix COLXV-B fingerprint to guide motor axon navigation

Emilie Guillon (1), Sandrine Bretaud (1), Florence Ruggiero (1)
1. IGFL, ENS de Lyon- CNRS- UCBL, Lyon, France

The extracellular matrix (ECM) provides local positional information to guide motoneuron axons towards their muscle target. Collagen XV is a basement membrane component mainly expressed in skeletal muscle. We have identified twozebrafish collagen XV gene paralogs, col15a1a and col15a1b thatdisplay distinct expression patterns. Here we show that col15a1b is expressed and deposited in the motor path ECM by slow muscle precursors also called adaxial cells. We further demonstrate that collagen XV-B deposition is both temporally and spatially regulated prior to motor axon extension from the spinal cord in such a way that it remains in this region after the adaxial cells have migrated towards the periphery of the myotome. Loss and gain of function experiments in zebrafish embryos demonstrate that col15a1b expression and subsequent collagen XV-B deposition and organization in the motor path ECM depend on a previously undescribed two-step mechanism involving Hedgehog/Gli and unplugged/MuSK signaling pathways. In silico analysis predicts a putative Gli binding site in the col15a1b proximal promoter. Using col15a1b promoter-reporter constructs, we demonstrate that col15a1b participates in the slow muscle genetic program as a direct target of Hedgehog/Gli signaling.Col15a1b knockdown or overexpression provokes pathfinding errors in primary and secondary motoneuron axons both at and beyond the choice point where axon pathway selection takes place. These defects result in muscle atrophy and compromised swimming behavior, a phenotype partially rescued by injection of a smyhc1:col15a1b construct. These reveal an unexpected and novel role for COLXV-B in motor axon pathfinding and neuromuscular development.

extracellular matrix, motor axon navigation, muscle progenitors, zebrafish, signaling pathway

Skeletal muscle development- #3261

P23- 358- Sine oculis homeobox (Six) genes and muscle stem cell environment

Maud WURMSER (1), Josiane DEMIGNON (1), Pascal MAIRE (1)
1. Institut Cochin, INSERM U1016, Paris, France

The environment in which stem cells behave all along their life allow them to retain their stemness properties and to renew their host tissue. In skeletal muscle, this environment is established during the fetal life, assembling all the components that will be essential for muscle stem cell homeostasis: vessels, nerves, extra cellular matrix... This niche is also rebuilt occasionally upon muscle repair after an injury or constantly in the case of muscle degeneration. How skeletal muscles are able to reorganize all the components of the niche is not totally understood. Sine oculis homeobox (Six) genes encodes for transcription factors that are essential for muscle development. We observed that in the absence of Six1 and Six4 proteins, muscle stem cells did not locate correctly in their niche at the end of the fetal life. Upon engraftment in an injured adult muscle, those fetal cells poorly regenerated the host muscle, only forming immature myofibers. We are trying to understand the role of Six homeoproteins in the establishment of the skeletal muscle stem cell niche, with in vivo and in vitro models of myogenic stem cells homing.

Sine oculis, satellite cells, niche, Pax7, Extra cellular matrix

P24 – Spinal muscular atrophy (including variants)- N° 359 to N° 377

Spinal muscular atrophy (including variants)- #2323

P24- 359- Pharmacologically-induced mouse model of adult spinal muscular atrophy to evaluate effectiveness of therapeutics after disease onset

Zhihua Feng (1), Karen K. Y. Ling (1), Xin Zhao (2), Chunyi Zhou (1), Gary Karp (3), Ellen M. Welch (3), Nikolai Naryshkin (3), Hasane Ratni (4), Karen S. Chen (5), Friedrich Metzger (4), Sergey Paushkin (5), Marla Weetall (3), Chien-Ping Ko (1)
1. Biological Sciences, University of Southern California, Los Angeles, CA, Etats-Unis
2. N/A, PTC Therapeutics, Inc., South Plainfield, NJ, Etats-Unis
3. N/A, PTC Therapeutics, Inc., South Plainfield, NJ , Etats-Unis
4. Pharmaceutical Research and Early Development, F. Hoffmann-La Roche Ltd., Basel, Suisse
5. N/A, SMA Foundation, New York City, NY, Etats-Unis

Spinal muscular atrophy (SMA) is a genetic disease characterized by atrophy of muscle and loss of spinal motor neurons. SMA is caused by deletion or mutation of the survival of motor neuron 1 (SMN1) gene and the nearly identical SMN2 gene fails to generate adequate levels of functional SMN protein due to a splicing defect. Currently several therapeutics targeted to increase

SMN protein are in clinical trials. An outstanding question in the field is whether initiating treatment in symptomatic older patients would confer a therapeutic benefit, which is an important consideration since the majority of patients with milder forms of SMA are diagnosed at an older age. An SMA mouse model that recapitulates the disease phenotype observed in adolescent and adult SMA patients is needed to address this important question. Recently, we have described small molecules that penetrate the blood-brain barrier and selectively correct SMN2 alternative splicing that results in elevated levels of full length SMN protein both in SMA patient cells and in SMA mouse models (Naryshkin et al., 2014, Science, 345, 688-693). These compounds correct pathology of ?7 mice, a model of severe SMA, in a dose-dependent manner, with a sub-optimal dose achieving partial rescue and prevention of disease progression. This observation prompted us to treat ?7 mice with a sub-optimal dose of SMN-C3, an SMN2 splicing modifier, to allow survival into adulthood. We demonstrate here that ?7 mice treated with a sub-optimal dose of an SMN2 splicing modifier survive into adulthood and display SMA disease-relevant pathologies. Increasing the dose of the splicing modifier after the disease symptoms further mitigates SMA histopathological features in sub-optimally dosed adult ?7 mice. In addition, inhibiting myostatin using intramuscular injection of AAV1-follistatin ameliorates muscle atrophy in sub-optimally dosed ?7 mice. Taken together, we have developed a new murine model of symptomatic SMA in adolescents and adult mice that is induced pharmacologically from a more severe model, and demonstrated efficacy of both SMN2 splicing modifiers as well as a myostatin inhibitor in mice at later disease stages. These data also suggest that enhancing muscle growth in SMA in later stages of development may be an additional therapeutic approach for the treatment of SMA, particularly its intermediate/mild forms.

spinal muscular atrophy, neuromuscular junction, muscle, myostatin, follistatin, motoneurons, SMN, therapeutics,

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Spinal muscular atrophy (including variants)- #2344

P24- 360- Effects of olesoxime on the maintenance of motor function in spinal muscular atrophy (SMA):Further analyses of a randomized, double-blind, placebo-controlled, multinational Phase II study of olesoxime in people with Type 2 or non-ambulatory Type 3 SMA

Thomas Blaettler (1), Eugenio Mercuri (2), Carole Vuillerot (3), Carol Reid (4), Patricia Sanwald Ducray (5), Eric Dessaud (6), Enrico Bertini (7)

1. *F. Hoffmann-La Roche Ltd, Basel, Suisse*
2. *Paediatric Neurology Unit, Catholic University, Rome, Italie*
3. *Hôpital Femme Mère Enfant, Centre Hospitalier Universitaire de Lyon, Lyon, France*
4. *Roche Products Limited, Welwyn Garden City ,*
5. *Roche Pharma Research and Early Development, Clinical Pharmacology, Roche Innovation Center Basel, Basal, Suisse*
6. *Trophos, Marseille, France*
7. *Unit of Neuromuscular Disorders, Laboratory of Molecular Medicine, Bambino Gesu' Children's Research Hospital IRCCS, Rome, Italie*

Preclinical studies suggest that olesoxime may promote the survival of motor neurons and maintain function via the modulation of mitochondrial-dependent apoptosis. The effects of oral, once-daily olesoxime on motor function over 2 years were assessed in people aged 3-25 years with Type 2 and non-ambulatory Type 3 SMA (NCT01302600). Here we report the independent re-analysis of this study, including exploratory analyses. We confirm primary findings1 and provide further evidence of the maintenance of motor function and reduction of disease-associated complications with olesoxime.
Study participants (n=165) were randomized in a 2:1 ratio to olesoxime 10 mg/kg or placebo administered as a liquid oral formulation. Primary endpoint was change from baseline at 2 years in MFM D1 + D2, a valid, sensitive and clinically relevant measure of motor function in SMA. Secondary endpoints included MFM total score and HFMS.
The full analysis set comprised 160 participants. MFM D1 + D2 treatment difference at 2 years between olesoxime (n=103) and placebo (n=57) arms was 2.00 (96%CI -0.25-4.25) in favor of olesoxime (P=0.0676; overall treatment effect P=0.0084). Those treated with olesoxime maintained motor function over 2 years (0.18-point improvement in MFM D1 + D2), whilst those treated with placebo worsened (1.82-point decline). Participants were ?stable or improved' if they had no decrease relative to baseline in MFM D1 + D2 (Month 24) or HFMS (Month 21). A greater percentage in the olesoxime group was ?stable or improved' compared with placebo on MFM D1 + D2 (54% vs 39%; P=0.0609) and HFMS (50% vs 28%; P=0.0091). The effects of olesoxime on motor function were also observed on MFM total score, and treatment benefit was consistent across gender, country, SMA type and baseline severity. Exploratory exposure-efficacy analysis indicated that participants with higher exposure to olesoxime had greater treatment benefit vs those with low exposure and separated further from the placebo group. Olesoxime was well tolerated and people with SMA receiving olesoxime had fewer disease-associated complications.
We provide further evidence of the maintenance of motor function by olesoxime over 2 years in people with Type 2 and non-ambulatory Type 3 SMA. Effects across SMA type, patient gender, country and baseline disease severity were consistent with primary findings. Olesoxime may represent a future therapeutic option for people with SMA.

Dessaud E, et al. Neurology 2014; 83:e37.

Spinal muscular atrophy, olesoxime, clinical trial

Spinal muscular atrophy (including variants)- #2369

P24- 361- Modified U1 core spliceosomal particles for SMA therapy

Mojca Tajnik (1), Malgorzata Rogalska (1), Franco Pagani (1)
1. Human Molecular Gentics, ICGEB, Trieste, Italie

Modified U1 snRNAs bound to intronic sequences downstream of the 5' splice site restore exon skipping caused by different types of mutations. In SMA cellular model systems, these Exon Specific U1 (ExSpe U1s) molecules promote Survival motor neuron 2 (SMN2) exon 7 splicing and stabilizes an SMN pre-mRNA intermediate. This selective effect on pre-mRNA stability resulted in higher levels of SMN mRNAs in comparison with those after treatment with an antisense oligonucleotide that targets corresponding intronic sequences. We have used the ExSpeU1 approach in order to improve SMN2 splicing in Smn1-deficient

severe SMA affected mice line, where a silent exonic transition in the SMN2 leads to exon 7 skipping. The therapeutic activity was achieved with crossing of two transgenic lines: severe SMA and transgenic line expressing ExSpeU1. ExSpeU1 expression in vivo did not result in obvious phenotypic abnormalities. ExSpeU1 introduced by germline transgenesis efficiently rescued the phenotype increasing SMN2 exon 7 splicing, SMN protein production and radically extending the life span. Compared to control animals, rescued ExSpeU1 transgenic SMA mice showed a reduced weight gain and ability to perform neuromuscular functional tests. This result indicates that modified U1 core spliceosomal particles, when introduced by germline transgenesis, can efficiently rescue SMA phenotype in a severe mouse model. The presented results, especially the significantly prolonged survival of ExSpeU1 SMA-affected animals, have a great therapeutic potential in treatment of SMA, as well as in other exon-skipping pathologies.

Spinal Muscular Atrophy , splicing , gene therapy,

Spinal muscular atrophy (including variants)- #2371

P24- 362- BBrm02, a Read-Through Repurposed Drug, Shows Proof of Efficacy in Spinal Muscular Atrophy (SMA) Treatment

Hagar Greif (1), Rina Rosin-Arbesfeld (2), Dalia Megiddo (3)
1. Director of preclinical studies , Bioblast Pharma, Tel Aviv, Israël
2. Prof. Department of Anatomy, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israël
3. Chief Development Officer, Bioblast Pharma, Tel Aviv, Israël

Background:
Administration of read-through agents (e.g. aminoglycosides) acting on the stop codon located at exon 8 of the SMN-del7 protein, was found to be effective in inducing higher level of functional SMN protein. Prior attempts to translate these agents to therapeutic candidates were hampered by prohibitive toxicity. Bioblast Pharma develops a proprietary therapy for Spinal Muscular Atrophy (SMA), using FDA-approved macrolide drugs as read-through agents, known as the BBrm family.
Results:
BBrm02, intrathecal formulation of Azithromycin, increased SMN protein expression levels and function (nuclear GEMs presence) in SMA patients' cell lines. Intracerebroventricular (ICV) administration of BBrm02 to the well-known delta7 mouse model caused an increase in SMN expression levels in brain, spinal cord and muscle at 2.1, 2.4 and 5.7 fold, respectively, above vehicle-treated animals. The unique PK profile of BBrm02 enabled sustained effect in this model on body weight, motor function and increased survival, following a single administration, especially at low dose. Additional in vivo studies in the Regeneron C/C mouse model (the Jackson Laboratory), demonstrated statistically significant increase in both tail length and body weight, phenomena equivalent with effective intervention in this model, during 30 days following a single ICV administration of BBrm02. Toxicological studies with intrathecal administrations to rats and dogs were completed, establishing no observed adverse effect level (NOAEL).
Conclusions:
Our combined results demonstrate a proof of efficacy of the Bioblast approach for the treatment of SMA using its lead molecule, BBrm02. The completion of safety studies enables Bioblast to initiate Phase I clinical study in the near future.
Acknowledgements:
E Osman, C Washington, CL Lorson, University of Missouri, Columbia, MO, USA
M Osborne and C Lutz, Rare and Orphan Disease Center, The Jackson Laboratory, Bar Harbor, ME, USA

Spinal muscular atrophy (including variants)- #2432

P24- 363- Importance of high concentration of BDNF and NGF in neurite-inhibition effect when analyzing blood serum of patients with 2 type Spinal Muscular Atrophy in organotypic tissue culture

Mariia Sokolova (1), Valentina Penniyaynen (2), Sergei Lobzin (3), Anastasia Lobzina (4)
1. Ph.D. of Medicine, North-West StateMedical University n.a. I.I. Mechnikov, neurology chair n.a. acad. S.N.Davidenkov, Saint-Petersburg, Russie

2. Ph.D. of Biology, FSBU Institute of physiology n.a. I.p. Pavlov of RAS, Russia, Saint-Petersburg, Russie
3. Ph.D. of Medicine, professor, North-West StateMedical University n.a. I.I. Mechnikov, neurology chair n.a. acad. S.N.Davidenkov, Saint-Peterburg, Russie
4. student, North-West StateMedical University n.a. I.I. Mechnikov, neurology chair n.a. acad. S.N.Davidenkov, Saint-Petersburg, Russie

METHODS: 12 patients with SMA 2 type aged 8-12 years were examined. Study blood serum of the 12 patients was done in an organotypic tissue culture. Studies were performed on 600 explants sensory dorsal root ganglia of the chick embryo. The ganglia were cultured for three days on collagen supports in Petri dishes at 36.5 °C and 5% CO2. CO2 incubator (Sanyo, Japan) was used. In experimental dishes, blood serum of patients with 2 type SMA was added to the cultural medium. The growth of explants in tissue culture was controlled on vital preparations using a confocal laser scanning microscope LSM-710 (Carl Zeiss, Germany). Neurites outgrowth was quantified using the Image J program. The area index was used to estimate the neurite outgrowth. All values were taken as 100% in the control experiments. Level Beta-NGF and BDNF in 12 patients (SMA 2 type) was determined in serum using Beta-NGF ELISA Kit and BDNF ELISA Kit (RayBiotech, Inc). The statistical analysis was carried out using STATISTICA 8.0 package (StatSoft®, Inc., USA).
RESULTS: According to enzyme immunoassay a concentrations of the NGF and BDNF in blood serum of patients with 2 type SMA was statistically significantly (?>0,001) higher than in the control group. Concentration of the NGF in the blood plasma in the control group was within the range from 110 to 2237 pg/ml, in patients with 2 type SMA- from 1387 up to 5411 pg/ml . Concentration of BDNF (36653±3606 pg/ml) in blood serum of SMA 2 patients was significantly (? >0,05) higher than in control group (27313±7260 pg/ml). We studied the influence of blood serum of patients with 2 type SMA on growth of neurites of sensory ganglia of 10-12 days old chicken embryos in organotypic culture. Blood serum was researched in a wide range of dilution (1:100-1:2). The plasma of patients in a dilution of 1:2 -1:50 completely blocked the growth of sensory ganglion neurites.

AI of experimental explants cultured in the medium containing blood serum in dilution 1:70 was 25% less than the control value. The correlation analysis has shown strong negative association between inhibition phenomenon and high concentration of NGF and BDNF.
SUMMARY: For the first time it was shown that blood serum of patients with 2nd type SMA inhibits the growth of neurites of dorsal root ganglia in dose-dependent manner. Apparently, neurite-inhibitory effect is caused by high concentration of NGF and BDNF in patient serum.

SMA of type 2, blood serum, BDNF, NGF, organotypic tissue culture, neurite-inhibitory effect

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Spinal muscular atrophy (including variants)- #2475

P24- 364- Baseline data from a European Prospective and Longitudinal Natural History of patients with type 2 and 3 Spinal Muscular Atrophy- NatHis-SMA

Aurelie Chabanon (1), Mélanie Annoussamy (1), Aurélie Phelep (1), Jean-Yves Hogrel (2), Pierre Carlier (3), Emmanuel Fournier (4), Ricardo Hermosilla (5), Guillemette Ramey (6), Christian Czech (7), Anne Marquet (7), Omar Khwaja (7), Linda Lowes (8), Yann Pereon (9), Armelle Magot (9), Claude Cances (10), Jean-Marie Cuisset (11), Vincent Laugel (12), Carole Vuillerot (13), Aurore Daron (14), Nathalie Goemans (15), Liesbeth De Waele (15), Ulrike Schara (16), Thomas Voit (17), Laurent Servais (1)
1. Institut I-Motion, Association Institut de Myologie, Paris, France
2. Laboratoire de Physiologie et Evaluation Neuromusculaire, Association Institut de Myologie, Paris, France
3. Laboratoire RMN, Association Institut de Myologie, Paris, France
4. Unité clinique de pathologie neuromusculaire, Association Institut de Myologie, Paris, France
5. Roche Innovation Center, Roche Pharmaceutical Research and Early Development, Basel, Suisse
6. Institut Roche de Recherche et de Médecine Translationnelle, Paris, France
7. Roche Innovation Center, Roche Pharmaceutical Research and Early Development, Basel, France
8. Nationwide Children's Hospital, Columbus, Ohio, Etats-Unis
9. Centre de référence Maladies Neuromusculaires Nantes-Angers, Hôpital Hôtel-Dieu, Nantes, France
10. Unité de neurologie pédiatrique, Hôpital des Enfants, Toulouse, France
11. Service de neuropédiatrie, Hôpital Roger Salengro, Lille, France
12. Neuropédiatrie, CHU Strasbourg Hautepierre, CHU Strasbourg Hautepierre, Strasbourg, France
13. Service de rééducation pédiatrique infantile ?L'Escale?, Hôpital Mère Enfant, CHU-Lyon, Lyon, France
14. Centre de référence neuromusculaire, CHR La Citadelle, Liège, Belgique
15. Reference center for neuromuscular diseases, University Hospitals Leuven, Leuven, Belgique
16. Paediatric neurology and neuromuscular center, University of Essen, Essen, France
17. Association Institut de Myologie, Paris, France

Spinal muscular atrophy (SMA) is the second most frequent autosomal recessive disorder worldwide affecting approximately 1 in 10,000 peoples. Clinical manifestations include muscle atrophy and varying degrees of weakness.
Several therapeutic strategies for treatment of SMA are under investigation including SMN2 splicing modifier and gene therapy. Recently, Trophos Company reported positive results in a phase III trial for olesoxime. However no effective treatment has currently received market approval.
Due to the variability in SMA patients' phenotypes, it is important to determine the best outcome measures to assess the efficacy of potential therapies and the prognostic factors for the disease. We present here the baseline data of a prospective study of the pathophysiology of type 2 and 3 SMA patients to characterize the disease course by using standardized evaluations. A total of 70 patients aged 2 to 30 years old have been to be enrolled in Belgium, France and Germany. Visit frequency and assessments are adjusted to age, ambulatory and respiratory status. Evaluations include clinical exam, pulmonary function tests, electrophysiology and NMR measurements, strength and motor function assessments using upper limb-specific devices (MyoSet and Active-Seated), MFM scale and standard timed tests, activity monitoring using the Actimyo device and quality of life assessment. Blood samples are collected to determine the copy number of SMN2 gene, and to analyze SMN mRNA, SMN protein and exploratory biomarkers. Data from the patients' usual follow-up care are collected from their medical files including psychomotor development, respiratory function, feeding and orthopedic status. According to the fatigability of the patient, the assessments can be performed on one or two days.
The generated data will help to characterize the disease course and spectrum of SMA and may help to empower future therapeutic studies as well as to eventually substitute for placebo groups.

SMA

Spinal muscular atrophy (including variants)- #2485

P24- 365- Tweak/Fn14 pathway: at the crossroads of muscle atrophy and metabolic perturbations in spinal muscular atrophy and amyotrophic lateral sclerosis

Melissa Bowerman (1), Karin Meijboom (2), Gareth Hazell (1), Corinne A Betts (1), Emily McFall (3), Marc-Olivier Deguise (4), Daniel Anthony (1), Rashmi Kothary (3), Linda C Burkly (5), Matthew JA Wood (1)
1. University of Oxford, Oxford, Royaume Uni
2. University of Oxford, OXFORD, Royaume Uni
3. Ottawa Hospital Research Institute, Ottawa, Canada
4. Ottawa, Canada
5. Biogen Idec, Cambridge, Etats-Unis

Amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA) are neuromuscular disorders characterized by selective loss of spinal cord motoneurons and muscular atrophy. While motoneurons are undoubtedly the primary targets of these diseases, ALS and SMA muscle displays intrinsic defects and therapeutic strategies aimed at alleviating muscle pathology in pre-clinical models have lead to improved lifespan and/or phenotype. A combined therapeutic action at both neuronal and muscular sites can therefore be considered as the most pertinent approach towards these motoneuron diseases. TWEAK is a

cytokine of the TNF family, which predominantly binds the Fn14 receptor. Interestingly, the TWEAK/Fn14 interaction has been ascribed a critical role in the regulation of denervation-induced muscle atrophy as well as in muscle proliferation, differentiation, metabolism and atrophy. Seeing as neurodegeneration, muscle atrophy and metabolic perturbations typify ALS and SMA, we set out to investigate the expression profiles of Tweak and Fn14 in SOD1G93A ALS mice and Smn-/-;SMN2 SMA mice. We have thus uncovered an aberrant expression of both Tweak and Fn14 mRNA in various skeletal muscles of ALS and SMA mice. While we have previously addressed the contribution of TWEAK to ALS disease progression, the roles of Fn14 in ALS pathology and of TWEAK and Fn14 in SMA pathogenesis remain to be investigated. Furthermore, TWEAK and Fn14 have recently been reported to interact with and regulate the expression of PGC-1?. PGC-1? is an important mediator of atrophy and energy metabolism in skeletal muscle. Interestingly, our preliminary data in SMA muscle also show an aberrant regulation of PGC-1? mRNA, in agreement with the dysregulated Tweak and Fn14 levels. We thus hypothesize that an aberrant expression of the TWEAK/Fn14 pathway in ALS and SMA skeletal muscle promotes and exacerbates muscle atrophy and metabolic perturbations and that the modulation of this pathway may ameliorate disease progression. Endeavors are presently underway to further define the pathogenic roles of TWEAK and Fn14 in ALS and SMA muscle as well as assess the therapeutic potential of manipulating TWEAK and Fn14 expression via genetic and pharmacological approaches. The overall aim of this project is to highlight TWEAK and Fn14 as novel muscle-specific molecular targets for ALS and SMA therapy that could eventually be combined with strategies targeting the central nervous system.

spinal muscular atrophy, amyotrophic lateral sclerosis, Tweak, Fn14, muscle atrophy, metabolism

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Spinal muscular atrophy (including variants)- #2570

P24- 366- Development of a cell-penetrating peptide for the delivery of antisense oligonucleotides to peripheral and CNS tissues of spinal muscular atrophy mice

Melissa Bowerman (1), Frank Abendroth (2), Gareth Hazell (1), Karin Meijboom (1), Corinne Betts (1), Larissa Goli (1), Francesco Catapano (3), Haiyan Zhou (3), Francesco Muntoni (3), Michael Gait (2), Matthew Wood (1), Suzan Hammond (1)

1. *Oxford, Royaume Uni*
2. *Cambridge, Royaume Uni*
3. *London, Royaume Uni*

Spinal muscular atrophy (SMA) results from functional loss of the survival motor neuron 1 (SMN1) gene and is characterized by loss of lower motor neurons and muscular atrophy. Most eukaryotes have a single copy of SMN1. In humans however, a genomic duplication gave rise to a second gene, SMN2. Although nearly identical, the critical difference between SMN1 and SMN2 lies at position 6 of exon 7, where a C to T substitution in SMN2 leads to aberrant splicing of exon 7 and production of an unstable SMN?7 protein. Thus, SMN1 expresses the full-length (FL) SMN protein while SMN2 mostly produces the SMN?7 protein and only a small amount of FL protein. Many therapeutic interventions have thus focused on promoting SMN2 exon 7 inclusion. Recently, the use of antisense oligonucleotides (ASOs) that bind SMN2 mRNA, modify its splicing and induce exon 7 inclusion, has emerged as a viable therapy for SMA. ASOs show promise in SMA mice and successful improvements of patients in phase II clinical trials. However, they require invasive administration methods for adequate delivery to the CNS and do not provide systemic delivery to peripherally affected tissues. An alternate method is to covalently conjugate the ASO to a cell-penetrating peptide (CPP). We have developed such a peptide-conjugated ASO (Pip6a-PMO) that efficiently modulates splicing in various tissues when delivered via a less invasive intravenous injection. We have evaluated Pip6a-ASO in neonatal SMA mice and observe a significant upregulation of FL SMN2 in CNS and peripheral tissues, rescue in lifespan and overall improvement of neuromuscular phenotype. Pip6a-ASO also crosses the blood brain barrier in adult mice, upregulating FLSMN transcripts in the spinal cord and brain. Seeing as only a small fraction of possible CPP designs has been explored, our objective is to generate a novel CPP that can more effectively penetrate CNS and peripheral tissues in adult mice and display an enhanced favorable toxicity profile. Here, we present the CPPs evaluated in neonatal SMA pups and adult WT mice that express human SMN2. Our goal is to develop a novel clinically amenable and relevant CPP-ASO approach for SMA.

Spinal muscular atrophy, survival motor neuron, cell-penetrating peptides, antisense oligonucleotides, splicing modification

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Spinal muscular atrophy (including variants)- #2729

P24- 367- AAV9-mediated SMN expression in peripheral organs is crucial for SMA gene therapy in mice.

Aurore Besse (1), Marianne Roda (1), Stéphanie Astord (1), Thibaut Marais (1), Martine Barkats (1)

1. CNS Gene Transfer & Biotherapy of Motor Neuron Diseases, Centre de Recherche en Myologie, Paris, France

Spinal muscular atrophy (SMA) is a genetic devastating neuromuscular disease of childhood characterised by progressive loss of motor neurons (MNs), muscle denervation, weakness, and paralysis. Recently, a major breakthrough has been done in gene therapy for motor neuron diseases with our discovery that self-complementary adeno-associated-virus vector of serotype 9 (scAAV9) could mediate widespread and sustained transduction of MNs after intravenous (IV) delivery in both neonatal and adult animals. Importantly, this approach was successfully translated to SMA gene therapy using IV delivery of ubiquitous AAV9-PGK-SMN in neonatal SMN?7 mice.

As a continuation of this work, we showed that intracerebroventricular (ICV) delivery of the ubiquitous AAV9-PGK-SMN vector outperformed that of IV, suggesting a crucial role of SMN in MNs. However, ICV AAV9-PGK-SMN injection also mediated high and widespread SMN expression in peripheral organs such as heart and liver.

To determine the respective contribution of central and/or peripheral SMN expression in SMA mouse rescue, mice were ICV injected with an AAV9 vector expressing SMN under control of the neuron-specific Synapsin promoter (AAV9-SYN-SMNopti). Interestingly, mice injected at the « therapeutic dose » did not survive beyond 20 days of age. The transcriptional strength of the SYN promoter being possibly weaker than that of PGK, a second group of mice was injected with a high dose of scAAV9-SYN-SMN (3-fold the ?therapeutic dose?). Again, the median survival remained significantly lower than that of mice injected with AAV9-PGK-SMN (42 days for scAAV9-SYN-SMN injected mice versus 105 or 173 days for IV and ICV scAAV9-SMN injected

mice, respectively), although 70 to 90% of MNs expressed SMN. All but one scAAV9-SYN-SMN injected mice died before the age of 70 days, and displayed severe necrosis of the limb extremities.

Finally, co-injection of the scAAV9-PGK-SMNopti vectors into both the temporal vein and the brain ventricles (Co-IV/ICV) improved the efficacy of the treatment (significant delay in disease onset, and improvement of body weight loss and spontaneous motor activity)

Together, these results argue in favor of a crucial role of peripheral SMN expression for the treatment of SMA mice and provide Co-IV/ICV delivery as an optimal route for SMN gene therapy.

Gene therapy, SMA, AAV

Spinal muscular atrophy (including variants)- #2735

P24- 368- Differential atrophy mechanisms operate in different muscles of mouse models of SMA

Marc-Olivier Deguise (1), Emily McFall (1), Armin Yazdani (1), Yves De Repentigny (1), Justin Boyer (1), Rashmi Kothary (1)
1. Ottawa Hospital Research Institute, Ottawa, Canada

Spinal muscular atrophy (SMA) is characterized by selective loss of motor neurons in the spinal cord and eventual skeletal muscle atrophy. Most research to date has focused on understanding why motor neurons are preferentially affected in the disease. In contrast, the contribution of defective mechanisms in skeletal muscles to SMA pathogenesis has been largely overlooked. Regardless, emerging evidence is demonstrating that muscles and other organs are also implicated in the overall clinical picture of SMA. We therefore investigated the molecular pathways implicated in the reduction of muscle fiber size in mouse models of SMA, and we further examined how these defects can be reversed. We observed that although proteosomal degradation is present in skeletal muscles of both severe (Smn-/-;SMN2) and less severe (Smn2B/-) mouse models of SMA, autophagosomal degradation is only observed in skeletal muscles of Smn2B/- mice. Autophagy regulation in skeletal muscles is complex and could be mediated by multiple pathways. Our data suggest that the FoxO transcription factor family may play a role in this process. We also demonstrate that the histone deacetylase inhibitor trichostatin A (TSA) completely reversed the effect on protein degradation pathways in muscles from Smn2B/- mice, independent of an increase in the SMN protein. We have extended our analysis to cardiomyocytes since they have been shown to be smaller in size. Interestingly, we observed different patterns of expression in atrophic genes in the hearts of both mouse models. Taken together, these results provide evidence that different molecular mechanisms can intervene in different mouse models of SMA, dependent on disease severity. Additionally, we showed that TSA can provide beneficial effects on muscles that are non-SMN mediated. Further research will concentrate on understanding pathways that are specifically targeted by TSA in SMA pathogenesis.
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spinal muscular atrophy, HDAC inhibitors, Trichostatin A, autophagy, proetosomal degradation

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Spinal muscular atrophy (including variants)- #2739

P24- 369- Innovative method for motor functions evaluation in SMA type 2 and 3 patients

Justine Coton (1), Dominique Vincent-Genod (2), Julien Veytizou (1), Guillaume Thomann (1), Carole Vuillerot (2)
1. Collaborative Design, Laboratory G-SCOP, Grenoble, France
2. Rééducation pédiatrique, Hospices civils de Lyon, Hôpital Femme Mère Enfant, Lyon, France

Considering the current progress being made in terms of research and clinical practice in neuromuscular diseases (NMD), interest in developing valid outcome measures to assess the evolution of motor function in order to precisely describe the effects of treatments tested in international therapeutic trials has recently greatly increased. Hence, several scales as the Motor Function Measure (MFM) have been developed with the aim to measure validly, reproducible and sensitive motor function of patients with NMD.
Focusing in type 2 and 3 Spinal Muscular Atrophy (SMA), the originality of our approach is to measure quantitatively the children's motor function by a low-cost, non-invasive and plug and play technological tools that avoid the hazardous characteristic of the human evaluation and increase children's compliance for the evaluation. This project aims to propose more precise and reproducible tools to be used as an outcome measure for the MFM protocol in clinical trial.
Firstly, to analyze the relevance of the 32 items in our context (MFM for SMA), MFM's items were subjected to experts through a Delphi process. This process has enabled the extraction of specific items for SMA patients. Secondly, for each specified items, the technological needs in terms of detection and analysis were identified leading to the Microsoft Kinect as a preferential sensor which presents interesting measurements and utilities advantages.
Thirdly, this sensor associated with a software developed by our research team, KiMe2 (Kinetic Medical Measurement), permits to automatically score MFM's items. Experiments were proposed to adjust and validate the relevance of the KiMe2?s score. The first experiments made on selected item from the MFM with 5 SMA volunteer's patients showed good correlation between the score generated by our tool and the practitioners' evaluation. The current tool may lack precision and ability to score specific cases. The project's future is to improve this tool in a twofold perspectives: (1) to be able to fully deal all items of the MFM, and (2) to take advantage of the Kinect gaming features in order to develop entertaining activities for the MFM's practice.

Spinal Muscular Atrophy (SMA), Motor Function Measure (MFM), Kinect

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Spinal muscular atrophy (including variants)- #2970

P24- 370- Study of correlation between DNA methylation levels of DYNC1H1, SLC23A2 and CDK2AP1 genes and spinal muscular atrophy severity

Marianna Maretina (1), Galina Zheleznyakova (2), Anna Egorova (3), Kristina Lanko (4), Vladislav Baranov (5), Anton Kiselev (6)
1. PhD student, D.O. Ott Research Institute of Obstetrics and Gynecology RAMS, Saint-Petersbourg, Russie
2. PostDoc, Karolinska Institute, Stockholm, Suède

3. PostDoc, D.O. Ott Research Institute of Obstetrics and Gynecology RAMS, Saint-Petersbourg, Russie
4. student, Saint-Petersburg State Technological Institute, Saint-Petersbourg, Russie
5. head of laboratory of Prenatal Diagnostics of Inherited Diseases, D.O. Ott Research Institute of Obstetrics and Gynecology RAMS, Saint-Petersbourg, Russie
6. senior scientist, D.O. Ott Research Institute of Obstetrics and Gynecology RAMS, Saint-Petersbourg, Russie

Spinal muscular atrophy (SMA) is an autosomal recessive disorder characterized by degeneration of motor neurons of the spinal cord caused by mutations within the SMN1 gene. The SMN1 gene has a nearly identical copy- the SMN2 gene- that produces about 10% of full-length SMN transcript and is a principal disease modi?er. SMA is subdivided into 4 clinical groups depending on the age of onset and disease severity. The SMN2 copy number correlate with symptoms severity, however, involvement of additional factors is also suggested. Previously we showed that DNA methylation level influences disease phenotype. Genome-wide methylation analysis for SMA patients compared to healthy individuals was performed and a significant difference in the methylation levels between two groups was found for the CpG islands of 40 genes. Genes most probably involved in SMA pathogenesis have been chosen for further analysis: DYNC1H1, SLC23A2 and CDK2AP1 that encode proteins implicated in axonal transport, neuritogenesis and chromatin remodeling respectively. For methylation profiles determination of CpG islands we used high-resolution melting procedure with bisulphite-modified DNA. Analysis of melting profiles of PCR products was carried out on 78 DNA samples of patients with severe SMA type I (40) and mild form III-IV (38). Methylation level was estimated by the method of interpolating polynomials using fluorescence values of DNA standards with known percentage of methylation. There was no significant difference in methylation of 5'-UTR of SLC23A2 and CDK2AP1 genes and DYNC1H1 exon 41 between patients with severe and mild SMA forms. However, patients with mild phenotype have significantly higher methylation level of DYNC1H1 exon 37 compared to those with severe SMA form. Previously it was reported that gene body methylation is necessary for normal gene expression; also mutations of DYNC1H1 gene was shown to be associated with development of neuromuscular diseases caused by impaired axonogenesis, such as ALS and dominant SMA. Thus our results are consistent with involvement of this gene into SMA pathogenesis caused by degeneration of motor neuron axons.

spinal muscular atrophy, DNA methylation, epigenetic modifiers

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Spinal muscular atrophy (including variants)- #2984

P24- 371- Identification and characterization of a drug candidate for spinal muscular atrophy

Dephine Sapaly (1), Matthieu Dos Santos (1), Gwendoline Querol (1), Olivier Biondi (1), François Girardet (1), Perrine Delers (1), Kevinee Khoobarry (1), Frédéric Charbonnier (1), Suzie Lefebvre (1)
1. INSERM UMR_S1124, CICB-Paris, Université Paris Descartes, Paris, France

Proximal spinal muscular atrophy (SMA) is an incurable autosomal recessive neurodegenerative disease characterized by the degeneration of spinal motor neurones and progressive muscular atrophy. The disease is due to mutations of the survival motor neuron 1 gene (SMN1), leading to reduced levels of the ubiquitously expressed SMN protein. SMN is present in both the cytoplasm and nucleus, where it concentrates in Cajal bodies (CBs). There are functional links between CBs and small nuclear ribonucleoprotein (snRNP) and small nucleolar (sno)RNP biogenesis. Whereas more is known about the cytoplasmic role of the SMN complex in the assembly of the splicing snRNPs, its CB-associated function remains elusive. By studying the defects of the CB composition in SMA patient fibroblast cells, we screened chemical libraries and identified a small-molecule able to promote both SMN protein and snRNP localization into CBs. We then addressed the question whether this hit has any SMA-relevant activities in a mouse model of SMA. We show that survival increases while muscle atrophy decreases in the Taiwanese SMA mouse model. Moreover, SMN protein is re-localized to CBs in motor neurones of SMA mice upon treatment. Ongoing work is directed at studying the mode of action of the molecule both in vitro and in vivo. Hopefully, these studies will allow a better understanding of SMA disease and help design therapeutic strategies.

spinal muscular atrophy, neurodegeneration, SMN protein complex, Cajal bodies, ribonucleoproteins

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Spinal muscular atrophy (including variants)- #2985

P24- 372- The use of cell-permeable peptides to delineate Gemin8 function in Cajal bodies formation and SMN protein complex localization in SMA

Benoit Coddens (1), Perrine Delers (1), Mounira Kharoubi (1), Margherita Di Pisa (1), Solange Lavielle (1), Alphonse Garcia (1), Suzie Lefebvre (1)
1. INSERM UMR_S1124, CICB-Paris, Université Paris Descartes, Paris, France

The neurodegenerative disease spinal muscular atrophy (SMA) is caused by mutations in the survival motor neuron (SMN1) gene that results in the reduction of the SMN protein levels. The SMN complex is involved in the assembly of the small nuclear RNAs (snRNAs) into core splicing small nuclear ribonucleoprotein (snRNP) in the cytoplasm. These core complexes are then imported into the nucleus and targeted to the Cajal bodies (CBs) for the final maturation steps. SMN is also found in the CBs but its CB-associated function remained elusive. Phosphorylation is regulating the sub-cellular localization of the SMN complex. We previously showed that protein phosphatase PP1 gamma modulates snRNP biogenesis by the formation of the complex and its localisation to CBs through an interaction between PP1 gamma and SMN-complex component Gemin8 protein. But how does this interaction coordinate the assembly of the SMN complex to promote the biogenesis of snRNPs? To address this question we use here cell-permeable peptides (CPPs) that are short amino acid (aa) sequences entering into living cells very quickly. Using drug phosphatase technology (DPT) CPPs derived from PP1-interacting domain of Gemin8, we characterize the formation of CBs in HeLa and SMA patient fibroblast cells. Our findings form the basis to explore the mechanisms by which the gemin8-derived DPT-peptides mediate the formation of CBs and the recruitment of SMN. Moreover, if SMN has any SMA-relevant function in CBs, the DPT-peptides might be useful in the future development of a therapeutic strategy against SMA.

spinal muscular atrophy, SMN protein complex, Cajal bodies, ribonucleoproteins, protein phosphatases, cell-permeable peptides

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Spinal muscular atrophy (including variants)- #2997

P24- 373- Interest of the ratio ?thoracic diameter / head circumference? in children with spinal muscular atrophy type 1

Juliette ROPARS (1), Isabelle DESGUERRE (2), Christine BARNERIAS (2), Sara BRANDT (3)
1. service de pédiatrie et génétique médicale, CHRU Brest, BREST, France
2. Service de neurologie pédiatrique, centre hospitalier Necker Enfants Malades, PARIS, France
3. department of mental health, Mount Sinai Hospital New York, NEW YORK, Etats-Unis

Spinal muscular atrophy type 1 (SMA 1) is a neuromuscular disease usually fatal before the age of 1 year, mainly related to severe respiratory impairment. With the recent development of new therapies, identification of outcome measures for this disease is essential.
The objective of the study was to compare anthropometric parameters of a group of children with SMA 1 to healthy control children of the same age in order to identify outcomes measures of disease.
The anthropometric parameters (i.e. weight, height, thoracic diameter (TD), head circumference (HC)) of 17 children with SMA 1 were compared to 124 healthy control children of the same age.

The children SMA 1 had a TD, BMI and TD / HC ratio significantly lower than that of control children. Specifically, the TD / HC ratio was 1 (± 0.04) in control children; it was significantly decreased in children SMA 1 regardless of age. The TD / HC ratio decreased with age for the same SMA 1 child, although there was no statistically significant association between death time and the value of TD / HC ratio.
The TD reflects an abnormal chest growth in children SMA 1 due to the severe damage of the intercostal muscles. These data suggest that the TD / HC ratio eliminates the influence of age on the TD. In children with SMA 1, TD / HC ratio may reflect the evolution of this disease. The ratio TD / HC could be an objective measure, sensitive, simple and non-invasive for monitoring the disease. Further studies with larger sample size are nedded to confirm the clinical and prognostic relevance of this ratio.

spinal muscular atrophy, outcome measures, respiratory insufficiency

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Spinal muscular atrophy (including variants)- #3004

P24- 374- SMN rescue following tricyclo-DNA antisense oligonucleotides treatment

Valerie Robin (1), Graziella Griffith (1), branislav dugovic (2), Christian J. LEUMANN (3)
1. UFR Science de la santé, U1179-UVSQ, montigny le bretonneux, France
2. University of Berne, Synthena, berne, France
3. department of chemistry and biochemistry, University of Bern, montigny le bretonneux, France

Spinal muscular atrophy is a recessive disease caused by mutations in the SMN1 gene, which encodes a protein (SMN) involved in RNA processing whose absence dramatically affects the survival of motor neurons. In Man, the severity of the disease is alleviated relatively to the SMN2 gene copy number, which varies from individual to individual. SMN2 encodes the same SMN protein as SMN1. However, a single nucleotide change (C to T transition) affects the definition of exon 7 during splicing such that about 90% of SMN2 mRNAs lack this exon. One of the most promising therapeutic strategy for SMA aims at reincluding exon 7 using antisense oligonucleotides. In this study, we investigate in patient's cells the therapeutic potential of the tricyclo-DNAs (Tc-DNA), Tc-DNA antisense oligonucleotides annealing a nearby intron 7 splice silencer (ISS) of the SMN2 pre-mRNA. RT-PCR showed approximately 60% of SMN2 mRNAs rescued after treatment with Tc-DNA analogues targeting the ISS, leading to nearly normal levels of SMN, detected by Western blot. Immunostaining also confirmed that rescued SMN was correctly located in nuclear gems.

More importantly, we investigated also the therapeutic potential of Tc-DNA in vivo by weekly subcutaneous injections of Tc-DNA[ISS] in SMA type III mice. This study revealed efficient inclusion of exon 7 in all tissues analyzed, including in brain and spinal cord, confirming results obtained with Tc-DNA in mdx model. Tc-DNA treatment rescued the phenotype of SMN type III mice and prevented necrosis of tails, ears and toes in treated mice compared to controls. An improvement in respiration function has also been observed in plethysmography after Tc-DNA treatment.

Altogether, these results suggest the therapeutic potential of Tc-DNA for the systemic treatment of SMA.

tricyclo-DNA, spinal muscular atrophy

Spinal muscular atrophy (including variants)- #3175

P24- 375- Muscle stem cells in Spinal Muscular Atrophy: SMN regulates muscle satellite cell behavior

Nathalie DIDIER (1), Jordan MECCA (2), Stéphanie ASTORD (3), Thibault MARAIS (3), Martine BARKATS (4)
1. Biology of the Neuromuscular system- INSERM IMRB U955- Unité d'Ingénierie et de Thérapie Cellulaire, EFS Ile de France, Créteil, France
2. Centre de recherche en Myologie, UMRS 974 UPMC- INSERM- FRE 3617 CNRS- AIM, UPMC, Paris, France
3. Centre de recherche en Myologie, UMRS 974 UPMC- INSERM- FRE 3617 CNRS- AIM, AIM, Paris, France
4. Centre de recherche en Myologie, UMRS 974 UPMC- INSERM- FRE 3617 CNRS- AIM, INSERM, Paris, France

Spinal muscular atrophy (SMA) is a common human inherited disease characterized by progressive degeneration of motor neurons (MNs) and muscle atrophy. In most cases, this pathology is due to homozygous mutations in the Survival of Motor Neuron 1 gene (SMN1), coding for the ubiquitous SMN protein whose role in RNA metabolism has been largely studied in MNs.
We and others have reported that a single intravenous injection of SMN1-encoding self-complementary AAV9 vectors induced a tremendous rescue of SMNdelta7 mice, a common model of SMA. However, recent data from our group and from the literature suggest that this therapeutic effect could result not only from the restoration of SMN expression in MNs but also at the periphery. Among peripheral tissues affected by reduced levels of SMN, skeletal muscle fibers and stem cells (named satellite cells or SCs), may be particularly vulnerable.

In order to further investigate the role of SMN in muscle stem cell function, we characterized post-natal myogenesis of a severe mouse model of SMA (hSMN2). We observed that reduced levels of SMN lead to an increased number of proliferating SCs (Pax7+MyoD+) associated with a decreased number of self-renewing SCs (Pax7+MyoD-) in SMA mice as compared to littermate controls. Conversely, overexpression of SMN by intramuscular scAAV9-SMN1 injection in newborn wild-type mice, induced a strong increase of the quiescent SC pool and decreased fusion.

These data suggest for the first time that SMN may be involved in the transition between proliferative and quiescent states of muscle SCs. Therefore, SMN levels may have to be tightly regulated for proper post-natal growth and SC pool constitution, which could have major implications for the development of SMA gene therapy strategies.

SMA, Muscle, Muscle stem cells

Spinal muscular atrophy (including variants)- #4468

P24- 376- Skeletal muscle expression of insulin like-growth factor system elements in spinal muscular atrophy patients

Ay?e Ye?bek Kaymaz (1), Beril Talim (2), Gamze Bora Tatar (1), Hayat Erdem Yurter (1)
1. Department of Medical Biology, Faculty of Medicine, Hacettepe University, ANKARA, Turquie
2. Department of Paediatrics, Pathology Unit, Faculty of Medicine, Hacettepe University, ANKARA, Turquie

Spinal muscular atrophy (SMA) is a childhood neuromuscular disease characterized by motor neuron degeneration in the spinal cord and progressive skeletal muscle atrophy. Survival of Motor Neuron 1 (SMN1) gene mutations and resulting SMN protein deficiency cause the disease. Although ~94% of the patients have homozygous SMN1 exon 7/8 deletion type of mutations, substantial variation is observed among patients in the degree of skeletal muscle atrophy. It is known that insulin-like growth factor (IGF) system elements have role in skeletal muscle development, differentiation, regeneration after denervation and IGF system dysregulation has been reported in mouse models of SMA. It was hypothesized that IGF system elements might affect the degree of muscle weakness displayed in SMA patients. The aim of the study was to investigate the expression of IGF system elements in SMA patients. For this purpose, immunoflorescence analyses were performed for IGF1, IGF2, IGFBP5 (insulin-like growth factor binding protein 5) and IGF1R (IGF1 receptor) proteins in the frozen skeletal muscle biopsy specimens of 9 SMA patients, 4 healthy controls and 6 disease controls. The results revealed that immunostaining pattern was not different between SMA patients, healthy or disease controls. More intense immunostaining was detected in atrophic fibers in the skeletal muscle of SMA and other disease controls (including muscular dystrophy and fiber type disproportion) when compared to hypertrophic fibers. The results should be further investigated for the underlying cause of the increased IGF system proteins in the atrophic fibers in SMA and other muscle diseases.

Muscle atrophy, insulin-like growth factors, insulin-like growth factor binding proteins

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Spinal muscular atrophy (including variants)- #4471

P24- 377- Skeletal muscle expression of insulin like-growth factor system elements in spinal muscular atrophy patients

Ay?e Ye?bek Kaymaz (1), Beril Talim (2), Gamze Bora Tatar (1), Hayat Erdem Yurter (1)
1. Department of Medical Biology, Faculty of Medicine, Hacettepe University, ANKARA, Turquie
2. Department of Paediatrics, Pathology Unit, Faculty of Medicine, Hacettepe University, ANKARA, Turquie

Spinal muscular atrophy (SMA) is a childhood neuromuscular disease characterized by motor neuron degeneration in the spinal cord and progressive skeletal muscle atrophy. Survival of Motor Neuron 1 (SMN1) gene mutations and resulting SMN protein deficiency cause the disease. Although ~94% of the patients have homozygous SMN1 exon 7/8 deletion type of mutations, substantial variation is observed among patients in the degree of skeletal muscle atrophy. It is known that insulin-like growth factor (IGF) system elements have role in skeletal muscle development, differentiation, regeneration after denervation and IGF system dysregulation has been reported in mouse models of SMA. It was hypothesized that IGF system elements might affect the degree of muscle weakness displayed in SMA patients. The aim of the study was to investigate the expression of IGF system elements in SMA patients. For this purpose, immunoflorescence analyses were performed for IGF1, IGF2, IGFBP5 (insulin-like growth factor binding protein 5) and IGF1R (IGF1 receptor) proteins in the frozen skeletal muscle biopsy specimens of 9 SMA patients, 4 healthy controls and 6 disease controls. The results revealed that immunostaining pattern was not different between SMA patients, healthy or disease controls. More intense immunostaining was detected in atrophic fibers in the skeletal muscle of SMA and other disease controls (including muscular dystrophy and fiber type disproportion) when compared to hypertrophic fibers. The results should be further investigated for the underlying cause of the increased IGF system proteins in the atrophic fibers in SMA and other muscle diseases.

Muscle atrophy, insulin-like growth factors, insulin-like growth factor binding proteins

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P25 – Stem cells therapies- N° 378 to N° 387

Stem cells therapies- #2335

P25- 378- Intratissular distribution and fate of grafted muscle-precursor cells after injection in normal skeletal muscle of primates.

Daniel Skuk (1), Jacques p. Tremblay (1)
1. Axe Neurosciences, Research Center of the CHU de Quebec- CHUL, Quebec, Canada

Cell therapies depend on adequate knowledge of the behavior of the grafted cells after administration. To clarify the fate of muscle-precursor cells (MPCs) injected in skeletal muscles in a context extrapolable to the human, we conducted a study in nonhuman primates.
MPCs proliferated in vitro and labeled with ß-galactosidase and/or [14C]thymidine were auto- or allo-transplanted in muscle regions of 1cm3 in cynomolgus or rhesus monkeys. Cell culture and transplantation (Tx) protocols were similar to our recent