

**PW 31:  
Satellite cells  
and muscle homeostasis**

PW31-386	<p><b><u>EXPRESSION OF DLK1 AND MYOSTATIN IN SKELETAL MUSCLE AND SATELLITE CELLS</u></b>  SELLATHURAI J<sup>1</sup>, JOERGENSEN LH<sup>1</sup>, PETERSSON SJ<sup>1</sup>, JENSEN CH<sup>2</sup>, DHAWAN J<sup>3</sup>, SCHROEDER HD<sup>4</sup>  (1) Institute of Clinical Research, University of Southern Denmark, Odense, DENMARK. (2) Institute of Medical Biology, University of Southern Denmark, Odense, DENMARK. (3) Center for Cellular and Molecular Biology, Hyderabad, INDIA. (4) Institute of Clinical Research, University of Southern Denmark and Department of Clinical Pathology, Odense University Hospital, Odense, DENMARK.</p>
To contact the author:: jeeva.sellathurai@ouh.re gionsyddanmark.dk.	<p>Muscular dystrophies are a heterogeneous group of muscle degenerating diseases for which no effective treatment exist. A potential treatment strategy could be developed by studying DLK1 and myostatin regulation. DLK1 and myostatin are known to generate muscle hypertrophy phenotypes and therefore these may be potential targets for intervention or be used in gene therapeutic strategies of muscular disorders. DLK1 is involved in embryonic muscle development and it has been shown to induce muscle hypertrophy in transgenic mice and callipyge sheep. Myostatin negatively regulates myogenesis, and inactivation leads to heavy muscle growth.</p> <p>The expression of DLK1 and myostatin was studied in a set of experiments. In transgenic mice over expressing DLK1 it was found that myostatin mRNA levels were significantly lower compared to the controls.</p> <p>The effect on proliferation and differentiation were tested <i>in vitro</i> by transfecting C2C12 cells with full length mouse <i>Dlk1</i>. DLK1 did not affect the myogenic potential of the cells; but it appeared to keep the cells in a proliferative state for a longer time before initiation of differentiation. Like in the regeneration studies, myostatin mRNA was down regulated in DLK1-C2C12 cells. In addition we found decorin upregulated. Furthermore, the expression of DLK1 and myostatin was studied in human satellite cell cultures. A high level of DLK1 was observed in nonproliferating G0 cells but during proliferation and differentiation the level of DLK1 was highly down regulated. The expression of myostatin was down regulated in G0 and proliferating human satellite cell cultures, but was highly up regulated in differentiating cultures. Thus, the results again indicate that DLK1 interact with the expression of myostatin.</p> <p>The studies were made using Taqman® RT-qPCR and immunohistochemistry.</p>

PW31-387	<p><b>MUSCLE GROWTH STIMULATION AFTER MYOSTATIN BLOCKADE IN ADULT MUSCLE IS INDEPENDENT OF SATELLITE CELL ACTIVITY</b></p> <p>MOUISEL E<sup>1</sup>, VULIN A<sup>1</sup>, HOURDE C<sup>1</sup>, DUMONCEAUX J<sup>1</sup>, RELAIX F<sup>1</sup>, GARCIA L<sup>1</sup>, AMTHOR H<sup>1</sup></p> <p>(1) UMR S 787-Institut de Myologie, Paris, FRANCE.</p>
<p>To contact the author::  etienne.mouisel@chups.jussieu.fr.</p>	<p>Previously, we have shown that null mutation of myostatin resulted in muscle fibre hypertrophy that is independent of satellite cell activity. Here, we investigated the effect of myostatin blockade in mature mouse muscle. Myostatin was blocked after intramuscular injection of recombinant AAV-2/1 coding for the myostatin propeptide (AAV-prop) into tibialis anterior (TA) muscle of 6 weeks old female C57Bl6 mice. The local overexpression of myostatin propeptide resulted in a 28% increase of muscle weight after 28 days of treatment compared to the contralateral side that was injected with a AAV-Mseap control vector (<math>P &lt; 0.001</math>). Morphometric analysis revealed a 16% increase of mean single fibre area following AAV-prop injection compared to the controls (<math>P = 0.004</math>). The mean fibre number after AAV-prop vector injection was 3328 compared to 3420 after control vector injection (<math>P = 0.47</math>). Thus, excessive growth after myostatin blockade resulted exclusively from fibre hypertrophy.</p> <p>Next, the number of myonuclei was determined on cross sections of TA muscles. Muscle fibres contained on average 0.65 myonuclei after injection of AAV-prop compared to 0.63 myonuclei after control vector injection (<math>P = 0.77</math>).</p> <p>We finally determined the effect of myostatin blockade on satellite cell number. We injected AAV-prop vector into TA muscle of Myf5<sup>nlacZ/+</sup> mice, in which satellite cells express the transgene lacZ. One month after injection of AAV-prop, muscle fibres contained on average 32 satellite cells versus 25 satellite cells per 1000 fibres after control vector injection, a difference that was statistically not significant (<math>P = 0.31</math>). As Myf-5 is expressed in quiescent as well as in activated proliferating satellite cells, we can conclude that satellite cell activity is not increased after myostatin blockade.</p> <p>Above results confirm findings from previous analysis of constitutive myostatin knockout mice. Myostatin blockade in adult muscle resulted in fibre hypertrophy without concomitant increase in myonuclear number and without any evidence of increased satellite cell activity.</p>

PW31-388	<p><b><u>CHARACTERIZATION OF THE HUMAN SATELLITE CELLS SECRETOME DURING THE IN VITRO SENESCENCE</u></b>  LE BIHAN MC<sup>1</sup>, ROGOWSKA-WRZESINSKA A<sup>2</sup>, BIGOT A<sup>1</sup>, FURLING D<sup>1</sup>, COULTON G<sup>3</sup>, MOULY V<sup>1</sup>, BUTLER-BROWNE G<sup>1</sup>  (1) UMRS787 – Groupe Myologie; Inserm / UPMC-ParisVI; Institut de Myologie, Paris, FRANCE. (2) Department of Biochemistry &amp; Molecular Biology, University of Southern Denmark, Odense, DENMARK. (3) St. George's University of London, London, UNITED-KINGDOM.</p>
	<p>With age, there is a gradual decline in the effectiveness of the regenerative response of skeletal muscle to damage which is accompanied by muscle fiber atrophy and a general loss of muscle mass and function. Age-related muscle wasting, like the decline in the regenerative capacity may be due to the decrease in the number of muscle precursor cells (satellite cells) as well as to an age-related decline in satellite cell function. It has been suggested that the decreased level of the circulating trophic factors that occurs with age could also contribute to the decrease in muscle mass, force and regenerative capacity described in the elderly. The aim of this study was to identify secreted muscle proteins essential for the maintenance of the regenerative capacity of the resident satellite cells and to characterize differences in the global pattern of protein expression observed in a model of muscle ageing: the replicative senescence of human satellite cells <i>in vitro</i>. Conditioned medium from differentiating primary cultures at an early passage and at senescence were analyzed by a proteomic approach using 3 different expression profiling strategies: 1) 2D gel electrophoresis/mass spectrometry (2DE/MS); 2) Luminex based assay; 3) mass spectrometry (MS) based approach. A time course of myoblast secretion at early passage and late passage in "Differentiation medium" revealed a 3 to 4-fold decrease in the total amount of protein secreted during the senescence process associated with considerable qualitative and quantitative changes in the secretome. Preliminary data from 2DE/MS &amp; Luminex expression profiling has enabled us to identify 44 molecules differentially "secreted" during muscle ageing <i>in vitro</i>: senescent myoblasts expressed a similar panel of inflammatory cytokines as previously reported for senescent fibroblasts associated with an upregulation of key regulators of matrix remodelling. Results of the MS based approach will be obtained in the near future.</p>

PW31-389	<p><b><u>MUSCLE AGING AND CALCIUM-DEPENDENT PROTEOLYTIC SYSTEM.</u></b>  BRULE C<sup>1</sup>, DARGELOS E<sup>1</sup>, COTTIN P<sup>1</sup>, POUSSARD S<sup>1</sup>  (1) UPCDM universit� Bordeaux 1, TALENCE, FRANCE.</p>
To contact the author:: cedricbrule@hotmail.fr.	<p>The calcium-dependent proteolytic system is composed of cysteine proteases named calpains. They are ubiquitous or tissue-specific enzymes and the two best characterised isoforms are the ubiquitously expressed <math>\mu</math>- and m-calpains. Besides its regulation by calcium, calpain activity is tightly regulated by calpastatin (which is the specific endogenous inhibitor), binding to phospholipids, autoproteolysis and phosphorylation. Calpains are responsible for limited proteolytic events. Among the multitude of substrates identified so far are cytoskeletal and membrane proteins, enzymes and transcription factors. Calpains are involved in a large number of physiological processes such as muscle growth and differentiation, and pathological conditions such as muscular dystrophies.</p> <p>Aging is associated with a progressive and involuntary loss of muscle mass also known as sarcopenia. This condition represents a major public health concern. Although sarcopenia is well documented, the molecular mechanisms of this condition still remains unclear. The aim of this study was to determine if the proteolytic system could be involved in the phenotype associated with sarcopenia. Calpains and calpastatin levels, subcellular distributions and activities were compared between muscles from young (3 months) and old (24 months) rats. While the subcellular localisation of calpains did not change between young and old rat samples, their enzymatic activity significantly increase with age. In the meantime, calpastatin specific activity and protein level decreased in old samples. Altogether, our data showed an overall increase in calpain activity associated with muscle aging. These findings suggest that the calcium-dependent proteolytic system is indeed involved in sarcopenia.</p>

PW31-390	<p><b>ANALYSES OF METABOLIC AND CONTRACTILE PLASTICITY DURING IN VITRO MYOGENESIS OF NEW MURINE CELL LINES</b></p> <p>PELTZER J<sup>1</sup>, COLMAN L<sup>2</sup>, CEBRIAN J<sup>3</sup>, MUSA H<sup>2</sup>, MARTELLY I<sup>1</sup>, PECKHAM M<sup>2</sup>, KELLER A<sup>1</sup></p> <p>(1) Laboratoire CRRET, UMR CNRS 7149, Créteil, FRANCE. (2) Institute of Mol and Cell Biol, University of Leeds, Leeds, UNITED-KINGDOM. (3) Génomes et Cancers, FRE CNRS 2939, Institut Gustave Roussy, Villejuif, FRANCE.</p>
To contact the author:: keller@univ-paris12.fr.	<p>Our general objective is to contribute to the elucidation of factors involved in coordinated regulations of metabolic and contractile pathways in myofibres. For that purpose we have established myogenic clones derived from satellite cells of the transgenic <i>immortomouse</i> capable of expressing adult fast-glycolytic and/or slow-oxidative markers in myotubes. We have investigated the expression, by such clones, of metabolic enzymes and contractile markers, indicative of coordinated modulations, as a function of time and differentiation stage. The WTt clone, that differentiates into myofibres of a mixed fast and slow phenotype was chosen for attempts to direct its phenotype towards a more homogeneous slow-oxidative <b>or</b> fast-glycolytic phenotype. To reach a slow-oxidative phenotype, we have selected stably infected WTt clones over-expressing the PPAR delta transcription factor known to be involved in the balance of oxidative energy metabolism. PPAR delta over expression enhanced the slow-contractile phenotype of the WTt myogenic clone. To reach a fast-glycolytic phenotype, we have attempted to stabilize the HIF-1alpha protein of WTt cells by using cobalt chloride (CoCl<sub>2</sub>). This pseudo-hypoxic treatment induces HIF-1alpha stabilisation, which in turn activates target genes, including enzymes of the glycolytic pathway. Interestingly, a short CoCl<sub>2</sub> treatment (24 hours) favoured the accumulation of fast MHC isoforms. Only longer treatments (96 hours) also induced a decrease in expression of the slow phenotypic markers, suggesting that HIF-1alpha might regulate fast-glycolytic and slow-oxidative myofibre phenotypes through independent pathways. Our data support the idea that modifying oxidative or glycolytic metabolism will induce coordinated changes in contractile phenotype of muscle cells. It will be worth investigating whether the sequences we found on MHC genes, carrying putative binding sites for PPAR delta and HIF-1alpha transcription factors, might be active.</p>

PW31-391	<p><b>DYSTROGLYCAN, TKS5 AND SRC MEDIATED ASSEMBLY OF PODOSOMES IN MYOBLASTS</b></p> <p>THOMPSON O<sup>1</sup>, KLEINO I<sup>2</sup>, CRIMALDI L<sup>3</sup>, GIMONA M<sup>3</sup>, SAKSELA K<sup>2</sup>, WINDER S<sup>1</sup>  (1) University of Sheffield, Sheffield, UNITED-KINGDOM. (2) University of Helsinki, Helsinki, FINLAND. (3) Mario Negri Sud, Santa Maria Imbaro, ITALY.</p>
<p>To contact the author::  s.winder@sheffield.ac.uk</p>	<p>Dystroglycan is an essential component of the dystrophin glycoprotein complex of skeletal muscle (DGC), an important mediator of connections to the actin cytoskeleton and a scaffold for signalling molecules in a number of other cell types. In addition dystroglycan is also an important polarity determinant and is dysregulated in the majority of epithelial-derived tumours. We have examined the role of dystroglycan in the early stages of myoblast adhesion and spreading and found that dystroglycan initially associates with other adhesion proteins in small puncta that precede the formation of true focal adhesions in these cells. The complement of proteins localised in these puncta have all the hallmarks of podosomes. Using a phage display library of all 296 human SH3 domains we identified Tks5, a key regulator of podosomes, as interacting with <math>\alpha</math>-dystroglycan. We verified the interaction by immunoprecipitation, GST-pulldown and immunofluorescence localisation. Both proteins localise to small puncta during early phases of spreading, but importantly following stimulation by PDBu also localise to structures indistinguishable from podosomes. Furthermore the interaction between <math>\alpha</math>-dystroglycan and Tks5 relies of the tyrosine phosphorylation of <math>\alpha</math>-dystroglycan by Src. Dystroglycan depletion by siRNA, not only reduces overall cell adhesion and cell motility but also the formation of podosomes and recruitment of Tks5 and cortactin to these structures. Interestingly dystroglycan overexpression also inhibited podosome formation, but mutation of Tyr892, previously identified as a Src substrate, restored podosome formation. We therefore propose that Src-dependent phosphorylation of <math>\alpha</math>-dystroglycan drives the SH3-mediated association between dystroglycan and Tks5 which together regulate podosome formation.</p>

PW31-392	<p><b><u>CHANGES IN GLYCOSAMINOGLYCANS DURING SKELETAL MUSCLE REGENERATION AND SATELLITE CELL DIFFERENTIATION <i>IN VITRO</i>.</u></b>  <b>OUZGHIR M<sup>1</sup>, BARBOSA I<sup>2</sup>, ZIMOWSKA M<sup>3</sup>, JENNISKENS G<sup>4</sup>, DUCHESNAY A<sup>5</sup>, PAPY-GARCIA D<sup>5</sup>, MARTELLY I<sup>5</sup></b>  (1) Faculty of Sciences, Cady Ayyat University, marrakech, MOROCCO. (2) CRRET laboratory, present address Sanofi-Aventis, Vitry, FRANCE. (3) Department of Cytology, Faculty of Biology, University of Warsaw, Warsaw, POLAND. (4) ModiQuest Company, Nijmegen, THE NETHERLANDS. (5) CRRET laboratory, University Paris-Est, Faculté de Sciences et Technologie, Paris 12, Créteil, FRANCE.</p>
To contact the author:: martelly@univ-paris12.fr.	<p>Together with other extracellular matrix and membrane-associated components that contribute to the cellular environment of muscle cells, proteoglycans (PG) have been implicated in numerous physiological and pathological processes including regulation of enzymes and growth factor bioavailability, cellular growth and differentiation. The known effects of PG are mainly due to the sulphated glycosaminoglycan (GAG), namely dermatan sulphate/chondroitin sulphate (DS/CS) and heparan sulphate (HS). It is generally accepted that unique tissue specific HS sequences are generated by biosynthetic enzymes that produce this type of molecules, key regulators of cell signaling. It is therefore of special interest to analyze the spatio-temporal pattern of GAG expression during skeletal muscle regeneration.</p> <p>We have used a crush-induced muscle regeneration model that generates differences in rat fast EDL and slow Soleus regenerating muscles. Using a simple sensitive method of GAG concentration measurement developed in our laboratory, GAGs were quantitated during regeneration of these muscles. The study was further extended to <i>in vitro</i> differentiation of primary cultures of satellite cells. Different GAG epitopes were visualized using specific antibodies applied on regenerating muscle sections and on myoblasts differentiating <i>in vitro</i>.</p> <p>We have demonstrated changes in the composition of GAG extracted from EDL and Soleus muscles during regeneration. In both muscle types, total GAG amounts were collapsed one day after crush, then increased during muscle repair. HS that was less abundant than DS/CS during the first week after crush, and became the most important GAG species starting at the second week of muscle reconstruction. Similar changes in GAG composition were observed during <i>in vitro</i> satellite cell differentiation. Time-dependent specificities were revealed that depended on muscle of origin (EDL or Soleus), in both <i>in vivo</i> and <i>in vitro</i> studies. We propose that changes in GAG environment of myogenic cells might alter signalling events associated to myogenesis.</p>

PW31-393	<p><b>OPPOSITE ROLES OF CONTROLLED EXPRESSION OF THE INITIATION FACTOR EIF3-F IN SKELETAL MUSCLES.</b>  CSIBI A<sup>1</sup>, LAGIRAND-CANTALOUBE J<sup>1</sup>, OFFNER N<sup>1</sup>, LEIBOVITCH MP<sup>1</sup>, BARBOIRON C<sup>2</sup>, PICARD B<sup>2</sup>, LEIBOVITCH S<sup>1</sup>  (1) Laboratoire de Génomique Fonctionnelle et Myogénèse, UMR 866 DCC, INRA, Campus INRA/Supagro, Montpellier, FRANCE. (2) URH Theix, INRA, Clermont-Ferrand, FRANCE.</p>
To contact the author:: csibi@supagro.inra.fr.	<p>Skeletal muscle size depends upon a dynamic balance between anabolic and catabolic processes. The E3 ubiquitin-ligase MAFbx/Atrogin-1 is upregulated during muscle atrophy caused by a variety of conditions, including cancer, AIDS, stress, diabetes and starvation. However, its precise function in muscle wasting is not yet completely elucidated. With the aim to identify new MAFbx targets during atrophy, we screened a human adult skeletal muscle library yeast two-hybrids and identified eIF3f-p47 as an interacting partner. MAFbx also targeted eIF3f for its ubiquitylation and subsequent proteasome degradation. Conversely, blocking MAFbx expression by shRNAi prevented eIF3f degradation during muscle atrophy. To address the question of how functionally relevant is eIF3f in muscle homeostasis, we used a controllable protein knockout method by using full-length eIF3f-antisense-RNA. Genetic activation of eIF3f caused hypertrophy and blocked atrophy in myotubes, whereas blocking eIF3f expression induced atrophy. Finally, overexpression of eIF3f showed an increase of sarcomeric proteins and hypertrophy in both myotubes and mouse skeletal muscle, as confirmed by comparative proteomics. Our results indicate that eIF3f is a key target for MAFbx during atrophy and plays a major role in skeletal muscle hypertrophy.</p>

PW31-394	<p><b>ASB2BETA, A NOVEL ACTOR OF MUSCLE DIFFERENTIATION</b>          BELLO NF<sup>1</sup>, HEUZÉ ML<sup>1</sup>, MÉTAIS A<sup>1</sup>, DUPREZ D<sup>2</sup>, MOOG-LUTZ C<sup>1</sup>, LUTZ PG<sup>1</sup>          (1) Institut de Pharmacologie et de Biologie Structurale, UMR 5089 CNRS, Université Paul Sabatier, Toulouse, FRANCE. (2) UMR 7622 CNRS, Université Pierre et Marie Curie, Paris, FRANCE.</p>
To contact the author:: Nana.Bello@ipbs.fr.	<p>The covalent linkage of a polyubiquitin chain to a protein and its subsequent targeting to the 26S proteasome is one of the major mechanisms for controlled proteolysis. The specificity of this degradation pathway is due to E3 ubiquitin ligases involved in the recruitment of specific substrate(s). Ubiquitin-mediated protein degradation is crucial for muscle development and for maintenance of muscle homeostasis. The ubiquitin-proteasome pathway also regulates the rapid proteolysis associated with muscle wasting which is induced by metabolic or catabolic diseases. However, few E3 ubiquitin ligases involved in these processes and their specific substrates have been described so far.</p> <p>The ankyrin repeat-containing protein with a suppressor of cytokine signaling box 2 (ASB2) gene that we originally identified as induced during differentiation of myeloid leukaemia cells encodes the specificity subunit of a multimeric E3 ubiquitin ligase complex. This suggests that ASB2 regulates the stability of specific proteins <i>via</i> their polyubiquitination and proteosomal degradation. We provide the first evidence that a novel ASB2 isoform, ASB2<math>\square</math>, is expressed in muscle cells during embryogenesis and in adult tissues. ASB2<math>\square</math> expression is also induced during <i>in vitro</i> muscle differentiation and appeared with the differentiation commitment of C2C12 myoblasts. Its inhibition by shRNAs during induced-differentiation of C2C12 cells delayed myotube formation and expression of muscle contractile proteins. Moreover, ASB2<math>\square</math> is upregulated during induced-atrophy of C2C12 myotubes suggesting that it might be also involved in this catabolic state. We also showed that ASB2<math>\square</math> can assemble with the Elongin BC complex and a Cullin5/Rbx2 module to reconstitute an active E3 ubiquitin ligase complex.</p> <p>Altogether, our results suggest that ASB2<math>\square</math> is involved in muscle differentiation likely through the targeting of crucial muscle proteins to destruction by the proteasome.</p>

PW31-395	<p><b><u>CYTOKINES ALTER NUMBER AND FUNCTION OF CELL POPULATIONS RELEVANT TO SKELETAL MUSCLE HOMEOSTASIS</u></b>  COLETTI D<sup>1</sup>, BERARDI E<sup>1</sup>, AULINO P<sup>1</sup>, MORESI V<sup>1</sup>, PRISTERÀ A<sup>1</sup>, SASSOON D<sup>2</sup>, MOLINARO M<sup>1</sup>, ADAMO S<sup>1</sup>  (1) Sapienza University, Rome, ITALY. (2) Groupe Myologie, Paris, FRANCE.</p>
To contact the author:: dario.coletti@uniroma1.it	<p>Chronic exposure to tumor necrosis factor-alpha (TNF) triggers muscle wasting reminiscent of cachexia (1), a debilitating syndrome characterized by skeletal muscle wasting (2). In addition to TNF-treated muscle we exploited tumor (C26)-bearing mice as a model of cachexia to study whether stem cell number and function are altered in muscle wasting. We observed that in the presence of elevated levels of cytokines several populations of cells with myogenic potential are stable or increased in muscle, including satellite (Pax7-expressing) cells, hematopoietic stem (Sca1- CD45-expressing) cells and muscle interstitial stem (Sca1- CD34-expressing) cells also characterized by PW1 expression (3,8). PW1 is involved in myogenic cell differentiation, fiber size control and p53-mediated apoptotic pathways (4-7). The increase in myogenic cells in cachectic muscle suggests an attempt to cope with wasting by recruitment and/or activation of a myogenic response. Nonetheless, we observed that the regenerative capacity of skeletal muscle is reduced by cytokines (1, 8). To characterize the molecular mechanisms underlying stem cell impairment in cachexia, we treated injured muscle with TNF (8). TNF negatively affected the onset of regenerating fibers, characterized by centrally located nuclei, without exacerbating fiber death following the initial trauma. Several cells showed caspase activity during regeneration and the number of caspase activated cells was markedly increased by TNF, concomitant with an inhibition in regeneration. Caspase activation did not involve caspase-3 nor led to apoptosis. Inhibition of caspase activity improved muscle regeneration either in the absence or presence of TNF, suggesting a non apoptotic role for this pathway in the myogenic program. Cells with caspase activity, were localized in the interstitial compartment and could be identified by the expression of Sca1, CD34 and PW1. Perturbation of PW1 activity blocked caspase activation and improved regeneration indicating a pivotal role for PW1 in controlling stem cell function and muscle regeneration (8).</p> <p>REFERENCES  1) Coletti D. et al. Genesis. 2005;43(3):120-8.  2) Coletti D. et al. Basic Appl Myol. 2006; 16(5&amp;6):131-139.  3) Berardi E. et al., Neurol Res., in press.  4) Coletti D. et al. EMBO J. 2002;21(4):631-42.  5) Schwarzkopf M. et al. Genes Dev. 2006; 20(24):3440-52.  6) Relaix F. et al. Proc Natl Acad Sci U S A. 2000;97(5):2105-10.  7) Relaix F. et al. Dev Biol. 1996;177(2):383-96.  8) Moresi et al. Stem Cells, in press.</p>

PW31-396	<p><b><u>MOLECULAR MECHANISMS REGULATING SKELETAL MUSCLE HOMEOSTASIS: EFFECTS OF V1A VASOPRESSIN RECEPTOR OVER-EXPRESSION</u></b>  SCICCHITANO B<sup>1</sup>, TOSCHI A<sup>1</sup>, MURFUNI I<sup>1</sup>, MOLINARO M<sup>1</sup>, ADAMO S<sup>1</sup>  (1) Sapienza University, Rome, ITALY.</p>
To contact the author:: bianca.scicchitano@uniroma1.it.	<p>The maintenance of a working skeletal musculature is conferred by its remarkable ability to regenerate after mechanical or pathological injury. However muscle atrophies are characterized by the progressive loss of muscle tissue due to alterations of skeletal muscle homeostasis. In particular cachexia is a severe syndrome consisting of marked skeletal muscle atrophy, characterized by a dramatic loss of muscle mass associated with a compromised regenerative ability. Arg-vasopressin (AVP) is a potent myogenesis promoting factor and activates both the calcineurin and CaMK pathways, whose combined activation leads to the formation of transcription factor complexes <i>in vitro</i> (2, 3). The local over-expression of the V1a AVP receptor (V1aR) in injured muscle results in enhanced regeneration. V1aR over-expressing muscle exhibits: early activation of satellite cells and regeneration markers, accelerated differentiation, increased cell population expressing hematopoietic stem cell markers and its conversion to the myogenic lineage. Here we investigate the role of V1aR over-expression in animals undergoing cachexia as a result of muscle over-expression of a specific cytokine (TNF) (1). In these conditions, the local V1aR over-expression counteracts the negative effects of TNF on muscle, as demonstrated by morphological and biochemical analysis. In particular, the presence of V1aR results in increased Pax-7, myogenin and myosin expression levels both in control and in cachectic muscles. We demonstrate that V1aR over-expressing muscle increases calcineurin and IL-4 expression levels, and induces the phosphorylation of FOXO transcription factors, inhibiting the expression of atrophic genes. This study highlights a novel <i>in vivo</i> role for the AVP-dependent pathways which may represent a potential gene therapy approach for many diseases affecting muscle homeostasis.</p> <p style="text-align: center;">Reference List</p> <p>1 -Coletti,D., Moresi,V., Adamo,S., Molinaro,M., Sassoon,D. (2005). Tumor necrosis factor-alpha gene transfer induces cachexia and inhibits muscle regeneration. <i>Genesis</i>. <b>43</b>, 120-128.</p> <p>2 -Scicchitano,B.M., Spath,L., Musaro,A., Molinaro,M., Adamo,S., Nervi,C. (2002). AVP induces myogenesis through the transcriptional activation of the myocyte enhancer factor 2. <i>Mol.Endocrinol.</i> <b>16</b>, 1407-1416.</p> <p>3 -Scicchitano,B.M., Spath,L., Musaro,A., Molinaro,M., Rosenthal,N., Nervi,C., Adamo,S. (2005). Vasopressin-dependent Myogenic Cell Differentiation Is Mediated by Both Ca<sup>2+</sup>/Calmodulin-dependent Kinase and Calcineurin Pathways. <i>Mol.Biol.Cell.</i> <b>16</b>(8):3632-41.</p>

PW31-397

**GENE EXPRESSION CHANGES IN ISOLATED MYOFIBRES**

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<p>PW31-398</p>	<p><b><u>GROWTH CHARACTERISTICS OF SATELLITE CELLS IN MIXED CULTURES AND SINGLE CLONES FROM THE SAME INDIVIDUAL.</u></b>  MAIER A<sup>1</sup>, COHEN R<sup>1</sup>, BLOM J<sup>1</sup>, WESTENDORP R<sup>1</sup>  (1) Leiden University Medical Center, Department of Gerontology and Geriatrics, Leiden, THE NETHERLANDS.</p>
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Sarcopenia is defined as a decrease in skeletal muscle mass that is particularly caused by satellite cells being unable to proliferate. The number of satellite cells, as well as the proliferative capacity of isolated satellite cells *in vitro* decreases with increasing chronological age. However, even at the end of human lifespan, myoblast cultures can be established out of muscle biopsies even in the presence of sarcopenia. As satellite cell characteristics have predominantly been assessed using mixed cultures and not compared to studies using single clones, the importance of these *in vitro* characteristics are uncertain.

We established a mixed myoblast culture and three clonal myoblast cultures out of the same muscle biopsy obtained from a middle aged man and cultured these cells for 100 days. We found a significantly lower replicative capacity of the myoblast clones when compared to the mixed culture. Replicative capacity was inversely related to the beta-galactosidase activity after exposure to oxidative stress when cultures were tested at an earlier passage. Remaining replicative capacity was greatly enhanced in all four cultures when carnosine was supplemented to the medium, whereas the beta-galactosidase activity at the early replicative stage remained unchanged.

We conclude that proliferative capacity of satellite cell in mixed cultures *in vitro* do not reflect characteristics of single clones. The *in vitro* characteristics of a mixed culture is likely to represent few dominant clones. The impact of the huge variation in replicative potential of each single cell on the *in vivo* situation remains to be established.