ANNUAL REPORT - ***DEPARTMENT OF TRANSLATIONAL MEDICINE - PhD PROGRAM IN SCIENCES & MEDICAL BIOTECHNOLOGY - XXIX cycle*** *(2014-2017)*
Coordinator: Prof. Emanuele Albano

Role of Ghrelin in Skeletal Muscle Regeneration

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SCIENTIFIC BACKGROUND

Skeletal muscle is the most abundant tissue in the vertebrate body and is composed by contractile cellular units, the myofibers, formed by hundreds of post-mitotic nuclei. Growth and repair of muscle are sustained by a pool of myogenic precursors, located between the basal lamina and the myofiber, called satellite cells (SCs) (Zammit *et al.*, 2004). In the adult skeletal muscle, SCs are in a quiescent state and express the transcription factor paired-box 7 (Pax7) (Seale *et al.*, 2000). After injury or exercise, several growth factors and cytokines trigger the activation of SCs, inducing the expression of myogenic genes, such as the myogenic factor 5 (Myf5) and the myoblasts determination factor (MyoD), thus the SCs enter the cell cycle as a transient amplifying population, referred to as myoblasts (Singh and Dilworth, 2013). After several cellular divisions, myoblasts turn off Pax7 expression and start to express other key genes that orchestrate the final stages of differentiation. At this stage, myoblasts exit the cell cycle and fuse into existing myofibers or fuse to each other to form *de novo* myofibers (Yin *et al.*, 2013).

During the regeneration process, the pool of SCs is maintained for a huge number of regeneration cycles, through the mechanism of self-renewal (Collins *et al.*, 2005). In particular, it has been elegantly demonstrated that a small portion of SCs, at the first division, generates two distinct cell daughters: one committed progenitor, and the other with stem-like features, through asymmetric division (Kuang *et al.*, 2007). Indeed, SCs are present in the adult skeletal muscle in a hierarchical pool, composed by muscle stem cells and committed progenitors (Kuang *et al.*, 2008).

Despite the extraordinary ability of skeletal muscle to regenerate itself, in some pathological conditions, such as dystrophies, the regeneration process is impaired (Rahimov and Kunkel, 2013). In this context, skeletal muscle undergoes continuous rounds of degeneration and regeneration. This leads to gradual depletion of SCs and to deposition of fat and fibrotic tissue, resulting in loss of structure and function of skeletal muscle (Jiang *et al.*, 2014). There are several proposed approaches to treat dystrophies, such as cell-based therapies. However, these approaches have some limitations, including the poor engraftment of the transplanted cells (Sirabella *et al.*, 2013). There is currently no cure for these pathologies, thus the investigation of factors that could improve the regeneration process and could enhance the engraftment of donor cells is crucial (Briggs and Morgan, 2013).

Acylated ghrelin (AG) is a peptide hormone released by the stomach in fasting condition. AG binds and activates the GHSR-1a (growth hormone secretagogue receptor 1a) inducing growth hormone release and appetite (Kojima *et al.*, 1999; Asakawa *et al.*, 2001).

The unacylated form of ghrelin (unacylated ghrelin - UnAG), does not bind to GHSR-1a and has been considered for many years the inactive product of AG catabolism (Chen *et al.*, 2009). However, AG and UnAG share several biological activities mediated by a not yet identified receptor, including inhibition of apoptosis in cardiomyocytes and endothelial cells, and impairment of skeletal muscle atrophy in mice (Baldanzi *et al.*, 2002; Porporato *et al.*, 2013). In addition, AG and UnAG induce differentiation and fusion of

myoblasts (Filigheddu *et al.,* 2007). Recently, it has been demonstrated that UnAG promotes regeneration of skeletal muscle following hindlimb ischemia (Togliatto *et al*., 2013).
These effects on skeletal muscle prompted us to investigate the role of UnAG on regeneration. Our preliminary data show that ghrelin gene (*Ghrl*) is upregulated in skeletal muscle after a necrotic damage, in accordance with previous observations (Guarriaran-Rodriguez *et al*., 2013). Together these data suggest that UnAG could promote regeneration of skeletal muscle.
To verify this hypothesis we use transgenic mice over-expressing the ghrelin gene, characterized by high levels of plasmatic UnAG (Porporato *et al*., 2013). After 7 days from a necrotic injury, these mice display an advanced regenerative status compared to wild type (WT) mice. Moreover, the analysis of skeletal muscle in basal conditions (not-injured) reveals that transgenic mice have a greater number of SCs compared to WT mice, suggesting that UnAG could have an effect on proliferation and/or self-renewal of SCs.
To evaluate the direct effect of UnAG on SCs, we isolated SCs from WT and we treated them *ex vivo* with UnAG. When plated, primary SCs undergo activation and start to proliferate. After 5 to 7 days, the majority of proliferating myoblasts exits the cell cycle and differentiates into myosin heavy chain (MHC) positive myotubes. While some of them do not differentiate and become quiescent Pax7+ cells (referred to as reserve cells). These cells define a reserve population that reflects satellite cell self-renewal (Danoviz and Yablonka-Reuveni, 2012). We note that UnAG increases the number of the reserve cells, suggesting that UnAG could improve self-renewal of SCs.

AIMS OF THE PROJECT

- To investigate the effect of UnAG on activation, proliferation, and self-renewal of SCs.
- To verify *in vivo* the ability of UnAG to induce proliferation and self-renewal of SCs.
- To assess the UnAG activity on skeletal muscle regeneration in dystrophic mice.
- To verify *in vivo* if UnAG could improve engraftment of transplanted SCs.

EXPERIMENTAL PROCEDURES

Mice and *in vivo* treatment

All experiments were conducted on young adult males, matched for age and weight. FVB and C57/BL6 *Myh6/Ghrl* transgenic mice express *Ghrl* gene under the control of the cardiac-specific promoter of alpha-myosin heavy chain (*Myh6*) gene. These transgenic mice have high levels (50 folds compared to wild type) of plasmatic UnAG (Porporato *et al*., 2013). *Ghrl* knock-out mice (*Ghrl-/-*) were from Prof. Catehrine- Laure Tomasetto laboratory (Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Strasbourg, France).
C57BL/6-Tg(CAG-EGFP)1Osb/J (β-actin/GFP) mice express ubiquitously the green fluorescent protein was used as donors of SCs for the transplantation experiments.
To induce skeletal muscle damage and regeneration, left tibialis anterior muscle was injected with 30 µl of 10 µM cardiotoxin from *Naja mossambica mossambica* (Sigma Aldrich). The thymidine analogue 5-Bromo-2'-deoxyuridine (BrdU) (Sigma Aldrich) was injected intraperitoneally 6 mg/gram mouse. For continuous administration, BrdU was dissolved in drinking water (2.5 mg/ml) and given for 7 days, as previously described (Shea *et al*., 2010).

Myofibers isolation and staining

Single myofibers were isolated from extensor digitorum longus (EDL) muscle as previously described (Shefer and Yablonka-Reuveni, 2005). Briefly, EDL muscles were extracted from FVB wild type mice and

digested in 0.2 mg/ml of collagenase type 1 (Sigma Aldrich) for 1h30' at 37°C. After digestion, released intact myofibers were washed three times in serum-free medium. Myofibers were maintained in low proliferation medium (DMEM supplemented with 1% penicillin-streptomycin, containing 10 % horse serum, 0.5 % chick embryo extract), in the presence or absence of 10 nM of UnAG, for up to 4 days.
At specific time points, myofibers were fixed in 4% paraformaldehyde for 10' then processed for immune-staining as previously described (Beauchamp *et al.*, 2000). Briefly, myofibers were permeabilized in 0.5% Triton X-100 in PBS and blocked in 4% BSA. Primary antibodies used were mouse monoclonal anti-Pax7 (Developmental Studies Hybridoma Bank) and rabbit polyclonal anti-MyoD (Santa Cruz Biotechnology). Secondary antibodies used were goat anti-mouse 488 (Alexa Fluor) and goat anti-rabbit 568 (Alexa Fluor).

Satellite cells isolation and transplantation

Satellite cells (SCs) were isolated from hind limb muscles and diaphragm as previously described (Danoviz and Yablonka-Reuveni, 2012). Briefly, muscles were minced and digested for 1h in 0.1% Pronase at 37°C, then mechanically triturated in serial passages trough Pasteur pipette. The cell suspension was filtered in a 40 µm cell strainer and then pre-plated in a 60 mm^2 dish for 1h and 30' at 37°C, in order to remove fibroblasts. After this incubation, cells were counted and plated in 35 mm^2 gelatin-coated dishes. For the transplantation procedure, 10^5 cells isolated from β-actin/GFP mice were injected in the central region of the pre-damaged tibialis anterior with an insulin syringe.

RESULTS

UnAG induces activation, proliferation, and self-renewal of SCs.

The enhanced regeneration observed in Myh6/Ghrl transgenic mice, could reflect the effect of UnAG on differentiation *in vitro* previously demonstrated (Filigheddu *et al.*, 2007). However, we also note that Myh6/Ghrl transgenic mice have a greater pool of SCs, suggesting that UnAG could have an effect on proliferation and/or self-renewal of satellite cells. Thus, we investigated the effects of UnAG treatment on single myofibers isolated from EDL muscle of Wt mice. At 6h after incubation of myofibers in the presence or absence of 10 nM of UnAG, we observed that UnAG increases the portion of activated (MyoD+) SCs (fig. 1A). Moreover, treatment with UnAG increases the number of myoblasts per cluster at 72h, indicating that UnAG is able to induce SCs proliferation (fig. 1B).
At 96h of incubation, the portion of Pax7+/MyoD- SCs is enhanced in UnAG-treated myofibers (fig. 1C), suggesting that UnAG induces self-renewal of SCs.

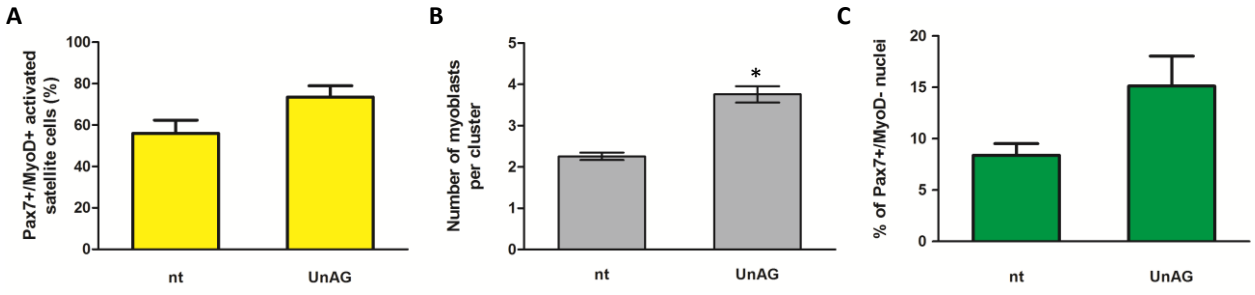

Figure 1. UnAG treatment of SCs on single myofibers induces activation, proliferation, and self-renewal.
Treatment with 10 nM of UnAG induces activation of SCs, seen as percentage of Pax7+/MyoD+ nuclei (A). After 72h, the number of myoblasts (Pax7 and/or MyoD positive nuclei) per cluster is increased by treatment with 10 nM of UnAG (B). At 96h, the percentage of Pax7+/MyoD- self-renewing SCs is higher in UnAG-treated myofibers than in the untreated ones (C). N=3 for each time point. *p<0.05.

UnAG increases self-renewal of SCs *in vivo*.

After activation and proliferation, a portion of SCs eventually undergo quiescence and maintain the stem pool. To investigate the putative effect of UnAG on self-renewal of SCs *in vivo*, we injected tibialis anterior of Wt and Myh6/Ghrl mice with CTX and we administrated BrdU for the first 7 days of regeneration. BrdU is incorporated in every cell that enters the S-phase. After 50 days from CTX injection, quiescent Pax7-expressing SCs that have incorporated BrdU are the self-renewing cells (Shea *et al.*, 2010) (fig. 2A). We noted that the number of Pax7+/BrdU+ SCs is higher in Myh6/Ghrl transgenic mice compared to WT (fig. 2C), and this demonstrate that high levels of UnAG enhance SCs self-renewal *in vivo*.

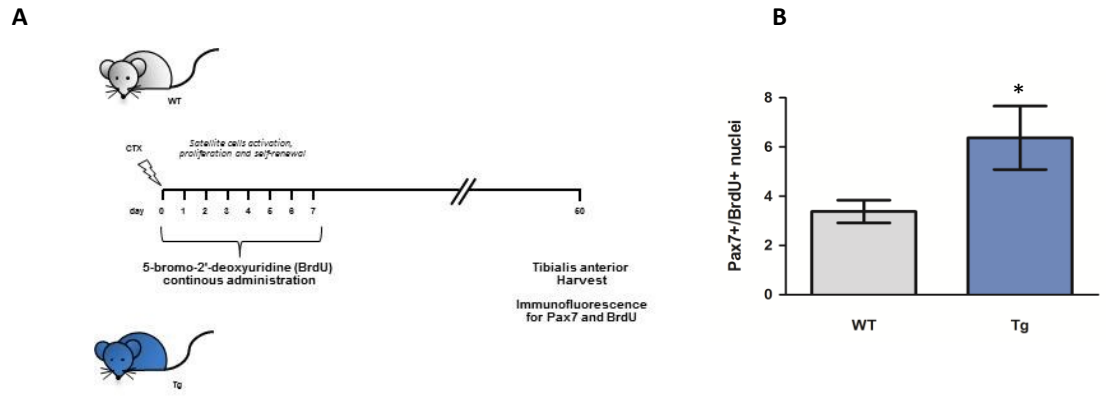

Figure 2. High levels of UnAG in mice increase self-renewal of SCs.

Experimental design: cardiotoxin (CTX) was injected in left tibialis anterior (TA) of wild type (WT) and Myh6/Ghrl transgenic mice (Tg). During the first 7 days, BrdU was administered to label the cycling cells. After 50 days, left and right TA were harvested and stained for Pax7 and BrdU **(A)**. Myh6/Ghrl transgenic mice (Tg) have a greater number of Pax7+/BrdU+ nuclei compared to wild type (WT), normalized to the contralateral number of SCs **(B)**. N=7, *p<0.05 (B).

Upregulation of UnAG improves SCs transplantation outcome.

Cell-based therapies are promising approaches to treat muscular dystrophies, but there are several limitations that impair their efficiency, such as the low engraftment of transplanted precursors (Briggs and Morgan, 2013). As UnAG promotes proliferation and differentiation of SCs we postulate that high levels of this peptide in recipient mice could improve the transplantation engraftment. To test this hypothesis, we isolated SCs from β-actin/GFP mice and we transplant those cells in the pre-damaged tibialis anterior muscles of Myh6/Ghrl and WT mice. After 30 days, the number of GFP positive myofibers is higher in My6/Ghrl mice compared to WT (fig. 3A and 3B). This data indicates that high levels of UnAG improve the SCs transplantation outcome in recipient mice.

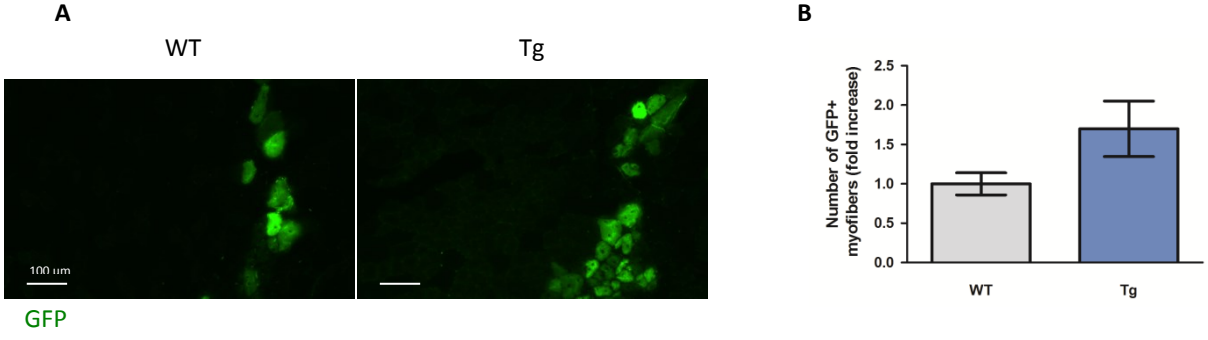

Figure 3. High levels of UnAG in mice enhance SCs transplantation outcome.
Representative images of green fluorescent protein (GFP) epifluorescence of transplanted tibialis anterior section of wild type (WT) and Myh6/Ghrl transgenic mice (Tg) **(A)**. Number of GFP+ myofibers in WT and Tg mice after 30 days from transplant (fold increase) N=7 **(B)**.

ACKNOWLEDGMENTS
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REFERENCES

Asakawa, A., Inui, A., Kaga, T., Yuzuriha, H., Nagata, T., Ueno, N., Makino, S., Fujimiya, M., Niijima, A., Fujino, M.A., Kasuga, M. (2001). Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology. 120, 337-345.

Baldanzi, G., Filigheddu, N., Cutrupi, S., Catapano, F., Bonissoni, S., Fubini, A., Malan, D., Baj, G., Granata, R., Broglio, F., Papotti, M., Surico, N., Bussolino, F., Isgaard, J., Deghenghi, R., Sinigaglia, F., Prat, M., Muccioli, G., Ghigo, E., Graziani, A. (2002). Ghrelin and des-acyl ghrelin inhibit cell death in cardiomyocytes and endothelial cells through ERK1/2 and PI 3-kinase/AKT. J Cell Biol. 159, 1029-1037.

Beauchamp, J.R., Heslop, L., Yu, D.S., Tajbakhsh, S., Kelly, R.G., Wernig, A., Buckingham, M.E., Partridge, T.A., Zammit, P.S. (2000). Expression of CD34 and Myf5 defines the majority of quiescent adult skeletal muscle satellite cells. J Cell Biol. 151, 1221-1234.

Briggs, D., Morgan, J.E. (2013). Recent progress in satellite cell/myoblast engraftment - relevance for therapy. FEBS J. 280, 4281-4293.

Collins, C.A., Olsen, I., Zammit, P.S., Heslop, L., Petrie, A., Partridge, T.A., Morgan, J.E. (2005). Stem cell function, self-renewal, and behavioral heterogeneity of cells from the adult muscle satellite cell niche. Cell. 122, 289-301.

Chen, C.Y., Asakawa, A., Fujimiya, M., Lee, S.D., Inui, A. (2009). Ghrelin gene products and the regulation of food intake and gut motility. Pharmacol Rev. 61, 430-481.

Danoviz, M.E., Yablonka-Reuveni, Z. (2012). Skeletal muscle satellite cells: background and methods for isolation and analysis in a primary culture system. Methods Mol Biol. 798, 21-52.

Filigheddu, N., Gnocchi, V.F., Coscia, M., Cappelli, M., Porporato, P.E., Taulli, R., Traini, S., Baldanzi, G., Chianale, F., Cutrupi, S., Arnoletti, E., Ghè, C., Fubini, A., Surico, N., Sinigaglia, F., Ponzetto, C., Muccioli, G., Crepaldi, T., Graziani, A. (2007). Ghrelin and des-acyl ghrelin promote differentiation and fusion of C2C12 skeletal muscle cells. Mol Biol Cell. 18, 986-994.

Gurriarán-Rodríguez, U., Santos-Zas, I., Al-Massadi, O., Mosteiro, C.S., Beiroa, D., Nogueiras, R., Crujeiras, A.B., Seoane, L.M., Señarís, J., García-Caballero, T., Gallego, R., Casanueva, F.F., Pazos, Y., Camiña, J.P. (2012). The obestatin/GPR39 system is up-regulated by muscle injury and functions as an autocrine regenerative system. J Biol Chem. 287, 38379-38389.

Jiang, C., Wen, Y., Kuroda, K., Hannon, K., Rudnicki, M.A., Kuang, S. (2014). Notch signaling deficiency underlies age-dependent depletion of satellite cells in muscular dystrophy. Dis Model Mech. 7, 997-1004.

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K. (1999). Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 402, 656-560.

Kuang, S., Gillespie, M.A., Rudnicki, M.A. (2008). Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell. 2, 22-31.

Kuang, S., Kuroda, K., Le Grand, F., Rudnicki, M.A. (2007). Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell. 2Jun 1;129(5):999-1010.

Porporato, P.E., Filigheddu, N., Reano, S., Ferrara, M., Angelino, E., Gnocchi, V.F., Prodam, F., Ronchi, G., Fagoonee, S., Fornaro, M., Chianale, F., Baldanzi, G., Surico, N., Sinigaglia, F., Perroteau, I., Smith, R.G., Sun, Y., Geuna, S., Graziani, A. (2013). Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest. 123, 611-622.

Rahimov, F., Kunkel, L.M. (2013). The cell biology of disease: cellular and molecular mechanisms underlying muscular dystrophy. J Cell Biol. 201, 499-510.

Seale, P., Sabourin, L.A., Girgis-Gabardo, A., Mansouri, A., Gruss, P., Rudnicki, M.A. (2000). Pax7 is required for the specification of myogenic satellite cells. Cell. 102, 777-786

Singh, K., Dilworth, F.J. (2013). Differential modulation of cell cycle progression distinguishes members of the myogenic regulatory factor family of transcription factors. FEBS J. 280, 3991-4003.

Sirabella, D., De Angelis, L., Berghella, L. (2013). Sources for skeletal muscle repair: from satellite cells to reprogramming. J Cachexia Sarcopenia Muscle. 4, 125-136.

Shea, K.L., Xiang, W., La Porta, V.S., Licht, J.D., Keller, C., Basson, M.A., Brack, A.S. (2010). Sprouty1 regulates reversible quiescence of a self-renewing adult muscle stem cell pool during regeneration. Cell Stem Cell. 6, 117-129.

Shefer, G., Yablonka-Reuveni, Z. (2005). Isolation and culture of skeletal muscle myofibers as a means to analyze satellite cells. Methods Mol Biol. 290, 281-304.

Togliatto, G., Trombetta, A., Dentelli, P., Cotogni, P., Rosso, A., Tschöp, M.H., Granata, R., Ghigo, E., Brizzi, M.F. (2013). Unacylated ghrelin promotes skeletal muscle regeneration following hindlimb ischemia via SOD-2-mediated miR-221/222 expression. J Am Heart Assoc. 2.

Yin, H., Price, F., Rudnicki, M.A. (2013). Satellite Cells and the Muscle Stem Cell Niche. Physiol Rev. 93, 23–67

Zammit, P.S., Golding, J.P., Nagata, Y., Hudon, V., Partridge, T.A., and Beauchamp, J.R. (2004). Muscle satellite cells adopt divergent fates: a mechanism for self-renewal? J. Cell Biol. 166, 347-357.

PUBBLICATIONS

Porporato, P.E., Filigheddu, N., Reano, S., Ferrara, M., **Angelino, E**., Gnocchi, V.F., Prodam, F., Ronchi, G., Fagoonee, S., Fornaro, M., Chianale, F., Baldanzi, G., Surico, N., Sinigaglia, F., Perroteau, I., Smith, R.G., Sun, Y., Geuna, S., Graziani, A. (2013). Acylated and unacylated ghrelin impair skeletal muscle atrophy in mice. J Clin Invest. 123, 611-622.

SEMINARS 2014 (department of translational medicine, NOVARA)

Oral presentation of PhD students of XXVII CYCLE

-20/10/2014 15:00 Famà Rosella
"The Krüppel-like factor 2 transcription factor is a novel tumor suppressor gene recurrently mutated in Splenic Marginal Zone Lymphoma"

-1/10/2014 14:00 Ciardullo Carmela "Clonal evolution andclinical relevance of subclonal mutations in chronic lymphocyticleukemia"

The Borghese Sessions Steven R Ellis

-September 8
10:00 Clinical case – Skin as an organ
11:00 Layers of skin, cell types, developmental origins

-September 9
10:00 Cell-Cell Interactions – anchoring junctions
11:00 Cell-Cell Interactions – occluding junctions, tight junctions

-September 10
10:00 Cell Matrix Interactions – basal lamina
11:00 Epithelial-mesenchymal transition

-September 11
10:00 Angiogenesis
11:00 Innervation

-September 15
10:00 Basal layer stem cells, symmetric versus asymmetric divisions, transient amplifying cells
11:00 Solar radiation, nucleotide excision repair

-September 16
10:00 Basal and squamous cell carcinomas
11:00 Melanoma – biology

-September 17
10:00 Melanoma - treatment
11:00 Contact dermatitis

-September 22
10:00 Other skin disorders
11:00 Other components of skin
-21/7/14 Dr Maria Giuseppina Miano "a functional link between arx and kdm5c genes linked to neuronal diseases defines a crucial epigenetic path"

-16/07/2014 at 14.30 Prof. John F. McDonald "The potential of small regulatory RNAs for the treatment of ovarian cancer

-15/07/2014 ore 14.30-16 Prof.ssa Follenzi "applicazioni terapia genica''

-30/06/2014 at 14-16 Dott. Cotella ''the C-value paradox, junk DNA and ENCODE''

-27/06/2014 at 14 Manuela Sironi Has nature done the experiment for us? Evolutionary insights into infection susceptibility and autoimmunity

-26/06/2014 at 14 Prof Gianni Del Sal "Disarming mutant P53 in cancer"

-19/06/2014 at 12-13.30 Prof.ssa Follenzi ''terapia genica''

-12/06/2014 at 14 Gianni Cesareni "Metformin rewires the signaling network of breast cancer cells and changes their sensitivity to growth and apoptotic stimuli"

-11/06/14 at 14 Prof. Fabrizio Loreni "Ribosome alteration in cancer: effect or cause?"

-9/06/2014 at 14 Dott Iacopo Baussano "Assessment of cervical cancer control in Rwanda and Bhutan''

-5/05/2014 ore 12 Prof. Vittorio Colombo e Dr. Matteo Gherardi, ''atmospheric pressure plasma sources ad processes for biomedical and surface treatment applications''

-19/03/2014 14.30 Prof Emilio Hirsch ''role of phosphoinositides-3-kinase C2-alph, a Class II PI 3-kinase, in development and cancer''

-19/02/2014 14 Prof. Salvatore Oliviero Università di Torino ''epigenetic modifications that control stem cell differentiation''

CONGRESS

"Stem cell therapy: hype or hope?". Lugano (CH). University of Italian Switzerland, 29 marzo 2014.

"NextStep: la giovane ricerca avanza". Milan (IT). Universita' degli Studi di Milano, 3 giugno 2014.

POSTERS

Elia Angelino, Simone Reano, Michele Ferrara, Omar Sabry, Andrea Graziani, Nicoletta Filigheddu. Ghrelina deacilata promuove la rigenerazione del muscolo scheletrico. Giornata del dottorato. Alessandria, Italy. September 26, 2014.

Ferrara M, Sabry O, Reano S, **Angelino E**, Ciraolo E, Coletti D, Hirsch E, Costelli P, Filigheddu N, Graziani A. A GHSR1a-independent signaling contributes to ghrelin anti-cachectic activity in skeletal muscle and is reciprocally regulated by PI3-kinase beta and gamma isoforms. 2nd cancer cachexia conference. Montreal, Canada. September 26-28, 2014.

Reano S, **Angelino E**, Sabry O, Ferrara M, Graziani A, Filigheddu N. Ghrelin promotes skeletal muscle regeneration. 7th Cachexia Conference. Kobe, Japan. December 9-11, 2013.

Ferrara M, Reano S, **Angelino E**, Sabry O, Coletti D, Filigheddu N, Graziani A (2013). Acylated and Unacylated Ghrelin protect the skeletal muscle from atrophy by acting directly on the muscle fiber, independently on GHSR1a-mediated orexigenic and anabolic activities. 7th Cachexia Conference. Kobe, Japan. December 9-11, 2013.

Filigheddu N, Reano S, **Angelino E**, Ferrara M, Sabry O, Graziani A. Role of ghrelin in skeletal muscle regeneration. Regenerative medicine: From biology to therapy. Cambridge, England. October 30 – november 1, 2013.

Reano S, Ferrara M, **Angelino E**, Sabry O, Coletti D, Filigheddu N, Graziani A (2013). Role of acylated and unacylated ghrelin in skeletal muscle regeneration. EMBO Workshop – Molecular mechanisms of muscle growth and wasting in health and disease, Ascona, Switzerland. September 15 – 20, 2013.

Simone Reano, **Elia Angelino**, Michele Ferrara, Omar Sabry, Andrea Graziani, Nicoletta Filigheddu. Role of ghrelin in skeletal muscle regeneration. Gordon Research Conference, Lucca, Italy. July 7 – 12, 2013.

Nicoletta Filigheddu, Simone Reano, Michele Ferrara, Paolo Ettore Porporato, **Elia Angelino**, Viola Gnocchi, Yuxiang Sun, Andrea Graziani. Acylated and Unacylated Ghrelin impair skeletal muscle atrophy without inducing hypertrophy and indipendently of GHSR1, the only known ghrelin receptor. Society for muscle biology, New York, USA. June 4 – 8, 2012.

Nicoletta Filigheddu, Simone Reano, Michele Ferrara, Paolo Ettore Porporato, **Elia Angelino**, Giulia Ronchi, Stefano Geuna, Yuxiang Sun, Andrea Graziani. Unacylated ghrelin and ghrelin counteract skeletal muscle atrophy. ABCD meeting 2011, Ravenna, Italy. Semptember 8 – 10, 2011.