

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**

Ayşe Yeşbek Kaymaz (1), Beril Talim (2), Gamze Bora Tatar (1), Hayat Erdem Yurter (1)

1. Department of Medical Biology, Faculty of Medicine, Hacettepe University, ANKARA, Turkiye

2. Department of Paediatrics, Pathology Unit, Faculty of Medicine, Hacettepe University, ANKARA, Turkiye

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, immunofluorescence 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*

Spinal muscular atrophy (including variants)- #4471

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

Ayşe Yeşbek Kaymaz (1), Beril Talim (2), Gamze Bora Tatar (1), Hayat Erdem Yurter (1)

1. Department of Medical Biology, Faculty of Medicine, Hacettepe University, ANKARA, Turkiye

2. Department of Paediatrics, Pathology Unit, Faculty of Medicine, Hacettepe University, ANKARA, Turkiye

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, immunofluorescence 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*

### **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

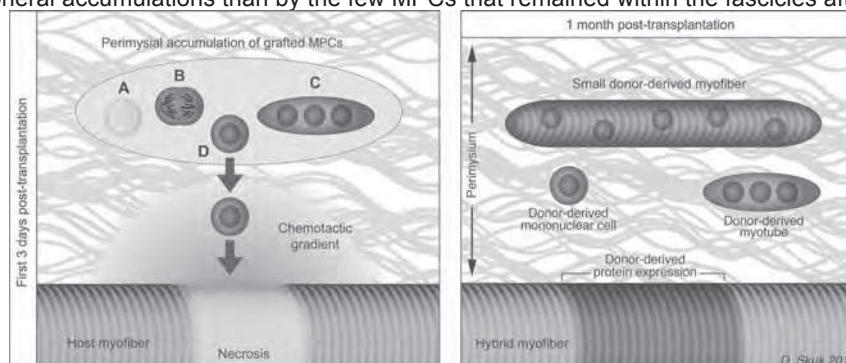
Cell therapies depend on adequate knowledge of the behavior of the grafted cells after administration. To clarify the fate of muscle-precursor cells (MPCs) injected in skeletal muscles in a context extrapolable to the human, we conducted a study in nonhuman primates.

MPCs proliferated in vitro and labeled with  $\beta$ -galactosidase and/or [14C]thymidine were auto- or allo-transplanted in muscle regions of 1cm<sup>3</sup> in cynomolgus or rhesus monkeys. Cell culture and transplantation (Tx) protocols were similar to our recent

clinical trials. The MPC-grafted regions were biopsied at post-Tx periods of 1 hour, 1, 3, 7, 14 days and 1 month, and analyzed by histology or radiolabel quantification.

One hour post-Tx, grafted cells formed compact accumulations of variable size, located randomly in the perimysium and to a lesser extent in the epimysium. Only a few cells were observed in the trajectories of injection into the muscle fascicles. At day 1, necrosis was observed in several grafted cells (by immunodetection of intracellular complement deposition) while immunodetection of active caspase 3 suggested that some cells undergo apoptosis. Proliferating cell nuclear antigen immunodetection showed significant proliferation in the grafted-cell collections during the first week post-Tx. The radiolabel analysis showed a cell death dynamics in the grafted cells during the first week following allo- and auto-Tx, relatively similar to what we previously reported in mice grafted with primary-cultured MPCs.

We interpret that during injection the cell suspension leaks from the muscle fascicles and dissects cleavage plans in the surrounding connective tissue, where it accumulates. There, the cells may have different fates (figure, left). Some die by necrosis and probably by apoptosis (A). At least some surviving MPCs proliferate (B) and either fuse among them to form myotubes (C) or migrate to participate in segmental myofiber regeneration towards the myofibers damaged (e.g., by the injection) in the surrounding fascicles (D). After 1 month (figure, right), the process is complete: the compact accumulations of grafted cells have disappeared leading to few graft-derived elements in the perimysium: mononuclear cells, myotubes and small myofibers (longitudinally or transversally oriented). Hybrid myofibers within the fascicles occur more likely by MPCs that migrated from the peripheral accumulations than by the few MPCs that remained within the fascicles after the injection.



*Cell therapy, nonhuman primates, regenerative medicine, skeletal muscle.*

Stem cells therapies- #2406

# **P25- 379- Myoblasts but not pericytes of nonhuman primates are myogenic after transplantation in immunodeficient mice.**

*Daniel Skuk (1), Jacques p. Tremblay (1)*

*1. Axe Neurosciences, Research Center of the CHU de Quebec- CHUL, Quebec, Canada*

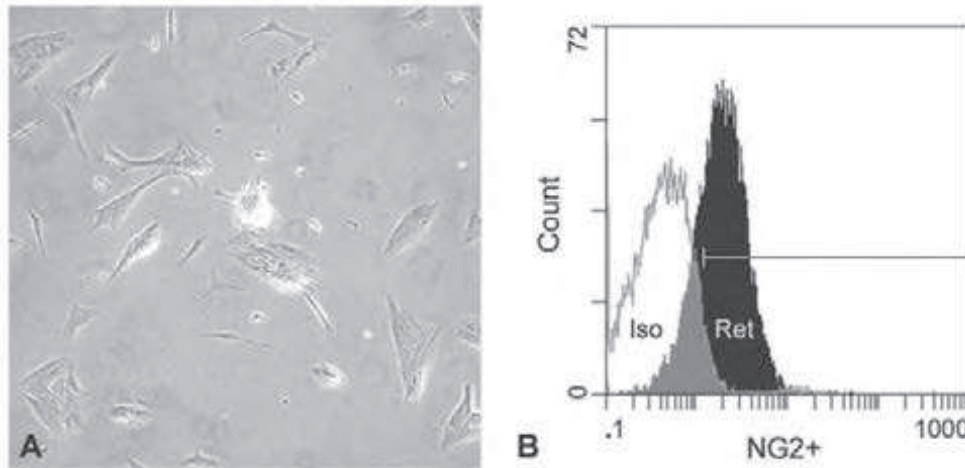
Some teams have assigned significant stem cell and myogenic capacity for cell therapies in myology to pericytes/mesoangioblasts, regardless of the tissue of origin. The retina is a tissue of choice to obtain pericytes (PCs) for these studies, due to the density of small vessels with high amounts of PCs in the absence of contamination by satellite cells. In fact, a group reported that perivascular cells from the retina, mostly PCs, were myogenic in vitro. Given the importance of nonhuman primates (NHPs) for preclinical translational research, we assessed the in vivo myogenicity of PCs of NHPs by transplantation in immunodeficient mice, testing for potential later studies of allotransplantation in NHPs. Additionally, this was the first analysis of the myogenicity of NHP myoblasts in immunodeficient mice.

Retinas were obtained from cynomolgus monkeys euthanized for reasons unrelated to this study, and we used a protocol to culture retinal PCs of large mammals. By flow cytometry, 76%- 78% of the cultured retinal cells were positive for NG2, a marker of PCs (figure). Myoblasts were proliferated in vitro from a muscle biopsy made in another cynomolgus monkey (99% of these cultured cells were CD56+).

Both Tibialis anterior (TA) muscles of 4 SCID mice were X-ray irradiated (20 Gy). A week later, 1 x 10<sup>6</sup> cells resuspended in saline with 10-µg/ml cardiotoxin (to induce muscle regeneration) were implanted per TA. Myoblasts were injected in the right TA and PCs in the left TA. Mice were killed 1 month later and the TAs were sampled and analyzed by histology.

There were abundant nuclei from NHP origin both in the left and right TAs, as detected with an anti-human/NHP lamin A/C antibody. This showed that a large amount of grafted cells survived in the muscles of the SCID mice both for myoblasts and PC grafts. In the muscles transplanted with myoblasts, these nuclei were in large regions with numerous myofibers recently regenerated and expressing human/NHP dystrophin. Several NHP nuclei expressed Pax7, generally in the periphery of regenerated myofibers. Conversely, in the PC-grafted TAs, NHP nuclei were present in areas of fibrosis in muscles with poor regeneration. Neither NHP-derived myofibers nor NHP-derived nuclei expressing Pax7 were detected by immunohistochemistry in the PC-grafted muscles.

In conclusion, NHP myoblasts, but not PCs, were capable of regenerate myofibers and potential satellite cells in vivo after transplantation in immunodeficient mice.



*Cell therapy, muscle regeneration, myofiber formation, NG2, Pax7.*

Stem cells therapies- #2503

**P25- 380- EOM satellite cells: high performance myo-engines for muscular dystrophy therapy.**

Pascal Stuelsatz (1), Andrew Shearer (1), Zipora Yablonka-Reuveni (2)

1. Department of Biological Structure, University of Washington, Seattle, Etats-Unis

2. Department of Biological Structure, University of Washington, Seattle, Etats-Unis

Extraocular muscles (EOMs) comprise a group of highly specialized skeletal muscles controlling eye movements. The EOMs represent a unique skeletal muscle phenotype based on a range of properties, including specialized patterns of innervation, diversity of expressed sarcomeric myosin isoforms and their altered susceptibility to certain disease. Especially intriguing for muscular dystrophy research, EOMs are uniquely spared in Duchenne muscular dystrophy and other dystrophies associated with impairments in the dystrophin complex. Specific traits of myogenic progenitors may be determinants of this preferential sparing, but very little is known about the myogenic cells in this muscle group. To this end, we have been investigating lineage origin, antigen signature and function of satellite cells (the adult myogenic progenitors) from mouse EOM vs. the prototypic limb muscles. We showed that EOMs harbor satellite cells that share a common signature with their limb counterparts, but are remarkably endowed with a high proliferative and renewal potential as revealed in cell culture assays. Freshly isolated EOM satellite cells also retain higher performance in cell transplantation assays in which donor cells were engrafted into the tibialis anterior muscle of host dystrophin-null mice. Experiments are now in progress to determine first the best conditions for the ex vivo expansion of EOM satellite cells, and then to analyze the engraftment outcomes of their in vitro progeny. Impressively, when cultured in vitro, EOM satellite cells establish numerous renewal cells that maintain vigorous expansion ability upon re-culturing. Collectively, our results provide a comprehensive picture of EOM myogenic progenitors promoting consideration of their use as a source for donor myoblasts that could allow bypassing the limited expansion capacity encountered with limb satellite cells, and meeting the demand for a high number of progenitors essential for clinical applications.

*satellite cells, extraocular muscles, stem cells, cell-based therapy, Duchenne muscular dystrophy, mdx mouse*

Stem cells therapies- #2612

**P25- 381- Human MuStem cells: a cell-based therapy candidate for Duchenne Muscular Dystrophy, with immunomodulatory properties**

Judith Lorient (1), Nicolas Jaulin (2), Isabelle LEROUX (1), Céline ZUBER (1), Cindy SCHLEDER (1), Marine CHARRIER (1), Blandine LIEUBEAU (3), Yann PEREON (4), Armelle MAGOT (4), Antoine HAMEL (5), Oumeya ADJALI (2), Karl ROUGER (1)

1. INRA UMR703 PAnTher, LUNAM Université, Oniris- Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation, Nantes, France

2. Atlantic Gene Therapies, INSERM UMR1089 Centre Hospitalier Université Hôtel Dieu, Nantes, France

3. INRA USC1383 Université EA4644 IECM, LUNAM Université, Oniris- Ecole Nationale Vétérinaire Agroalimentaire et de l'Alimentation, Nantes, France

4. Centre de référence des maladies neuromusculaires rares Nantes-Angers, Service des Explorations Fonctionnelles, Centre Hospitalier Université Hôtel-Dieu, Nantes, France

5. Service de chirurgie infantile, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France

Allogeneic cell transplantation protocols are highly limited by graft rejection. To avoid this effect, immunosuppressive regimens are given to patients, improving cell engraftment but return causing major secondary effects. Over the last years, various adult stem cell populations including mesoangioblasts and mesenchymal stem cells (MSC) were shown to display immunomodulation properties in vitro and in vivo (Cossu et al., 2012; English et al., 2013). In the context of cell-based therapy of Duchenne Muscular Dystrophy (DMD), these original features could increase stem cell ability to engraft in an allogeneic recipient despite the absence of strong immunosuppression and then improve muscle repair. In addition, administration of immunomodulatory cells may be beneficial in the context of chronic inflammation that characterizes DMD tissues and negatively impacts the muscle regenerative potential. We have previously demonstrated that allogeneic muscle-derived delayed adherent stem cells (that we called MuStem cells) are able to phenotypically and clinically correct the Duchenne dystrophic canine model (Rouger et al., 2011; Robriquet et al., 2015). Recently, we isolated human MuStem cells (hMuStem cells) from Paravertebralis muscle of 9 to 15-years old patients free of known muscle disease. The aim of our present study was to assess whether hMuStem cells exhibit an immune privilege behavior. Interestingly, our preliminary data show the ability of hMuStem cells from different donors to modulate allogeneic T cell proliferation and to express immunomodulatory molecules such as prostaglandin-E2, indoleamine-2,3-deoxygenase-1, Tumor Growth Factor beta 2, Heme oxygenase-1 and inducible nitric-oxide synthase-1.



Finally, our data suggest that hMuStem cells are able to interact with the complement system by inhibiting complement-mediated lysis of erythrocytes. This effect seems to be mediated by factor H, an alternative inhibitory complement pathway. Overall, our study is critical for the understanding of the interaction between MuStem cells and the immune system, as well as the design of safe and efficient allogeneic stem cell-based therapy for the treatment of muscle dystrophies.

*MuStem cells, Duchenne Muscular Dystrophy, Allogeneic transplantation, Immune system, Immunomodulation*

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Stem cells therapies- #2634

**P25- 382- Human MuStem cell, a cell therapy candidate for Duchenne Muscular Dystrophy (DMD): From research to clinical-grade production**

Charlotte SAURY (1), Sabrina VIAU (2), Isabelle LEROUX (3), Cindy SCHLEDER (3), Lucie CHABRAND (2), Yann PEREON (4), Armelle MAGOT (4), Antoine HAMEL (5), Laëtitia GUEVEL (6), Bruno DELORME (7), Karl ROUGER (8)

1. INRA UMR703 PAnTher ; LUNAM Université, Oniris, Ecole nationale vétérinaire, agro-alimentaire et de l'alimentation Nantes-Atlantique, Macopharma, Biotherapy division, Nantes, Mouvaux, France

2. Macopharma, Biotherapy division, Mouvaux, France

3. INRA UMR703 PAnTher ; LUNAM Université, Oniris, Ecole nationale vétérinaire, agro-alimentaire et de l'alimentation Nantes-Atlantique, Nantes, France

4. Centre de référence des maladies neuromusculaires rares Nantes-Angers, Service des explorations fonctionnelles, Centre hospitalier Universitaire Hotel-Dieu, Nantes, France

5. Service de Chirurgie Infantile, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes, France

6. INRA UMR703 PAnTher ; LUNAM Université, Oniris, Ecole nationale vétérinaire, agro-alimentaire et de l'alimentation Nantes-Atlantique ; Université de Nantes, Nantes, France

7. Macopharma, Biotherapy division ; Equal contribution, Mouvaux, France

8. INRA UMR703 PAnTher ; LUNAM Université, Oniris, Ecole nationale vétérinaire, agro-alimentaire et de l'alimentation Nantes-Atlantique ; Equal contribution, Nantes, France

Allogeneic delayed adherent muscle-derived stem cells, previously called cMuStem cells, are able to phenotypically and clinically improve the DMD dog model (Rouger et al., 2011 ; Robriquet et al., 2015). Recently isolated in human, hMuStem cells revealed similar phenotype and myogenic potential to cMuStem cells. Their positioning as potential cell product for clinical application to DMD notably implies the setting-up of production processes that should meet Good Manufacturing Practices (GMPs) requirements. Indeed, translation of conventional into clinical-grade cell expansion requires precise definition and standardization of all procedural parameters.

Fetal bovine serum, widely used as nutritive supplement for cell culture, is giving rise to concerns about ethical issues and xenogenic risks. As an alternative to animal-origin component, we showed that human serum allows isolation and greater expansion of hMuStem cells while preserving their phenotype of perivascular mesenchymal progenitor cells and myogenic-committed ones as well as their differentiation potential in adipocytes and osteoblasts. We also showed that xeno-free CELLstart™ Substrate, proposed as ?animal-free origin? alternative to the classically-used gelatin that is required for cell adhesion and growth, promotes hMuStem cell proliferation without alteration of myogenic potential. Then, GMP-grade tissue and cell dissociation enzymes were included in hMuStem cell isolation and expansion processes, allowing better isolation/amplification yield without affecting cell viability. Then, we demonstrated our ability to isolate at least 1,3.10<sup>5</sup> cells at P1/gr of tissue and to obtain of about 30 cumulative population doublings on 40 days of culture. Because hMuStem cells are adherent cells, seeding density appears to be a crucial parameter for optimal expansion rate and preservation of cell features. Comparative study of cell densities revealed higher amplification yield for lower tested densities (1000 cells/cm<sup>2</sup>) without alteration of cell differentiation potential. Overall, we demonstrated that hMuStem cells can be successfully generated and expanded under GMP compatible culture conditions, enabling their use in pre-clinical studies required to determine their therapeutic potential with the aim of the implementation of clinical trial. For this purpose, the contribution of expanded hMuStem cells for muscle fiber regeneration will be rapidly investigated in vivo under dystrophic context.

*Human MuStem cell, Good Manufacturing Practices, Ancillary Medicinal Substances*

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Stem cells therapies- #2667

**P25- 383- Reprogramming of Adult Muscle Satellite Cells to Pericyte-Like Cells via Notch and PDGF Signalling**

Louise Moyle (1), Matt Gerli (1), Martina Ragazzi (1), Hiroshi Sakai (2), Ekin Ucuncu (3), Chrystalla Constantinou (1), Shahrarim Tajbakhsh (2), Giulio Cossu (4), Francesco Saverio Tedesco (1)

1. University College London, London, Royaume Uni

2. Institut Pasteur, Paris, France

3. University college London, London, Royaume Uni

4. University of Manchester, Manchester, Royaume Uni

Satellite stem cells are responsible for post-natal skeletal muscle maintenance and regeneration; upon activation they proliferate as transient amplifying myoblasts, most of which fuse into myofibres. Despite the encouraging outcomes obtained from transplanting myoblasts into dystrophic animals and patients with localised forms of muscular dystrophy, results achieved in clinical trials with more severe forms of muscle diseases, such as Duchenne muscular dystrophy, show limited efficacy. In addition to satellite cells, pericyte-derived mesoangioblasts can contribute to muscle regeneration and give rise to satellite cells. Crucially, when injected systemically they migrate through the vascular endothelium, circumventing the requirement for local intra- muscular injections. These cells have recently undergone clinical experimentation in a phase I/II first-in-human trial in five Duchenne patients (EudraCT no. 2011-000176-33). We hypothesised that by modulating Notch and PDGF signalling, involved in pericyte specification in the embryo, we might reprogram adult satellite cells into pericyte-like cells. Here we show that skeletal-to-smooth muscle lineage reprogramming can be induced by exposing adult satellite cells to Dll4 and PDGF-BB. These reprogrammed cells acquire perivascular markers and functional properties, such as stabilisation of capillary networks.

Interestingly, treated satellite cells also show up-regulation of Pax7, a marker normally found in quiescent satellite cells. This implies that treated cells acquire an intermediate stem cell phenotype between satellite cells and pericytes. Importantly, preliminary in vivo data shows increased engraftment of reprogrammed cells upon both intramuscular and intra-arterial delivery in dystrophic mice. These results extend our understanding of smooth/skeletal muscle lineage choice and provide evidence of a druggable pathway with potential clinical relevance, by enabling the systemic delivery of myoblasts to treat muscle diseases.

\*L. Moyle and M. Gerli contributed equally to this work.

*myoblast, pericyte, cell reprogramming*

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Stem cells therapies- #2949

#### **P25- 384- Combinative methods to evaluate human cells in xenografts**

*Elisa Negroni (1), Anne Bigot (1), Pierre Klein (1), Capucine Trollet (1), Soraya Chaouch (1), Ingo Riederer (2), Vincent Mouly (1)*

*1. Sorbonne Universités UPMC Univ Paris 06, Inserm, CNRS, Centre de Recherche en Myologie (CRM), GH Pitié Salpêtrière, 47 bld de l'hôpital, Paris 13, France, Paris, France*

*2. Laboratory on Thymus Research, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, Brazil, Rio de Janeiro, Brésil*

Cell-based treatment is one of the innovative therapeutic strategies for genetic diseases, including muscular dystrophies. These are a heterogeneous group of disorders, characterized by progressive muscle wasting and weakness. Cell therapy for muscular dystrophies is based on the delivery in muscle tissue of precursor cells that will contribute to regeneration and tissue repair. To optimize such treatments, preclinical steps in immunodeficient mouse models and accurate methods to evaluate human cells in xenotransplantation are needed. Therefore, quantification of human cells in vivo is an essential part of these pre-clinical steps. Here we describe a combined immunofluorescence and real time quantitative PCR-based method to analyse and quantify the fate of human myoblasts after intramuscular injection in regenerating muscles of immunodeficient mice. Both techniques allow the quantification of the number of engrafted human cells. Immunofluorescence approach- using human-specific antibodies recognizing engrafted human cells- adds complementary information about human myoblasts' behaviour and localization within the host tissue, with the inconvenient of being more time consuming. Real time quantitative PCR-based method provides an accurate quantification of human cells but does not document their localization. We propose that the combination of these two approaches provides an accurate and fast way of analysing human cells engraftment after xenotransplantation and represents a useful system to compare results obtained on different types of human myogenic stem cells and/or between different research groups.

*Skeletal muscle, Cell therapy, Transplantation, Myoblast, Immunodeficient mouse model*

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Stem cells therapies- #3035

#### **P25- 385- Muscle stem cell therapy in a preclinical model of DMD: noninvasive tracking of cells by NIS transgene expression**

*David Mauduit (1), Isabel Punzon (1), Inès Barthélémy (2), Jean-Thomas Vilquin (3), Mattia Quattrocchi (4), Maurilio Sampaolesi (4), Stéphane Blot (2)*

*1. UMR U955 Equipe 10 BNMS Neurobiologie, Maisons Alfort, France*

*2. UMR U955 Equipe 10 BNMS Neurobiologie, Maisons Alfort, France*

*3. 2Sorbonne Universités UPMC Univ Paris 06, Inserm UMRS974, CNRS FRE3617, Myology Research Center, Pitié Salpêtrière Hospital, Paris, France*

*4. Stem Cell Institute Leuven, KU Leuven, Leuven, Belgique*

Cell therapy for the treatment of Duchenne Muscular Dystrophy disease (DMD) offers substantial hopes with ongoing clinical trials. Several cells have been proposed as candidates for therapy, however there is a lack of consensus of which cell type to use and which features must own this therapeutic cell. We propose a noninvasive tool to decipher the best cell candidate for cell therapy in a relevant model of DMD, the Golden Retriever Muscular dystrophy dog (GRMD). We have already proved the efficiency of mesoangioblast (MABs) to restore dystrophin expression and to improve muscle function when delivered systemically in GRMD dogs. To face the problem of tracking cells in therapeutic approaches, we decided to use the Sodium Iodide Symporter (NIS), a symporter of iodine-sodium on cells' membrane. NIS allows a noninvasive and long-term in vivo tracking of live cells by injection of radioisotope. To overcome the limited in vitro expansion of MABs until senescence, and for genetic modifications (for autologous grafts), we decided to use iPSC. For the proof of principles we first obtained human iPSC. We demonstrated that overexpression of canine NIS (cNIS) in iPSC does not alter iPSC properties and that cells are able to uptake radioactivity in vitro. The in vivo visualization of iPSC (expressing NIS) after teratoma formation in immunodeficient mice was evidenced by small animal PET and Cerenkov luminescence imaging. We have started the obtaining of canine iPSC derived from canine MABs, to then introduce the cNIS gene in a safe locus by CRISPR/Cas9 genome editing (to avoid insertional mutagenesis). We have already selected and cloned guided sequences (targeting 1st intron of PPP1R12C gene) in a pX330-U6-CBh-hSpCas9 plasmid. For the homology directed integration of cNIS (flanked by 800bp homology arms) a pCAG-cNIS-IRES-GFP was developed. We propose the study of canine iPSC-NIS+ (committed to MABs like cells) features as therapeutic cells in GRMD dogs: in vivo quantification, distribution and homing to skeletal muscle and to undesirable organs. This method will allow the comparative study of different iPSCs derived muscle progenitor in a standard animal model of DMD. A follow-up study, both by imaging and histology in combination with our functional studies will help to determine the therapeutic properties of cells: muscle colonization, dystrophin expression, beneficial effects, and influences of cells' environment on their migration and differentiation ability.

*iPSC, CRISPR/Cas9, Regeneration*

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**P25- 386- Induction of mouse embryonic stem cells differentiation using Mimic miRNAs- functional analysis in co-culture with the C2C12 myoblasts**

Maciej Daniszewski (1), Joanna Bem (1), Maria A Ciemerych (1), Iwona Grabowska (1)

1. Department of Cytology, Institute of Zoology, Faculty of Biology, University of Warsaw, Warsaw, Pologne

Proper functioning of skeletal muscles requires precise spatiotemporal control of growth, development, and tissue regeneration. All of these processes are controlled by microRNAs (miRNAs), i.e. small, single-stranded RNA molecules either initiating degradation of mRNAs or inhibiting target gene expression. Recent research demonstrated which miRNAs, both muscle-specific (myo-miRNA) or expressed in other tissues, take part in the regulation of myogenic differentiation and muscle development. In vitro experiments conducted by us aimed to determine whether the increase in the levels of selected miRNAs impacts myogenic differentiation of pluripotent mouse embryonic stem cells (ESCs). First, we determined fusion index of C2C12 myoblasts co-cultured with ESCs that were transfected with miRNA Mimics. Next, we estimated the ability of such ESCs to create hybrid myotubes in co-culture with the C2C12 myoblasts. Finally, we analysed the expression level of transcription factors and miRNAs that secure pluripotency and those associated with myogenic differentiation. Over-expression of miRNA, such as miR-1, miR-133a/b, and miR-145 enhanced the ESCs ability to fuse with myoblasts. miR-1 increased the proportion of ESCs undergoing fusion with C2C12 myoblasts and hybrid myotubes. Transfection of ESCs with miR-145 increased the levels of miR-133a as well as miR-133b and miR-1 increased the level of let-7. It did not impact, however, at the expression of mRNAs encoding myogenic markers. On the other hand, transfection of ESCs with miR-133b or miR-145 induced the expression of genes involved in the mesoderm formation and myogenic differentiation, such as Pax3 and Kdr. Thus, by using transient expression of selected miRNA one can induce myogenic differentiation of mouse ESCs in vitro.

*ES cells, skeletal muscles, myoblasts, cell differentiation, miRNA***P25- 387- Directed Embryonic stem cells differentiation- the use of mesodermal and myogenic miRNAs.**

Joanna Bem (1), Iwona Grabowska (1), Dorota Zawada (1), Maria Ciemerych (1)

1. University of Warsaw, Warsaw, Pologne

miRNAs are known regulators of embryonic stem cells (ESCs) pluripotency and differentiation. Lentiviral miRNAs overexpression have been reported to induce ESCs differentiation towards cardiomyocytes and other cell types. We tested if transient miRNA driven by mimic miRNAs overexpression could affect mouse ESCs differentiation. ESCs transfected with exogenous miRNAs were differentiated in embryoid bodies, i.e. ESCs aggregates which serve as a model for ectoderm, endoderm, and mesoderm formation, cultured under conditions supporting myogenic differentiation. Several miRNAs were tested, among them molecules specific for mesodermal lineages or muscles. Transient miRNAs overexpression lead to the decreased expression of mRNAs encoding pluripotency factors and increased expression of those encoding such myogenic markers as Pax3, Pax7, MyHC, suggesting differentiation into myogenic precursor cells or skeletal myoblasts. Meanwhile introduction of other miRNAs inhibited differentiation of skeletal myoblasts, as it decreases expression of mRNAs encoding MyoD, Myf5, MyoG. Importantly, transient miRNA overexpression turned out to be relatively stable suggesting that this technique could be used for directed stem cells differentiation into other lineages.

*embryonic stem cells, miRNAs,***P26 – Young Investigator Poster- N° 388 to N° 423****P26- 388- A new role for the transcriptional coactivator CREB Binding Protein (CBP) during myogenesis**

Sarah Diouf (1), Karim Azzag (1), Aurélie Quillien (1), Lucas Fauquier (1), Carine Duboé (1), Gilles Carnac (2), Laurence Vandel (1)

1. University Paul Sabatier- Toulouse 3, UMR5547 CNRS, Centre de Biologie du Développement, 118 route de Narbonne, 31062 Toulouse, France

2. INSERM U1046, CHU Arnaud de Villeneuve, 34295 Montpellier, France

CBP (CREB Binding Protein) is a protein involved in many physiological processes, including proliferation, differentiation and apoptosis. As a transcriptional coactivator, it is recruited by transcription factors to activate polymerase II-dependent transcription mainly through its enzymatic acetyltransferase activity. SiRNA experiments performed in the laboratory demonstrated that CBP is important for differentiation of human primary myoblasts into myotubes. While analyzing CBP expression and localization in human primary myoblasts, we found that CBP was partly localized in mitochondria upon differentiation. Biochemical subcellular purification of mitochondria confirmed the presence of CBP in this organelle. As CBP is a transcriptional co-activator in the nucleus, we asked whether it could similarly act on the mitochondrial transcriptional machinery. Indeed, we found that CBP co-localizes with the mitochondrial TFAM protein, an important component for mtDNA transcription and replication. ChIP experiments performed on mitochondrial DNA (mtDNA) showed a binding of CBP on the non-coding region of mtDNA that regulates transcription and replication. Altogether, our data suggest an unexpected role of the nuclear coactivator CBP on mtDNA transcription/maintenance that will be discussed.

*myogenesis, CBP, human primary myoblast, gene regulation***P26- 389- Impact of an antioxidant treatment on recombinant adeno-associated vector-mediated gene transfer in a mouse model of Duchenne muscular dystrophy**