

**PW 5:  
Myogenic cells and  
myostatin**

PW5-056	<p><b><u>PERTURBATION OF CALCIUM HANDLING IN CD133+ STEM CELLS ISOLATED FROM DMD BLOOD</u></b>  PAROLINI D<sup>1</sup>, MEREGALLI M<sup>1</sup>, BELICCHI M<sup>1</sup>, FARINI A<sup>1</sup>, RAZINI P<sup>1</sup>, MACIOTTA S<sup>1</sup>, TORRENTE Y<sup>1</sup>  (1) Stem Cell Laboratory, Department of Neurological Sciences, Fondazione IRCCS Ospedale Maggiore Policlinico, Centro Dino Ferrari, University of Milan, Milan, ITALY.</p>
To contact the author:: daniele.parolini@unimi.it.	<p>Duchenne muscular dystrophy (DMD) is a progressive neuromuscular disease due to a deficiency in dystrophin, a 427kDa protein located at the sarcolemma and acting as a linker between cytoskeleton and extracellular matrix.</p> <p>Several observations suggest that an increase in intracellular calcium concentration could be involved in the aetiology of muscle fibre injury in DMD. Particularly, previous studies showed that store-operated calcium (SOC) entries can be activated in muscle fibres and that the activity of store-operated calcium channels (SOCCs) was increased in fibres from dystrophic mice. Normal and dystrophic blood-derived CD133<sup>+</sup> cells showed an unexpected expression of the B-cell marker CD20, a protein that can play a direct role in the SOC influx and in the modulation of intracellular calcium release through signalling pathways activation. Measurements of intracellular Ca<sup>2+</sup> ([Ca<sup>2+</sup>]<sub>i</sub>) performed on normal and dystrophic blood-derived CD133<sup>+</sup> cells revealed a higher calcium concentration in DMD than normal cells, thus opening the prospective of a calcium impairment possibly involving CD20 activity. Since no natural agonist for CD20 has been identified until now, we considered the possibility that CD20 channel or signal transduction activity can be indirectly modulated by cytokines or growth factors after activation of signalling cascades. By means of ELISA we checked for growth factors and cytokines released by normal and DMD blood-derived CD133<sup>+</sup> cells. Supernates from CD133<sup>+</sup> stem cells isolated from DMD blood showed a high concentration of brain derived neurotrophic factor (BDNF). Here we showed that a CD20-related signalling pathway able to induce an increase in [Ca<sup>2+</sup>]<sub>i</sub> is activated after BDNF stimulation of blood-derived CD133<sup>+</sup> cells, supporting the assumption of a “CD20-related calcium impairment” affecting circulating DMD CD133<sup>+</sup> cells. Presented findings represent the starting point toward the expansion of knowledge about molecules and pathways involved in the pathology of DMD and in the behaviour of dystrophic blood-derived CD133<sup>+</sup> cells.</p>

PW5-057	<p><b><u>AN EFFICIENT WAY TO DIFFERENTIATE HUMAN EMBRYONIC STEM CELLS INTO SKELETAL MUSCLE CELLS</u></b></p> <p>B HUOT N<sup>1</sup>, LAPOINTE E<sup>1</sup>, FUJII I<sup>2</sup>, MAKOTO M<sup>2</sup>, P. TREMBLAY J<sup>1</sup>  (1) Centre de Recherche du CHUL, Québec, CANADA. (2) Laboratory of Clinical Pharmacology and Therapeutics, Kumamoto, JAPAN.</p>
To contact the author:: nicolas.b-huot.1@ulaval.ca.	<p>Human embryonic stem cells (hESC) have a self-renewal capacity and can differentiate into all cells found in the body. For this reason, they represent an unlimited source of cells for the treatment of degenerative disease, such as Duchenne Muscular Dystrophy (DMD). Previous studies have reported the derivation of skeletal muscle cells from hESC but the techniques used in these studies were long and had a low efficiency. Here we report a new method to differentiate hESC into skeletal muscle cells using an adenovirus expressing the master gene MyoD under the CAG promoter (Ad.CAGMyoD). This adenovirus is very efficient and five days after the infection nearly 50% of the cells stained positive for desmin, a well known myogenic marker. Immunocytochemistry also confirmed that these cells expressed MyoD. Thereafter, we tested the <i>in vitro</i> functionality of the resulting myogenic precursors by measuring their fusion potential. When these cells were cultured in a low serum medium, they fused and formed myotubes. This differentiation was confirmed by immunostaining for the myosin heavy chain, a myotube marker. These preliminary results indicate that the Ad.CAGMyoD is an efficient way to differentiate hESC into skeletal muscle cells that are functional <i>in vitro</i>. Further <i>in vivo</i> experiments are underway to determine if these cells can be used for cellular therapy.</p>

PW5-058	<p><b><u>TRPC1 REGULATES SKELETAL MYOBLASTS MIGRATION AND DIFFERENTIATION.</u></b>          LOUIS M<sup>1</sup>, ZANOU N<sup>1</sup>, GAILLY P<sup>1</sup>          (1) University of Louvain, Brussels, BELGIUM.</p>
To contact the author:: gailly@fycl.ucl.ac.be.	<p>Myoblasts migration is a key step in myogenesis and in regeneration. It allows myoblasts alignment and fusion into myotubes. This process has been shown to involve m- or <math>\mu</math>-calpains, two calcium-dependent cysteine proteases. Indeed, growth factor-induced migration is accompanied by an increase of calpains expression and the rate of migration is reduced in transfected cells overexpressing calpastatin, an endogenous inhibitor of calpains. In the present paper, we measured calpain activity in situ (fluorimetric measurements) and show, for the first time, a peak of activity at the beginning of the differentiation process. Besides, we recently reported that, in adult skeletal muscle fibres, calpains were specifically activated by a store operated entry of calcium (possibly mediated by TRPC1 channel). In the present study, we therefore repressed the expression of TRPC1 in myoblasts and studied its influence on differentiation. Expression of TRPC1 was reduced by 60 to 80 % in myoblasts expressing a shRNA anti-TRPC1 in comparison to myoblasts expressing a control shRNA. These cells presented a largely reduced store-operated entry of calcium. When these myoblasts were maintained in a differentiation medium, their fusion into myotubes was significantly slowed down due to an absence of alignment of myoblasts. This observation was corroborated by the fact that myoblasts transfected with shRNA anti-TRPC1 also presented a significantly reduced speed of migration after wounding. This effect could be mimicked by applying 2 to 5 <math>\mu</math>M GsMTx4 toxin, an inhibitor of TRPC1. In parallel, we found that the peak of calpain activity observed at the beginning of differentiation was abolished in cells repressing TRPC1. We therefore suggest that an entry of calcium through TRPC1 channel induces a transient activation of calpains, allowing in its turn, myoblasts migration and fusion.</p>

PW5-059	<p><b><u>A SYNTHETIC MECHANO GROWTH FACTOR E PEPTIDE ENHANCES MYOGENIC PRECURSOR CELL TRANSPLANTATION SUCCESS</u></b></p> <p>MILLS P<sup>1</sup>, DOMINIQUE JC<sup>2</sup>, LAFRENIÈRE JF<sup>3</sup>, BOUCHENTOUF M<sup>4</sup>, TREMBLAY JP<sup>5</sup></p> <p>(1) Université Laval, Québec, CANADA. (2) Université Laval, Québec, CANADA. (3) Université Laval, Québec, CANADA. (4) Université Laval, Québec, CANADA. (5) Université Laval, Québec, CANADA.</p>
<p>To contact the author::  jean-christophe.dominique.1@ulaval.ca.</p>	<p>Duchenne muscular dystrophy (DMD) is the most frequent dystrophy by affecting 1 male on 3500 all around the world. Cellular therapy is one of the promising potential treatments for this disease although it faces some problems. Growth factors, such as the insulin like growth factor-1, are able to increase or decrease the proliferation, differentiation, migration and survival of cells. The mechano growth factor (MGF) is an IGF-1 isoform produced after a mechanical stress. The E peptide of MGF has been shown to play a role in the muscle physiology. Here, we use the predicted 24 amino acids (MGF-ct24E) from the E peptide of MGF to investigate its effects on the engraftment of human and mouse muscular precursor cells (MPCs) in the Tibialis anterior of SCID and mdx mice. The MGF-ct24E has been shown to modulate the proliferation and differentiation of C2C12 myoblasts. Our results showed, in vitro, the enhanced proliferation of MPCs treated with this peptide which could be due to the delayed fusion also observed. Moreover, this mechanism passes through another receptor than the IGF-1 receptor. In contrast with the IGF-1, the MGF-ct24E had no effect on the survival of human MPCs. In vivo, the pre-treatment and co-injection of the peptide with the MPCs showed no amelioration on the graft success established by the percentage of dystrophin positive fibers. However, when injected systemically or intra-muscularly, an improvement up to 1.6 fold was observed on the graft success. Together, those results demonstrate an interesting and non-invasive way to improve the engraftment of MPCs in DMD patients.</p>

PW5-060	<p><b><u>SKELETAL MUSCLE FIBROSIS CAN BE MODULATED BY MYOSTATIN</u></b>  LI Z<sup>1</sup>, KOLLIAS H<sup>1</sup>, WAGNER K<sup>1</sup>  (1) The Johns Hopkins University, Baltimore, USA.</p>
To contact the author:: zbli2001@yahoo.com.	<p>Myostatin is a TGF-<math>\beta</math> family member that negatively regulates muscle growth. Studies in a mouse model of Duchenne and Becker muscular dystrophy (mdx) showed that deletion of myostatin gene or treatment with a postnatal inhibitor of myostatin significantly reduced muscle fibrosis, in addition to enhancing regeneration of muscle. The current study was designed to determine if the observed reduction in muscle fibrosis is directly regulated by myostatin or is secondary to increased myofiber regeneration. We found that muscle derived fibroblasts expressed myostatin and the putative receptor ActRIIB, suggesting fibroblast could be directly targeted either by autocrine or paracrine production of myostatin. Studies in primary cultured muscle fibroblast <i>in vitro</i> showed that myostatin significantly increased fibroblast proliferation and extracellular matrix protein production, which were determined by <sup>3</sup>H-thymidine incorporation as well as expression of several fibroblast proteins. <i>In vivo</i> modulation of fibroblast growth by myostatin was demonstrated by injection of coated beads coated into mouse muscle yielding approximately 5 times more fibrosis surrounding myostatin coated beads than control. Collagen synthesis determined by incorporation of <sup>3</sup>H-proline was increased in fibroblasts from Mdx mouse muscle compared to those from myostatin null Mdx mice, suggesting that myostatin can directly modulate muscle fibrosis in muscular dystrophies. These results expand our understanding of the role of myostatin in muscle tissue, beyond regulation of myocyte growth to that of control of the composition of the tissue as a whole. Myostatin may be a therapeutic target to reduce fibrosis in muscle disease.</p>

PW5-061	<p><b>MYOSTATIN KNOCKOUT ALTERS TRANSCRIPTOMIC AND PROTEOMIC PROFILES OF QUADRICEPS MUSCLE</b></p> <p>CHELH I<sup>1</sup>, MEUNIER B<sup>1</sup>, PICARD B<sup>1</sup>, REECY J<sup>2</sup>, CHEVALIER C<sup>3</sup>, HOCQUETTE JF<sup>1</sup>, CASSAR-MALEK I<sup>1</sup></p> <p>(1) INRA, UR1213, Unité de Recherches sur les Herbivores, Equipe Croissance et Métabolisme du Muscle, Centre Clermont-ferrand/Theix, Saint-Genès-Champanelle, FRANCE. (2) Iowa State University, Animal Science Department, 2255 Kildee Hall, Ames, Iowa, USA. (3) PT transcriptome, Ouest Génopole, Institut du Thorax, Faculté de Médecine 1, Nantes, FRANCE.</p>
To contact the author:: ilham.chelh@clermont.inra.fr.	<p>Myostatin (MSTN), a member of the TGF-beta superfamily, is a negative regulator of skeletal muscle mass. Inactivating mutations of the MSTN gene are responsible for the development of a hypermuscular phenotype in mice (McPherron <i>et al.</i>, 1997) and cattle (Grobet <i>et al.</i>, 1997). The main objective of this study was to <i>identify molecular targets of MSTN action involved in the regulation of muscle mass.</i></p> <p>The proteomic profiles of Quadriceps muscles of 5-week-old MSTN-null mice (McPherron <i>et al.</i>, 1997) and their controls were analysed using two dimensional electrophoresis (4-7 pH gradient, SAM test, FDR&lt;5%) (Meunier <i>et al.</i>, 2005) and the transcriptomic analysis (SAM test, FDR&lt;5%) was carried out using myochips (West Genopole). The data were further analysed with bioinformatics tools (Genomatix, DAVID and String).</p> <p>Comparison of the protein profiles (20 up and 18 down-regulated proteins spots) confirmed that MSTN inactivation caused a glycolytic shift of the muscles (MyBP, MyHCII, H-FABP) and a differential abundance of survival/mortality factors (DJ-1 and TCTP/HSP9A).</p> <p>Comparison of the transcriptomic profiles (192 up and 245 down-regulated genes) revealed up-regulation of genes involved in insulin/IGF and protein kinase beta signalling, carbohydrate metabolism, and apoptosis/differentiation. Genes belonging to the canonical Wnt and calcium signalling pathways were down-regulated.</p> <p>In conclusion, these data showed a differential expression of genes and proteins related to the muscle energy metabolism and cell survival/apoptosis pathway (e.g. DJ-1, PINK1 and TCTP/HSP9A, GSK-3beta). This last finding suggests that <b><i>MSTN may be a modulator of cell survival, growth and differentiation</i></b> through the regulation of GSK-3beta activity and downstream signalling pathways; this action may implicate DJ-1 a regulator of the PI3K pathway.</p> <p>Our results allowed the identification of molecular networks that may be MSTN targets underpinning hypertrophy. The expected applications will be notably in clinical domains concerning the treatment of muscular dystrophy and muscle wasting associated with aging.</p>

PW5-062	<p><b><u>OVER-EXPRESSION OF FOLLISTATIN IN MYOBLASTS INCREASES THEIR PROLIFERATION AND DIFFERENTIATION, AND IMPROVES THE GRAFT SUCCESS</u></b></p> <p>BENABDALLAH BF<sup>1</sup>, BOUCHENOUF M<sup>2</sup>, ROUSSEAU J<sup>3</sup>, TREMBLAY JP<sup>4</sup>  (1) CHUL-Université Laval, Quebec, CANADA. (2) CHUL-Université Laval, Quebec, CANADA. (3) CHUL-Université Laval, Quebec, CANADA. (4) CHUL-Université Laval, Quebec, CANADA.</p>
	<p>Duchenne muscular dystrophy is caused by the absence of functional dystrophin protein, leading to the myofiber membrane instability and progressive muscle atrophy. Myoblast transplantation in dystrophic muscles is a potential therapy for the disease, as it permits the long term restoration of dystrophin expression in transplanted muscles. However, the success of this approach is limited by the short period of muscle repair which follows myoblast transplantation. Myostatin, known as a powerful inhibitor of muscle growth, is involved in terminating the period of muscle repair following injury by blocking myoblast proliferation and delaying myoblast differentiation. Follistatin forms a complex with myostatin preventing its interaction with its receptor and thus blocking the myostatin signal. Here, we used a lentivirus to over-express the follistatin protein in normal myoblasts to block the myostatin signaling. Our results first confirmed the over-expression of the human follistatin into lentivirus transduced myoblasts, and second showed that the over-expression of the follistatin protein in normal human myoblasts improved <i>in vitro</i> their proliferation rate by about 1,5 folds after 96 h and also their differentiation rate by about 1,6 and 1,8 folds respectively in the absence and in the presence of recombinant myostatin. Finally, our data demonstrated that the engraftment of those transduced human normal myoblasts with the follistatin lentivirus into SCID mouse muscles was enhanced by 2 folds.</p>

PW5-063

**PROTECTIVE EFFECT OF CYCLOSPORINE A AGAINST CALCIUM-DEPENDENT  
CELL DEATH IN DYSTROPHIN-DEFICIENT CELLS.**

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Evidence is presented for the involvement of IP3-dependent release calcium pathway in the contribution of calcium overload in dystrophin-deficient cells. Our previous results presented that global calcium release during stimulation ( $K^+$ -evoked calcium increase) was found higher in dystrophin-deficient SolC1(-) myotubes than in mini-dystrophin transfected SolD(+) myotubes. SolC1(-) myotubes also displayed higher activity of spontaneous calcium release as compared to SolD(+) myotubes. Moreover, dystrophin-deficient myotubes were naturally dying faster than mini-dystrophin transfected myotubes. These features prompted us to conduct experiments with cyclosporine A (CsA), known to, among other effects, modulate IP3 pathway, in order to investigate its effects on both cell death and calcium signaling. Cell survival assays, performed with MTT test, revealed a protective effect of both 2-APB (IP3R inhibitor) and CsA against natural cell death occurring in mature dystrophin-deficient myotubes. The area under the curve and kinetics parameters of  $K^+$ -evoked calcium increase were significantly reduced with the incubation of SolC1(-) myotubes with 2-APB. In the same way, 2-APB exposure was significantly decreasing spontaneous calcium release at rest in SolC1(-) myotubes. Interestingly, for both  $K^+$ -evoked calcium increases and spontaneous release events, similar results were obtained with CsA exposure with SolC1(-) myotubes. Furthermore, CsA reduced IP3-R1 mRNA levels in SolC1(-), leading to levels measured in mini-dystrophin transfected myotubes (SolD(+)) in control conditions. Taken together, these data suggest that CsA could modulate calcium releases in dystrophin-deficient cells by direct IP3Rs inhibition and/or by reduction of IP3R-1 mRNA expression via the calcineurin pathway, leading to the regulation of the calcium overload in dystrophin-deficient cells. On that account, these data underline a strong involvement of IP3 pathway in calcium-dependent cell death in dystrophin deficiency, and that a modulation of calcineurin pathway could be a therapeutic approach to counteract the calcium overload in dystrophin-deficient myopathies.

PW5-064	<p><b>REGULATION OF TRPC-DEPENDENT CALCIUM INFLUX BY ALPHA1-SYNTROPHIN: IMPLICATION IN DUCHENNE MUSCULAR DYSTROPHY</b>  SABOURIN J<sup>1</sup>, MAGAUD C<sup>1</sup>, LAMICHE C<sup>1</sup>, RIVET J<sup>1</sup>, SEBILLE S<sup>1</sup>, COGNARD C<sup>1</sup>, BOURMEYSTER N<sup>1</sup>, CONSTANTIN B<sup>1</sup>  (1) IPBC UMR 6187, POITIERS, FRANCE.</p>
<p>To contact the author::  jessica.sabourin@etu.univ-poitiers.fr.</p>	<p>Duchenne Muscular Dystrophy is a neuromuscular disease which leads to a progressive degeneration of muscles. This disease results from the lack of dystrophin, a protein localized to the cytoplasmic face of the sarcolemma. The lack of dystrophin leads to fiber necrosis and sustained calcium mishandling partly linked to increased calcium influx provoking an increase in the free calcium concentration under the sarcolemma. Store-operated cationic channels (SOCs) carried by TRPCs are over activated in dystrophic muscle cells generating elevated CCEs (Capacitative Calcium Entries). In accordance with this idea we demonstrated that CCEs are regulated by dystrophin. Moreover, we have demonstrated that TRPC1 forms a stable complex with dystrophin and alpha1-syntrophin and associates with the PDZ domain of alpha1-syntrophin. The reduction of alpha1-syntrophin at the sarcolemma in dystrophic muscle cells, could explain the increase of CCEs because, less TRPC1 was co-immunoprecipitated with alpha1-syntrophin from <i>mdx</i> muscle than from normal muscle. To explore the functional regulation of CCEs by alpha1-syntrophin independently to dystrophin, experiments were designed to repress or to overexpress alpha1-syntrophin in cultured myotubes. siRNA inhibition of alpha1-syntrophin expression disrupts the complex TRPC1/alpha1-syntrophin and also deregulates CCEs in normal cultured myotubes. Conversely, forced expression of alpha1-syntrophin reduced CCEs which are abnormally elevated in dystrophic cells.</p> <p>These results are in favour of the idea that the link of TRPC1 with alpha1-syntrophin could be essential for maintaining a normal regulation of SOC activity. Restoration of alpha1-syntrophin to the sarcolemma and pharmacological modulation of TRPC1 channels could be therapeutic approaches that need to be considered.</p>