Associazione di Biologia Cellulare e del Differenziamento

Cell Stress: Survival and Apoptosis

Organisers

Gaetano Cairo (Chair) - University of Milan

Sergio Giannattasio (vice-Chair) - CNR, Bari

Programme & Abstracts

Bertinoro, 30-31 May 2014

http://CSSA2014.azuleon.org

With the support of

PROGRAMME

Friday, 30 May

12:00 **Registration**

13:00 **Lunch**

14:30-14:45 **Welcome Addresses**

Gaetano Cairo & Sergio Giannattasio

14:45-15:30 **Plenary Lecture**

Julián Aragonés (Madrid, Spain)

Metabolic reprogramming through HIF oxygen sensing pathways in disease

15:30-16:30 **Session I: Cell Death and Autophagy**

Chairs: A. Montecucco & E. O'Brien

15:30-15:50 *Valeria Matranga (Palermo)*
Autophagy induction in sea urchin embryos exposed to gadolinium ions

15:50-16:10 *Federica Maccarinelli (Brescia)*
Mice lacking mitochondrial ferritin are more sensitive to doxorubicin-mediated cardiotoxicity

16:10-16:30 *Cristina Mazzoni (Rome)*
Role of GABA metabolism enzymes during stress, aging and apoptosis in budding yeast, *Saccharomyces cerevisiae*

16:30-17:00 **Coffee break and Poster viewing**

17:00-18:20 **Session II: Heat Shock and Protein Misfolding Response (I)**

Chairs: M.R. Woodford & V. Matranga

17:00-17:20 *Anna Maria Giuliodori (Camerino)*
Fine mapping of the Escherichia coli cold-shock protein CspA binding sites on its own mRNA

17:20-17:40 *Fabiana Geraci (Palermo)*
Autocrine role of extracellular Hsp70 in mesoangioblast migration capability

17:40-18:00 *Wei Li (Los Angeles, CA, USA)*
The non-Chaperoning function of secreted Heat Shock Protein-90a defines motility and invasiveness of breast cancer cells

18:00-18:20 *Edward O'Brien (Calgary, AB, Canada)*
Extracellular HSP27 attenuates atherogenesis by reducing plaque apoptosis and inflammation: insights into a novel signaling pathway for atheroprotection

18:20-19:00 **Poster Session**

19:00-19:30 **Business meeting and election of vice-chair**

20:30 **Dinner**

Saturday, 31 May

9:00-10:00 **Session III: Cell Stress and Cancer**

Chairs: W. Li & J. Aragonés

9:00-9:20 *Nicoletta Guaragnella (Bari)*
Silencing of the tumor suppressor BRCA2 decreases anoikis and its heterologous expression exacerbates acetic acid-induced programmed cell death in yeast cells

9:20-9:40 *Morena Catillo (Pavia)*
Metabolic stress controls alternative splicing programs: relevance to cancer biology

9:40-10:00 *Elia Ranzato (Alessandria)*
GRP78 and T-type Ca2+ channels: novel EGCG targets against malignant mesothelioma

10:00-11:20 **Session IV: Cell Stress and Genome Instability**

Chairs: D. Cavalieri & C. Mazzoni

10:00-10:20 *Valentina Tosato (Trieste)*
Post-translocational adaptation (PTA) to cellular stress

10:20-10:40 *Jade Quartararo (Parma)*
Mitochondria and lifespan extension in Saccharomyces cerevisiae: the longevity mutations sch9Δ and rei1Δ contribute to mitochondrial DNA stability

10:40-11:00 *Alessandra Montecucco (Pavia)*
Chronic DNA damage hampers cell morphology and motility

11:00-11:20 *Manuela Minguzzi (Bologna)*
GSK3β inhibition in osteoarthritic human primary chondrocytes determines oxidative stress and DNA damage response leading to a worse disease phenotype

11:20-11:40 **Coffee break and Poster viewing**

11:40-12:40 **SESSION V: HEAT SHOCK AND PROTEIN MISFOLDING RESPONSE (II)**

Chairs: M.C. Roccheri & A. Poletti

11:40-12:00 *Duccio Cavalieri (San Michele all'Adige)*
Epigenetics at work: phenotypic switching by Heat Shock Proteins and prions in yeast

12:00-12:20 *Mark Woodford (Syracuse, NY, USA)*
Post-translation modification crosstalk regulates Hsp90 chaperone cycle

12:20-11:40 *Riccardo Cristofani (Milan)*
Accumulation of motor neuron diseases associated misfolded proteins is counteracted by dynein inhibition

12:40-13:15 **CONCLUDING REMARKS - WINNER OF THE F. RITOSSA AWARD ANNOUNCED**

13:15 **LUNCH**

Oral Presentations

(in chronological order of presentation
presenting authors are shown underlined)

Metabolic reprogramming through HIF oxygen sensing pathways in disease

J. Aragonés
Research Unit, Hospital of Santa Cristina, Research Institute Princesa (IP), Autonomous Univ. of Madrid, Madrid, Spain

Local oxygen insufficiency is central of numerous pathologies such as ischemic episodes, tumor growth and more recently during white adipose tissue expansion. These oxygen fluctuations are sensed by the prolyl hydroxylase domain proteins (PHD-1, 2 and 3) oxygen sensors, which in turn regulate the hypoxia-inducible transcription factors (HIFs). One the biological responses controlled by the PHD-HIF-dependent transcriptional system is the reprogramming of cellular metabolism, which couple cell bioenergetics to oxygen supply. Our studies reveal that this oxygen-dependent metabolic rewiring is central in ischemia tolerance, obesity, and more recently have showed that also control tumor progression by regulating amino acid metabolism. The pathological impact of these oxygen-dependent axes affecting glucose, fatty acid and amino acid metabolism in disease will be discussed.

Autophagy induction in sea urchin embryos exposed to gadolinium ions

C. Martino[1,2], R. Chiarelli[1], M.C. Roccheri[1], V. Matranga[2]
[1]Dipartimento Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Univ. di Palermo, Palermo
[2]Consiglio Nazionale delle Ricerche, Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", Palermo

Sea urchin embryos are highly sensitive to several kinds of stressors, and able to activate different defense strategies. Gadolinium (Gd) is a metal of the lanthanide series of the elements: its chelates are employed as contrast agents for magnetic resonance imaging since the 1980s. Gd complexes are released in the aquatic environment, making Gd an emergent environmental pollutant. In this study we focused on the effects of Gd ions on sea urchin embryos development. The study was conducted looking at three different processes: general development, apoptosis and autophagy. At the whole morphological level, *Paracentrotus lividus* sea urchin embryos continuously exposed to Gd ions displayed morphological abnormalities and a significant inhibition of skeleton elongation and patterning. The study of apoptosis performed by immunofluorescence (IF) staining using an anti-cleaved-caspase-3 antibody on whole-mount embryos after 24h (gastrula) and 48h (pluteus) exposure showed no apoptotic induction. Autophagic processes were investigated by Western blot analysis of total lysates and IF staining (autophagosomes) on whole-mount embryos to detect LC3 protein and acridine orange (AO) vital staining to highlight the presence of acidic vesicular organelles (autophagolysosomes) in whole embryos. In particular, Western blots of embryos exposed to Gd showed a 2.6-fold increase relative to controls at 24h and a 4-fold increase at 48h, suggesting that the autophagic process is acting as a cell survival strategy to defend the developmental program. In agreement, AO vital staining and LC3 IF confirmed an increased number of autophagosomes and autophagolysosomes. Taken together, results show that autophagy is a molecular process activated in sea urchin embryos exposed to Gd ions.

Work supported by University of Palermo FFR to MCR and partially by PO-FESR 2007-2013 DeCroMed Project to VM. Authors thank Mr. M. Biondo for technical assistance.

Mice lacking mitochondrial ferritin are more sensitive to doxorubicin-mediated cardiotoxicity

F. Maccarinelli[1], E. Gammella[2], M. Regoni[1], M. Asperti[1], P. Buratti[2], I. Rybinska[2], L. Cornaghi,[2] E. Donetti[2] , S. Recalcati[2], G. Cairo[2], P. Arosio[1]
[1]Dept of Molecular and Translational Medicine, Univ. of Brescia, Italy
[2]Dept Biomedical Sciences for Health, Univ. of Milan, Italy

Mitochondrial ferritin (FtMt) is a functional ferritin that localizes in the mitochondria. It is expressed in the testis, heart, brain, and cells with active respiratory activity. Its overexpression in cultured cells protected against oxidative damage and reduced cytosolic iron availability. However, no overt phenotype was previously described in mice with inactivation of the FtMt gene. Here, we used the doxorubicin model of cardiac injury in a novel strain of FtMt-null mice to investigate the antioxidant role of FtMt. These mice did not show any evident phenotype, but after acute treatment to doxorubicin, they showed enhanced mortality and altered heart morphology with fibril disorganization and severe mitochondrial damage. Signs of mitochondrial damage were present also in mock-treated FtMt−/− mice. The hearts of saline- and doxorubicin-treated FtMt−/− mice had higher thiobarbituric acid reactive substance levels, heme oxygenase 1 expression, and protein oxidation, but did not differ from FtMt+/+ in the cardiac damage marker B-type natriuretic peptide (BNP), ATP levels, and apoptosis. However, the autophagy marker LC3 was activated. These results show that the absence of FtMt, which is highly expressed in the heart, increases the sensitivity of heart mitochondria to the toxicity of doxorubicin. This study represents the first in vivo evidence of the antioxidant role of FtMt.

Role of GABA metabolism enzymes during stress, aging and apoptosis in budding yeast, *Saccharomyces cerevisiae*

M. Stirpe[1], V. Palermo[1], A. Tramonti[2], E. Pennacchietti[3], G. Grassini[3], D. De Biase[3], C. Mazzoni[1]
[1]Dept of Biology and Biotechnology "C. Darwin", Pasteur Institute-Cenci Bolognetti, "Sapienza" Univ. of Rome, Italy
[2]Dept of Biology and Molecular Patology, CNR, Univ. of Rome "Sapienza", Italy
[3]Dipartimento di Scienze e Biotecnologie Medico-Chirurgiche, Pasteur Institute-Cenci Bolognetti Univ. of Rome "Sapienza", Latina, Italy

The γ-aminobutyric acid (GABA) is a non-proteic aminoacid synthesized by almost all living organisms.
GABA metabolism, known as "GABA shunt" involves three enzymes: glutamate decarboxylase (GAD), GABA aminotransferase (GAT) and succinate semialdehyde dehydrogenasi (SSADH). These three enzymes act in concert to convert glutamate (a-ketoglutarate) to succinate, bypassing two reactions of the tricarboxylic acid cycle.
The GABA shunt pathway is well conserved from bacteria, fungi and plants through vertebrates with different biological function in each organism.
In mammals, GABA has two different roles: inhibitory neurotransmitter and trophic factor during central nervous system (CNS) development. In plants, the GABA shunt appears to contribute to the control of cytosolic pH, balance between carbon and nitrogen metabolism and to stress response. In some of enteric bacteria GABA is a metabolic product inside the cell and it is involved in acid resistance system.
GABA has also been shown to play a potential signalling role in biological and developmental processes, i.e. it has been found as signalling molecule during host-pathogen interactions and during yeast colony development. In the yeast *Saccharomyces cerevisiae*, the deletion of *UGA3* gene -which encodes a zinc-finger transcription factor necessary for GABA-dependent induction of the *UGA1* (GABA aminotransferase), *UGA2* (succinate semialdehyde dehydrogenase), and *UGA4* (GABA permease) genes- extends replicative lifespan, as did deletion of *UGA1* and *GAD1* (glutamate decarboxylase); in contrast strains with *UGA2* or *UGA4* deletions exhibited no lifespan extensions. Therefore, two genes in the GABA metabolism pathway, *UGA1* and *GAD1*, were identified as aging genes. Nevertheless, the effects of these enzymes on chronological lifespan and stress response have not been studied yet. Data on the role of GABA shunt in budding yeast during CLS and under stress conditions will be presented.

Fine mapping of the *Escherichia coli* cold-shock protein CspA binding sites on its own mRNA

A.M. Giuliodori[1], R. Belardinelli[2], R. Garofalo[2], S. Marzi[3]
[1]School of Biosciences and Veterinary Medicine, Univ.of Camerino, Camerino, Italy
[2]Dept Physical Biochemistry, Max Planck Inst. for Biophysical Chemistry, Goettingen, Germany
[3]IBMC, Univ. of Strasbourg, CNRS, Strasbourg, France

CspA is a single-stranded RNA and DNA binding protein that is highly expressed during cold-shock in *Escherichia coli*. The *cspA* transcript is able to sense temperature downshifts, adopting alternative structures at 37°C and at the cold-shock temperature. The "cold-structure" is efficiently translated at low temperature while the "37 °C structure" is less active in translation because of the sequestration of SD and AUG codon by the mRNA structure.
The aim of our work is to characterize the interaction between CspA and its own mRNA and determine whether this interaction affects the alternative structures of *cspA* mRNA and/or the efficiency of their translation.
By crosslinking and high-resolution footprinting experiments we have identified four main binding sites localized along the whole *cspA* mRNA, which are flanked by weaker binding sites observed only at higher CspA concentration. Each of the main binding sites comprises one or two AAYNRG motifs, generally exposed in apical loops. Notably, one of these motifs is located immediately downstream the AUG triplet. Our experiments indeed demonstrate that at low temperature CspA promotes the *in vitro* translation of "the 37°C structure" of *cspA* mRNA but not translation of the "cold structure"; however, CspA does not stimulate the formation of the translation initiation complex with either conformations of the transcript. Therefore, we hypothesize that CspA binding might improve the translation elongation rate by weakening some local secondary structures present on its own mRNA, stabilized by the low temperature.

Autocrine role of extracellular Hsp70 in mesoangioblast migration capability

R. Tinnirello[1], P. Assenzo[1], A. M. Di Giovanna[1], I. Grillo[1], G. Sconzo[1], F. Geraci[1,2]
[1]Dept STEBICEF, Univ. of Palermo, Italy
[2]Euro-Mediterranean Institute of Science and Technology

Mouse mesoangioblast are vessel derived stem cells which are able to release membrane vesicles containing the two gelatinases MMP2/9. For the first time, we have demonstrated that these vesicles, and not exosomes, were also used to release in the extracellular environment Hsp70. Within vesicles this protein is localized in lipid raft as a transmembrane protein. To study its possible role on MMP2/9 regulation we compared mesoangioblast A6 cell clone with two other cell clones: NM3, partially knocked down for Hsp70 and a cell clone overexpressing it (T). To study whether or not Hsp70 is involved on cell migration we carried out both wound healing and transwell assays. We found that T cells showed a higher migrating capability than A6 and NM3. The role of Hsp70 on cell migration was confirmed by using in transwell assays methylene blue, a Hsp70 inhibitor. Western blot analysis on membrane vesicles isolated from the three cell lines showed that the extracellular Hsp70 levels depend on the intracellular amount of the protein. This result suggested us that MMP2/9 regulation could be related to the extracellular Hsp70. To confirm its involvement we also used specific inhibitors for three different Hsp70 receptors (i.e. CD91, TLR2/4). All these results demonstrated that mesoangioblasts have an intrinsic migration capability and that the extracellular Hsp70 plays a positive role on it. As mesoangioblasts release both gelatinases within membrane vesicles, we inhibited specifically MMP2 and MMP9 and transwell assays demonstrated that MMP2 has the greatest role in cell migration.

The non-chaperoning function of secreted Heat Shock Protein-90a defines motility and invasiveness of breast cancer cells

W. Li
Dept of Dermatology and the Norris Comprehensive Cancer Center, Univ. of Southern California Keck School of Medicine, Los Angeles, CA, USA

Heat shock protein-90 (Hsp90) is a molecular chaperone that maintains the stability and activity of numerous signaling proteins in normal and cancer cells. Higher levels of Hsp90 in cancer cells correlated with poor overall survival for cancer patients. Recent studies have shown that many cancer cells also constitutively secrete Hsp90 that in turn promotes invasion and metastasis. The important question is whether the inside or outside Hsp90 (including Hsp90a and Hsp90b) plays a direct role in supporting cancer progression. Here we show that Hsp90a gene knockout (by CRISPR/Cas9) had little effect on survival and growth of the breast cancer cells, MDA-MB-231, whereas Hsp90b gene knockout caused cell death. However, the Hsp90a-knockout cells have completely lost motility and invasiveness *in vitro*. Extracellular addition of recombinant Hsp90a fully rescued both motility and invasion of Hsp90a$^{-/-}$ cells. In contrast, neither over-expression of Hsp90b gene inside nor supplementing recombinant Hsp90b outside the cells was unable to rescue the defects in Hsp90a$^{-/-}$ cells. Finally, two newly raised monoclonal antibodies targeting two distinct 9-amino acid epitopes within the F-5 region of Hsp90a completely blocked the parental MDA-MB-231 cell migration and invasion. Since normal cells do not secrete Hsp90 under physiological conditions, the findings of this study suggest that inhibitors that target secreted Hsp90a will selectively block migration and invasion of cancer, but not normal, cells.

Extracellular HSP27 attenuates atherogenesis by reducing plaque apoptosis and inflammation: Insights into a novel signaling pathway for atheroprotection

E.R. O'Brien
Libin Cardiovascular Institute, Dept of Cardiac Sciences, Univ. of Calgary, Canada

Previously we showed that Heat Shock Protein 27 (HSP27) attenuates the acute (early) inflammatory phase of atherogenesis – either via short term experiments involving over-expression of the protein (HSP27$^{o/e}$ mice), transfer of bone marrow from HSP27$^{o/e}$ mice to atherosclerosis-prone apoE$^{-/-}$ mice or treatment with recombinant HSP27 (rHSP27). Moreover, we demonstrated that in patients, low serum HSP27 levels are predictive of adverse cardiovascular events. We now to explore the mechanisms by which HSP27 modulates more chronic inflammatory stages of atherogenesis. Using a model of advanced atherosclerosis in both male and female atherosclerosis-prone apoE$^{-/-}$ mice maintained on a high fat diet for 12 weeks, serum HSP27 levels rose more than 15-fold in apoE$^{-/-}$HSP27$^{o/e}$ mice. Relative to apoE-/- mice, female apoE-/-HSP27$^{o/e}$ mice showed reductions in aortic lesion area of 35% for *en face* and 30% for cross-sectional sinus tissue sections – with the same parameters reduced by 21% and 24% in male cohorts; respectively. Aortic plaques from apoE$^{-/-}$HSP27$^{o/e}$ mice showed almost 50% reductions in the area occupied by cholesterol clefts and free cholesterol, with fewer macrophages and reduced apoptosis but greater intimal smooth muscle cell and collagen content. In separate *in vitro* studies we note that HSP27 activates NF-kappa B resulting in an altered gene expression profile in macrophages, including increased GM-CSF expression. Moreover, we show *in vivo* that GM-CSF is required for rHSP27 atheroprotection, and speculate that it may impact on the survival and/or trafficking of mononuclear cells in the plaque.

Silencing of the tumor suppressor BRCA2 decreases anoikis and its heterologous expression exacerbates acetic acid-induced programmed cell death in yeast cells

N. Guaragnella[1], E. Marra[1], A. Galli[2], L. Moro[1], S. Giannattasio[1]
[1]Institute of Biomembranes and Bioenergetics, National Research Council (CNR), Bari-Italy
[2]Institute of Clinical Physiology, National Research Council (CNR), Pisa-Italy

Adhesion of normal epithelial cells to the extracellular matix is essential for survival and its loss induces a specific form of programmed cell death (PCD) known as anoikis. This process is not only important for maintaining tissue homeostasis but also plays an essential role in preventing dissemination and growth of misplaced cells at inappropriate locations, most notably during the metastatic process. The tumor suppressor BRCA2, whose mutations confer predisposition to different cancer types, has been implicated in the regulation of DNA repair, transcription, cell proliferation and apoptosis. So far, its potential role in the regulation of anoikis has not been addressed.
In this study we demonstrate that silencing of BRCA2 by shRNA promoted resistance to anoikis in prostate, breast and thyroid normal epithelial cells, which was accompanied by reduced caspase 3/7 levels and activity. In parallel, heterologous expression of human BRCA2 was assessed in a yeast model, where we found that *per se* it does not induce cell death, but can stimulate acetic acid-induced PCD (AA-PCD) by decreasing cell survival and increasing DNA fragmentation and phosphatidylserine externalization, which are typical apoptotic markers. A higher increase in ROS levels has been measured in BRCA2 expressing cells undergoing AA-PCD compared with non-expressing cells. Accordingly, BRCA2 knockdown anoikis-resistant human cells show a delay in the initial burst of ROS levels. Treatment with antioxidants (N-acetylcysteine or ascorbic acid) reduced sensitivity to anoikis and AA-PCD in BRCA2-expressing human and yeast cells, respectively.
Overall these results show a new function of BRCA2 as modulator of anoikis sensitivity through an evolutionarily-conserved mechanism involving regulation of ROS production and/or detoxification by BRCA2 during PCD processes.

Metabolic stress controls alternative splicing programs: relevance to cancer biology

M. Catillo, D. Pignataro, C. Ghigna, G. Biamonti
Institute of Molecular Genetics, Italian National Research Council, Pavia, Italy

Alternative splicing (AS) is a widespread mechanism of gene expression regulation that affects over 95% of human genes. AS programs are controlled by interactions of splicing regulators proteins (of the SR and hnRNP family) with silencer and enhancer elements in the primary transcripts. We have recently investigated AS in an in vitro model of EMT (epithelial to mesenchymal transition) based on cell density: at low-density (LD) adenocarcinoma SW480 cells have a typical mesenchymal phenotype while at high-density (HD) they acquire epithelial features. We have shown that splicing factor SRSF1, has a crucial role in the choice between EMT and its reversal MET. Intriguingly, SRSF1 expression is controlled at the post-transcriptional level through three AS events in the 3'UTR region, which promote transcript degradation via the NMD pathway. The identification of the signals and regulatory pathways that control AS of SRSF1 transcripts can shed new light on the biology of cancer cells.
We have found that cells grown at high-density are exposed to conditions mimicking those found in the tumor microenvironment, such as glucose and glutamine deprivation, low pH (medium acidification), which lead to the activation of the hypoxic pathway and other signaling cascades involved in the capacity of the cells to cope with stressing conditions. Interestingly metabolic stress appear to have a strong impact on AS profile of SRSF1 transcripts.

GRP78 and T-type Ca^{2+} channels: novel EGCG targets against malignant mesothelioma

E. Ranzato, S. Martinotti, B. Burlando
Dip. Scienze e Innovazione Tecnologica, Piemonte Orientale Univ., Alessandria, Italy

Malignant mesothelioma (MMe) is characterized by long latency period (20–30 years), poor prognosis, and limited effective therapies. Chemotherapy generally improves the quality of life and induces symptomatic relief, but the tumor is characterized by strong chemoresistance. Novel approaches, including gene therapy, vaccines and molecular target therapies are under evaluation; while the need of novel ideas in the therapeutic context of this serious disease is dramatically urgent.
Epigallocathechin-3-gallate (EGCG) is a flavan-3-ol polyphenol produced by green tea, which is known to exert antitumor activity in many types of cancer cells
We have elucidated that EGCG is selectively cytotoxic to MMe cells with respect to normal mesothelial cells. EGCG elicited H_2O_2 release in cell cultures, and exogenous catalase abrogated EGCG-induced cytotoxicity, apoptosis and necrosis. We have then demonstrated that sensitivity of MMe cells to EGCG is correlated with higher expression of $Ca_{v3.2}$ T-type Ca^{2+} channels, a new target of EGCG.
This latter involves the induction of T channel opening by H_2O_2, followed by $[Ca^{2+}]_i$ homeostasis impairment, induction of intracellular ROS, and eventually cell apoptosis or necrosis, depending on the intensity of the stimulus. These findings suggest the possible use of EGCG for MMe, and indicate T-type Ca^{2+} channels as a novel therapeutic target.
In addition, we have found that GRP78, the ER chaperone and signaling regulator, is up-regulated at the mRNA and protein levels in MMe cell lines, with respect to mesothelial cells. In addition, we have demonstrated that EGCG induces the increase of GRP78 expression in MMe cells. GRP78 can serve as both a downstream target and an upstream regulator of PI3K/AKT signaling. Hence, interfering GRP78 expression in tumors has the potential of being an accessible and useful method for assessing and planning treatment strategies. This would make GRP78 a preferential molecular target for MMe therapies.

Post-translocational adaptation (PTA) to cellular stress

V. Tosato, N. West, J. Sims, C.V. Bruschi
Yeast Molecular Genetics Laboratory, International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Chromosomal translocations represent a dramatic rearrangement of the genome and may provoke systemic gene expression alterations [1]. We developed a system named BIT (Bridge-Induced Translocations) that allows to link together two different chromosomes of *S. cerevisiae* exploiting the *RAD52*-homologous recombination system of the yeast cells [2, 3]. We found that the main consequence of BIT was an increase of the genetic expression within 100kb either side from the breakpoint. After BIT translocation, cells undergo an adaptation phase during which they acquire different gene expression patterns and complex genomic re-arrangements. Indeed, the same translocation event between two chromosomal loci can lead to translocated cells completely different by genotypic and phenotypic point of view [4, 5].
We decided therefore to select for a particular cellular phenotype after a translocation event. As hypothesis, we postulated that, since a BIT translocation might scramble a substantial part of gene expression, the following post-translational adaptation, if allowed to occur at high temperature, could result in a temperature resistance multi-genic phenocopy. With simple experiments we demonstrated that BIT-derived translocants, allowed to restore basic levels of transcription while being exposed to the selective condition, were able to grow at non-permissive temperatures and that, after chromosomal translocation, PTA produces a phenotypic variability upon which a particular environmental condition can successfully exert a selective pressure.

Bibliography:
[1] *Nikitin D, Tosato V, Zavec AB, and Bruschi CV (2008) PNAS, vol. 105, p. 9703-08*
[2] *Tosato V, Waghmare S, Bruschi CV (2005) Chromosoma vol. 114, p. 15-27*
[3] *Tosato V, Sidari S, Bruschi CV (2013) PLOS ONE 8(4):e60926*
[4] *Rossi B, Noel P, Bruschi CV (2010) GENETICS. 2010 186 p. 775-90*
[5] *Nikitin D, Bruschi CV, Sims J, Breitenbach M, Rinnerthaler M, Tosato V (2014) Eur J Cell Biol, accepted, doi: 10.1016/j.ejcb.2014.03.003.*

Mitochondria and lifespan extension in *Saccharomyces cerevisiae:* the longevity mutations *sch9Δ* and *rei1Δ* contribute to mitochondrial DNA stability

J. Quartararo, P. Goffrini, I. Ferrero
Dept of Life Sciences, Univ. of Parma, Parma, Italy

Mutations that reduce the activity of the nutritional signaling pathways TOR/SCH9 and RAS/PKA induce an increase in chronological lifespan (CLS) extension in different model organisms, redirecting cells towards a respiratory metabolism and promoting stress resistance.
To assess the relationship between mitochondrial function and ageing we investigated whether the increased CLS of well-known longevity mutants correlated with an increase in mitochondrial DNA stability. Among the longevity mutations analyzed only two, *sch9Δ* and *rei1Δ*, accumulate deletions on mtDNA at lower rate than the parental strain, suggesting that deletions on mtDNA may have a primary role in ageing and that maintenance of mtDNA is only one of the actors involved in the regulation of the ageing process.
Furthermore s*ch9Δ* and *rei1Δ* longevity mutants show high rate of respiratory activity accompanied by no significant difference in mtDNA amount. Different mechanisms through which the two longevity mutants promote lifespan extension and mtDNA stability have been identified. Deletion of *SCH9* leads to an increase of ROS production early during growth to promote an adaptive signal that stimulates lifespan extension and reduces oxidative damage in stationary cells, activating a stress response program mediated by Sod2p overexpression. Otherwise the reduced rearrangements on mtDNA and the increased respiratory activity in *rei1Δ* longevity mutant appear to rely on a direct stabilization of mitochondrial DNA through overexpression of nucleoid components.

Chronic DNA damage hampers cell morphology and motility

P. Cremaschi, M. Oliverio, R. Carriero, D. Sproviero, P. Tomaiuolo, S. Bione, G. Biamonti, A. Montecucco
Istituto di Genetica Molecolare, CNR, Pavia, Italy

Human cells experience low levels of DNA damage resulting from metabolic activities or sub-lethal doses of exogenous insults, which may eventually lead to cancer onset. We have exploited 46BR.1G1 cells as a model system to investigate the strategies used by the cells to cope with low levels of chronic DNA damage arising from replication stress. 46BR.1G1 cells are mutated in *LIGI* gene encoding replicative DNA ligase I, and show impaired maturation of newly synthesized DNA. This elicits a moderate activation of the ataxia telangiectasia mutated (ATM)-dependent checkpoint, including constitutive phosphorylation of H2AX and Chk2, without arresting cell cycle or activating apoptosis. DNA damage-initiated ATM signaling in 46BR-1G1 cells promotes changes in cell morphology, adhesion and migration suggesting that cell morphology and motility could be under the influence of DNA damage response programs. We are trying to trace the pathways involved comparing the transcriptome of 46BR.1G1 and wild type 7A3 cell lines.

GSK3β inhibition in osteoarthritic human primary chondrocytes determines oxidative stress and DNA damage response leading to a worse disease phenotype

M. Minguzzi[1,2], S. Guidotti[1,2], D. Platano[1,2], E. Olivotto[1], A. Facchini[3], L. Cattini[1], F. Flamigni[3], G. Trisolino[4], A. Facchini [1,2], R.M. Borzì[1]

[1]Laboratorio di Immunoreumatologia e Rigenerazione Tissutale/RAMSES, Dipartimento RIT, Istituti Ortopedici Rizzoli, Bologna, Italy

[2]Dipartimento di Scienze Mediche e Chirurgiche (DIMEC), Univ. di Bologna, Bologna, Italy

[3]Dipartimento di Scienze Biomediche e Neuromotorie (DIBINEM), Univ. di Bologna, Bologna, Italy

[4]Chirurgia Ricostruttiva Articolare dell'anca e del ginocchio, Istituti Ortopedici Rizzoli, Bologna, Italy

Osteoarthritis (OA) is a degenerative disease of the cartilage, characterized by altered phenotype of articular chondrocytes that loose their resting status to progress toward terminal differentiation. Cell stress is candidate as responsible of differentiative changes underlying OA, indeed increased apoptosis and senescence might be caused by elevated oxidative stress. Changes in OA are driven by deregulated differentiative pathways among which GSK3β and its regulatory activity on β catenin have been involved in chondrocyte differentiation in rodents. However, GSK3β inhibition probably impairs other cellular signals that determine OA. In fibroblasts, GSK3β inhibition exerts a direct blockage of respiration leading to reactive oxygen species production (ROS). Thus, we aimed to characterize the effects of GSK3β inhibition in human OA chondrocytes. Firstly, we confirmed GSK3β inhibition in human OA cartilage samples with an increased extent in the deep zones, associated with differentiation. Then, *in vitro* pharmacological inhibition of GSK3β in OA primary chondrocytes showed a negative effect on cell proliferation, with reduction in cell cycle progression and increased S phase. Thus, we investigated the involvement of intra-S DNA damage checkpoint and we found increased expression of γH2AX, GADD45β and p21 after GSK3β inhibition. GSK3β inhibition indeed determined ROS production as cause of DNA damage, as confirmed by 8-oxo-dG adducts formation, and leads to senescence of monolayer cultured chondrocytes. At the same time differentiating 3D human chondrocyte cultures showed cell death after GSK3β inhibition, possibly triggered by the increased terminal differentiation observed. Senescence was also assessed in 3D cultures suggesting that GSK3β inhibition and consequent ROS dependent DNA damage could determine different outcomes depending on context stimuli. Exposing OA chondrocytes to oxidative damage, GSK3β inhibition leads to a worse OA phenotype.

Epigenetics at work: phenotypic switching by Heat Shock Proteins and prions in yeast

V. Cappelletti[1], C. De Filippo[1], I. Stefanini[1], L. Berná[2], M. Ramazzotti[3], A. Cestaro[1], C. Romualdi[4], C. De Filippo[1], M. Kapushesky[5], I. Gut[6], A. Csikász-Nagy[1,7], D. Cavalieri[1]
[1]Dept of Computational Biology, Research and Innovation Centre, Fondazione Edmund Mach, Trento, Italy
[2]Unidad de Biología Molecular, Institut Pasteur Montevideo, Montevideo, Uruguay
[3]Dept of Experimental and Clinical Biomedical Sciences, Univ. of Florence, Florence, Italy
[4]Dept of Biology, Univ. of Padua, Italy
[5]EMBL Outstation-Hinxton, European Bioinformatics Institute, Cambridge, United Kingdom
[6]Centro Nacional de Análisis Genómico, Barcelona, Spain
[7]King's College London, United Kingdom

Colony formation is a fascinating trait described in unicellular organisms as a possible step towards multicellularity, one of the major *transitions* in the history of *life*. *Saccharomyces cerevisiae* has been shown to be a powerful model to deepen into this evolutionary process thanks to its ability to grow in multicellular structures in response to environmental changes. We applied a multi-level approach, ranging from genomics and transcriptomics to molecular microbiology in order to investigate yeast colony morphogenesis. We focused on a specific type of colony structure, called filigreed morphology, observed at low frequency in natural *S. cerevisiae* strains and recently associated to prion response. This phenotype was found in heterozygosity in the M28 natural *S. cerevisiae* strain. M28 sporal derivatives, showing a 2:2 Mendelian segregation of multicellular phenotype, represent a unique model to understand the inheritance of the nutritional response in the colony morphology context. Remarkably, we observed the ability of M28 meiotic segregants to switch from filigreed to smooth phenotype and *vice-versa*, with a reversion rate that is perturbed by prion removing agents, suggests an epigenetic control of this mechanism. In order to identify a gene expression profile associated to this phenotypic transition we performed a time course microarray transcriptional analysis. We observed an involvement of heat shock proteins, particularly from the Hsp70 family. Furthermore Illumina whole genome comparative analysis on 12 M28 sporal derivatives provide further insight in how the genotype is translated into the phenotype. These results draw our attention to the possibility of a prion based epigenetic switching mechanism that enables cells to respond to environmental changes and pass the phenotype to later generations. We are currently testing this hypothesis.

Post-translation modification crosstalk regulates Hsp90 chaperone cycle

M.R. Woodford[1,2,3], G. Bratslavsky[1,3], C. Prodromou[4], L. Neckers[5], M. Mollapour[1,2,3]
[1]Dept of Urology, [2] Dept of Biochemistry and Molecular Biology, [3]Cancer Research Institute, SUNY Upstate Medical University, Syracuse, NY, USA
[4]Genome Damage and Stability Centre, Univ. of Sussex, Brighton, UK
[5]Urologic Oncology Branch, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

Heat shock protein 90 (Hsp90) is an evolutionarily conserved molecular chaperone prominently involved in maintaining cellular homeostasis via the stabilization of a number of target proteins, termed "clients". Cancer cells are heavily reliant on Hsp90 due to the myriad stresses these cells encounter such as hypoxia, nutrient deprivation, and proteotoxicity. Because of this hyper-regulatory role, Hsp90 inhibition has proven to be an attractive target in cancer therapy.
The Hsp90 chaperone cycle is coupled to its ATPase activity and is tightly regulated by co-chaperones and post-translational modifications. Some of these modifications of Hsp90, such as phosphorylation, acetylation and *S*-nitrosylation, at specific amino acid residues are known to affect the rate of ATPase activity, binding of clients, and sensitivity to inhibitors. However the impact of SUMOylation on the Hsp90 chaperone cycle has only recently been elucidated.
We have shown that asymmetric SUMOylation of a single lysine residue K191 in human Hsp90 (yeast K178) helps recruit Aha1 to the co-chaperone complex and interestingly, improves binding of Hsp90 inhibitors. Importantly, steady-state N-domain SUMOylation of Hsp90 is increased in transformed cells, perhaps explaining the observed hypersensitivity of these cells to Hsp90 inhibitors. As lysine is also subject to acetylation and ubiquitylation, we are interested in teasing out the factors that determine the modification state of the critical K191 residue, as well as the crosstalk between these modifications in the Hsp90 homodimer and the effect on Hsp90 chaperone function.

Accumulation of motor neuron diseases associated misfolded proteins is counteracted by dynein inhibition

R. Cristofani[1], E. Giorgetti[1,2], V. Crippa[1], A. Boncoraglio[1,3], P. Rusmini[1], M.E. Cicardi,[1] A. Poletti[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari (DISFEB) e Centro di Eccellenza sulle Malattie Neurodegenerative (CEND), Univ. degli Studi di Milano, Milano, Italy
[2]Dept of Pathology, Univ. of Michigan, Ann Arbor, Michigan, USA
[3]Univ. Medical Center Groningen, Univ. of Groningen, Dept of Cell Biology, Groningen, The Netherlands

Spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are two motor neuron diseases (MNDs) characterized by selective death of motor neuron localized in cerebral cortex and in spinal cord. The hallmark of SBMA and ALS is the presence of aggregates of mutant proteins (androgen receptors (AR) and superoxide dismutase 1(SOD1), TDP43, etc. respectively) with aberrant conformation (misfolding). Altered protein quality control system cannot correctly remove toxic aggregates of misfolding proteins. These species may lead to cell death. Dynein motor complex seems play a crucial role to maintain an efficient removal of these toxic species by autophagy: i) through aggregate transport near MTOC, ii) by autophagosome nucleation, iii) by assisting the fusion of autophagosome to lysosome.
Treatment of NSC34 cell line with a selective inhibitor of dynein (EHNA), drastically reduce the autophagy marker LC3 when autophagy is induced by trehalose. In NSC34 transfected with mutant proteins: i) immunofluorescence (IF) analysis showed that dynein is sequestered into mutant AR aggregates. ii) EHNA treatment, unexpectedly, reduces aggregates of mutant AR, SOD1 and TDP43 retained on cellulose acetate membrane in filter retardation assay (FTA) also in presence of autophagy inhibitor (3-MA), but not in presence of proteasome inhibitor (MG132). iii) EHNA increases the solubility of mutated AR. iv) BAG1, which routes misfolding proteins to proteasome degradation or chaperone-mediated-autophagy, is increases after EHNA treatment. These data suggest that dynein impairment may induces proteasome targeting of misfolded proteins involve in MNDs.

GRANTS: Telethon; Fondazione AriSLA; AFM, France; Regione Lombardia; Università degli Studi di Milano; Ministero della Salute.

Posters

(presenting authors are shown underlined)

P1

Apoptosis rate of cumulus cells can be considered as an indicator for the selection of embryos to improve ongoing pregnancy and implantation rates

L. Bosco[1,3], M.C. Roccheri[1], E. Cittadini[2], M. Agnello[1], G. Ruvolo[2]
[1]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, Univ. degli Studi di Palermo, Palermo
[2]Centro di Biologia della Riproduzione, Palermo
[3]Istituto Euro-Mediterraneo di Scienza e Tecnologia (I.E.ME.S.T.), Palermo, Italy

Cumulus cells apoptosis rate an adjunct to morphology evaluation for the embryo selection on day 3 could be considered a new tool, compared with embryo selection by morphology alone, to select the embryos with higher implantation potential to increase the clinical outcomes after ICSI. Several studies have demonstrated a lower cumulus cell apoptotic rates in women who achieved pregnancy compared with women who did not become pregnant after ICSI.
A prospective randomized observational study on 76 ICSI patients was performed before Ovum Pick-Up. Patients were randomized into either the control group (embryo selection by morphology only, **A group**: 48 patients) or the treatment group (morphology plus cumulus cell apoptosis evaluation, **B group**: 28 patients). On Day 3, embryo transfer of a maximum of 3 embryo of grade A was performed.
Patient demographics and baseline characteristics were distributed equally over the two groups. No statistical differences were found between the group A vs group B in terms of FSH units for ovarian stimulation (1833 ± 754 vs 1927 ± 826), E_2 at hCG administration (1872 ± 788 vs 1787 ± 796), the numbers of oocytes collected (6.4 ± 2.1 Vs 6.7 ±3.7), the number of transferred embryos (A group: 126; B group: 69), the grade A transferred embryos (126 vs 69). No differences was found in the cumulative DNA fragmentation rate in the cumulus cells (16.39 ±12.9 vs 15.7 ± 11.3). Significative differences were found in ongoing pregnancy rate (33.3 vs 57.1) and implantation rate (23.1 vs 12.6).
Embryos selection according to cumulus cells apoptosis rate could help to identify competent embryos with higher implantation potential, suggesting a new diagnostic tool in IVF laboratories to increase the clinical outcomes reducing the number of embryos to transfer.

P2

CuNV1110 induces dissociation of the Hsp60-pro-caspase 3 complex and activation of apoptosis in tumor cells

C. Caruso Bavisotto[1,2], A.M. Gammazza[1,2], D. Nikolic[1], A. Palumbo Piccionello[2,3], G. Barone[2,3], A. Pace[2,3], F. Cappello[1,2], C. Campanella[1,2]
[1]Dept of Experimental Biomedicine and Clinical Neurosciences, Section of Human Anatomy "Emerico Luna", Univ. of Palermo, Palermo, Italy
[2]Euro-Mediterranean Institute of Science and Technology, Palermo, Italy
[3]Dept of STEBICEF, Section of Chemistry, Univ. of Palermo

Background Heat shock proteins (Hsps) are a group of molecules that assist protein folding, re-folding and degradation. Hsps are also responsible of apoptosis regulation, via direct or indirect pathways. Hsp60 is a mitochondrial protein that, in cancer cells, have either pro- and anti-apoptotic effects, depending on it accumulates in the cytosol with, or without, mitochondrial release, respectively. This effect is due to the induction, or not, of pro-caspase-3 cleavage.
As a consequence, Hsp60 is considered an important player in the delicate equilibrium that determinates the fate of a tumor cell. Foreseeing the fate of tumor cells is a main concern in the development of novel anticancer drugs. Recently, the biological activity of CuNV1110, a copper complex, was studied on cancer cells and it was demonstrated that it reduces the cell viability, in a dose and time dependent manner, and induces cell apoptosis.
Aims In this study we evaluated the possible mechanisms by which CuNV1110 induces cell apoptosis. In particular we looked at its effects on Hsp60 levels and caspase 3 activation. We used an in vitro model of a pulmonary mucoepidermoid carcinoma (NCI-H292 cells).
Results We firstly verified that also in our cells CuNV1110 reduces the cell viability, in a dose and time dependent manner, and induces cell apoptosis. Then, we performed western blotting analyses showing that Hsp60 levels decrease with the increasing concentrations of CuNV1110; by contrast, caspase 3 levels increased. Interestingly, we found by immunoprecipitation a complex between Hsp60 and pro-caspase 3 in untreated cells that dissociate with increasing doses of CuNV1110.
Discussion We show that CuNV1110 can dissociate the anti-apoptotic complex between Hsp60 and pro-caspase 3 in NCI-H292, in turn inducing apoptosis of tumor cells. If confirmed in other cell lines, CuNV1110 could be tested in vivo in some tumors in which has already been demonstrated Hsp60 accumulation in cytosol.

P3

Possible protective effects of hydroxytirosol and its derivates on UVB-induced keratynocyte apoptosis

F.M. Giordano[1], S. Salucci[1], S. Burattini[1], D. Curzi[1], F. Buontempo[2], A.M. Martelli[2], E. Falcieri[1,3], M. Battistelli[1]
[1]DiSTeVA, Univ. of Urbino Carlo Bo, Urbino, Italy
[2]Dept of Biomedical and Neuromotor Sciences, Univ. of Bologna, Bologna, Italy
[3]IGM, CNR, Rizzoli Orthopedic Institute, Bologna, Italy

Skin cells can respond to UVB-induced damage either by tolerating it, or restoring it through antioxidant activation and DNA repair mechanisms or, ultimately, undergoing programmed cell death, when damage is massive. Food antioxidants have potential role in new preventive, protective, and therapeutic strategies for chronic degenerative diseases, including skin inflammation and cancer. Hydroxytyrosol (HyT), a polyphenol present in virgin olive oil, shows a variety of pharmacological and clinical benefits such as anti-oxidant, anti-cancer, anti-inflammatory, and neuro-protective activities. These properties have been also reported for its synthetic derivatives. In a previous study we have demonstrated the anti-apoptotic effect of HyT and its esthers against pro-oxidant chemical agent in different cell models.
The possible protective effects of HyT and its derivatives against UV-induced apoptosis were investigated in HaCat cell line. Human keratinocyte were pre-treated with antioxidants before UVB exposure and their effects evaluated by means of ultrastructural and molecular analyses. After UVB radiation, a known cell death trigger, nuclear chromatin condensation, cytoplasm shrinkage and vacuolization can be observed at TEM. Moreover, while in control cells and in the case of HyT administration alone no labelled nuclei can be detected after TUNEL reaction, they diffusely appeared after UVB radiation exposure. An evident numerical decrease of ultrastructural apoptotic patterns and of TUNEL positive nuclei can be observed when antioxidants were supplied before cell death induction. These data have been confirmed by molecular analyses. In fact, after UVB radiation, both intrinsic and extrinsic pathways appear activated as well as PARP, a protein involved in the correlation between nuclear damage and apoptotic changes. Antioxidant compound administration before UVB-induced cell death shows an evident caspase involvement decrease, according to morphological analyses. In conclusion, our preliminary results demonstrate that these natural antioxidant compounds are able to prevent apoptotic cell death in human keratinocytes exposed to UVB, suggesting, for these molecules, a potential role in preventing skin damage and cancer.

P4

The mitotic cell death pathway elicited by a new class of microtubule inhibitors

A. Miele[1], E. Di Cesare[1], A. Verrico[1], M. Giubettini[1], G. La Regina[2], A. Coluccia[2], E. Cundari[1], R. Silvestri[2], P. Lavia[1]

[1]Institute of Molecular Biology and Pathology, CNR National Research Council, c/o Sapienza Univ. di Roma, Roma, Italy

[2]Istituto Pasteur – Fondazione Cenci Bolognetti, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Univ. di Roma, Roma, Italy

Higher eukaryotes have developed specific pathways of mitotic cell death as a strategy for eliminating mitosis-incompetent cells. This type of death is different from classical apoptosis and is is driven by poorly understood signals. Anti-mitotic drugs are commonly used to treat a variety of cancers because they prevent the organization or function of the mitotic spindle, hence blocking cell division and subsequently inducing cell death. However, the onset of resistance is frequent: in that case cancer cells do not activate the cell death machinery, but they survive erroneous mitotic divisions, acquiring a higher degree of genetic instability and ultimately generating aneuploid cells with chromosome gain or loss. This increases the genetic load of the surviving tumor cells and hence their degree of malignancy. It is therefore of primary importance to understand the molecular conditions that favor of disfavor mitotic cell death. Here we have used novel anti-tubulin compounds (arythioindoles, ATIs) as an experimental tool to induce mitotic damage and dissect the molecular features of mitotic cell death, then have imaged the response of individual cancer cells by time-lapse videorecording. We find that mitotic cell death depends on the extent and severity of tubulin inhibition, requires neither p53 nor spindle checkpoint function, but does require sustained arrest in mitosis and caspase-3 activity. These findings contribute to define the molecular requirement for mitotic cell death to occur and underscore the importance of single cell analysis to depict cell-to-cell variations that would be lost in conventional analyses of cell death.

P5

HSC70 as biomarker to monitor quality and shelf life of crustacean species of commercial importance

A. Pinsino, R. Bonaventura, C. Costa, R. Russo, F. Zito, V. Matranga
Institute of Biomedicine and Molecular Immunology "A. Monroy", CNR, Palermo, Italy

In the framework of the PO-FESR DeCroMed Project, we used a multi-parametric approach to study the quality of the shrimp *Parapeneus longirostris*, and the lobster *Nephrops norvegicus,* during post-thawing periods. Currently, the knowledge of the changes taking place during catching, handling, processing and storage of crustacean species of commercial importance caught in the Mediterranean Sea remains scant. The aim of this study was to obtain detailed information on the crustacean *post mortem* changes occurring after thawing procedures to monitor crustaceans freshness and shelf life over time. We measured the water-holding capacity of *P. longirostris* and *N. norvegicus* muscles and monitored changes in pH, protein content and composition of muscle exudates obtained from each specimen by centrifugation. In addition, we evaluated the levels of a few candidate marker proteins chosen from a list of biological markers validated to predict the quality of food products. We found that HSC70 protein levels are negatively related to: i) the increase of the water-holding capacity, ii) the formation of protein aggregates and protein degradation, iii) the pH of muscle exudates, and iv) the flesh softening of the crustacean muscle. In conclusion, we endorse the HSC70 protein as biomarker of crustacean freshness.

Work fully supported by PO-FESR 2007-2013 –SICILIA- DeCroMed "Pescato Mediterraneo: certificazione di qualità e valorizzazione dei crostacei decongelati" Linea di Intervento 4.1.1.1, GURS 28/10/2011, year 65, N° 45, CUP: G93F12000190004. Authors acknowledge the technical assistance of Mr. Mauro Biondo.

P6

Metallothionein genes in the sea urchin *Paracentrotus lividus*

M.A. Ragusa, V. Pezzino, S. Costa, F. Gianguzza
Dept STEBICEF, Univ. di Palermo, Italy

Metallothioneins (MTs) constitute a heterogeneous superfamily of cysteine rich proteins, which coordinate divalent (Zn^{2+}, Cd^{2+}) or monovalent (Cu^{+}) metal ions.
Several functions have been proposed for these peptides, ranging from toxic metal protection to physiological metal homeostasis, free radical scavenging, oxidative stress protection, antiapoptotic defense, control of the redox status of the cell and also a role during development.
Regarding the MT system in vertebrates' nearest kin, little information is available at present. Recently MTs were also characterized in cephalochordates.
Hence in order to shed some light on MT origin and functional differentiation through evolution, we studied MT genes in sea urchin *P. lividus*. Here we report the characterization of five different sea urchin MT genes (PlMT4 through PlMT8) and their regulation pattern during development.
By Southern blot hybridization using MT4 through MT8 cDNA fragments as probes (Ragusa et al, 2013), we determined the number of MT genes in the genome, showing the presence of at least two different PlMT8 genes.
Using primers based on *P. lividus* (v2.0) genome sequences, we amplified by PCR, cloned and sequenced the MT genes.
PlMT gene structures are different from both vertebrates and cephalochordates. The genes are composed of 4 exons separated by 3 introns, the last intron is into the 3'UTR. In PlMT7 gene there are two predicted polyadenylation signals and in fact two species of mRNA transcripts exist.
By RT-qPCR we showed that MT4 to MT6 are not expressed during development, while MT7 mRNA level rises throughout embryonic development and MT8 rises until gastrula stage and decreases thereafter.
Analyzing MT promoters *in silico*, a considerable number of transcription factor binding elements can be identified, comprising putative metal response elements (MRE), antioxidant response elements (ARE), but their copy number and positions are different between constitutive (MT7-8) and induced (MT4/6) genes.

P7

A pharmacological approach to counteract the motoneuronal cell stress in spinal and bulbar muscular atrophy

P. Rusmini[1], E. Giorgetti[1,2], V. Crippa[1], A. Boncoraglio[1], R. Cristofani[1], M.E. Cicardi[1], A. Poletti[1]
[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Univ. degli studi di Milano, Milano
[2]Dept of Pathology, Univ. of Michigan, Ann Arbor, Michigan

Several neurodegenerative disorders, as Parkinson's disease, amyotrophic lateral sclerosis and polyglutamine (polyQ) diseases that include spinal and bulbar muscular atrophy (SBMA), are characterized by protein misfolding and aggregate formation of the mutant proteins with a concurrent increase in cell stress. SBMA is a motoneuronal disorder caused by a polyQ expansion in the androgen receptor protein (ARpolyQ). The mutant and misfolded ARpolyQ activated by its ligand testosterone triggers different events that cause cellular stress and death. Stress-induce heat shock proteins facilitate the refolding of misfolded proteins and in addition cells can also degrade the misfolded proteins through the Ubiquitin Proteasome (UPP) and the autophagic pathways. In SBMA, these pathways seem to be overwhelmed and misfolded ARpolyQ accumulated into aggregates. To counteract the ARpolyQ neurotoxicity, we have tested the combined effects of two known compounds: Casodex, an AR antagonist, and trehalose, a well-known autophagic enhancer. Casodex is able to slow down the ARpolyQ nuclear translocation and to retain it into the cytoplasm, while trehalose enhances the ARpolyQ clearance. The Casodex and trehalose co-treatment reduces both the aggregates and the soluble forms of ARpolyQ with higher efficiency than that obtained with the single treatment. Analysing the autophagic markers LC3 and p62, we observed that only trehalose ameliorates the autophagic flux while casodex had no effects on the two markers. The Casodex and trehalose co-treatment offers the opportunity to retain in the cytoplasm the activated ARpolyQ and to strengthen its autophagic clearance leading to a more efficient removal of ARpolyQ. These data may represent the basis for future trials in SBMA mice models to test the therapeutic potential of these combined administration.

GRANTS: Fondazione AriSLA; AFM Telethon France; Regione Lombardia multicentric project; UNIMI

P8

An overview on *P. lividus* sea urchin embryo genes affected by UVB radiation

R. Russo, R. Bonaventura, F. Zito, A. Pinsino, V. Matranga
Consiglio Nazionale delle Ricerche, Istituto di Biomedicina e Immunologia Molecolare "Alberto Monroy", Palermo, Italy

Ultraviolet B (UVB) radiation that reaches the Earth's surface induces stress in marine organisms, altering the physiological functioning of cellular targets such as DNA and proteins. The *Paracentrotus lividus* sea urchin is considered a valid biosensor of stress, useful for ecotoxicological studies. It has fast embryogenesis and transparent embryos that are manageable for Whole Mount *In Situ* Hybridization (WMISH) and Immuno-HystoChemistry (IHC) studies. We had previously described the morphological impairment of sea urchin embryos exposed at cleavage stage to UVB radiation at the doses of 400 and 800 J/m^2 and the modulation in the expression of *Pl*-14-3-3epsilon mRNA. We extended our studies by analyzing the mRNA levels of genes probably activated upon stress response, such as those encoding for a DNA repair helicase (*Pl*-XPB/ERCC3), a few transcription factors (*Pl*-FOXO; *Pl*-jun; *Pl*-NF-kB), an adapter protein (*Pl*-14-3-3epsilon) and a target protein (*Pl*-MT). By QPCR, we found that some genes were responsive to UVB stimuli in a dose- and time- dependent manner. By WMISH we also analyzed the spatial expression of *Pl*-jun mRNA. The latter is normally localized in the skeletogenic cells (PMCs) at gastrula and pluteus stages, whereas we found it ectopically expressed in UVB exposed embryos. The Jun phospho-protein, detected by IHC on whole mount embryos showed a nuclear localization, confirming the presence of the active form of Jun protein both in controls and UVB exposed embryos. We conclude the involvement of many signaling pathways in the UVB stress response.

Work supported by 7FP EU-ITN Biomintec Project to VM, Contract N°215507 and by CNR Flagship Project POM-FBdQ 2011/2013. Authors acknowledge M. Biondo for technical assistance.

P9

Purkinje cell degeneration in Marinesco-Sjögren syndrome: role of cell stress and alterations of proteostasis

V. Capone[1], C. Ruggiero[1], M. grossi[1], F. Ornaghi[2], R. Chiesa[2], M. Sallese[1]
[1]Unit of Genomic Approaches to Membrane Traffic, Fondazione Mario Negri Sud, Santa Maria Imbaro (Chieti), Italy
[2]Dept of Neuroscience, Mario Negri Institute for Pharmacological Research, Milan, Italy

The Marinesco-Sjögren syndrome (MSS) is a rare, early-onset, autosomal recessive genetic disease caused by mutations in the SIL1 gene. One of the main symptoms of MSS is ataxia due to degeneration of Purkinje cells (PC). To date there is no treatment for MSS, and the medical care of the patients is essentially symptomatic. SIL1 encodes an ADP exchange factor for GRP78, the master operator of endoplasmic reticulum (ER) functions. Eukaryotic cells express a second ADP exchange factor, ORP150, potentially able to compensate for the loss of SIL1 function. However, ORP150 overexpression seen in mouse models of MSS (woozy), apparently is not sufficient to prevent PC apoptosis, suggesting that induction of ORP150 could be sufficient to rescue SIL1 loss only in some tissues.
As a model to study the MSS we used HeLa and SH-SY5Y cells. These cell lines express comparable levels of SIL1 while the basal expression of ORP150 is higher in SH-SY5Y. SIL1 knock down (KD) induces the UPR as revealed by the increased expression of several chaperons, including ORP150. Despite ORP150 induction, SIL1 KD cells show a reduced viability due to apoptosis. These data indicate that our cell models recapitulate key features of the MSS pathology. In addition, we have evidence that membrane trafficking is altered in SIL1 KD cells. Thus, SIL1 KD elicits a functional impairment that may affect the trafficking and distribution of ion channels and/or neurotransmitter receptors leading to PC dysfunction and degeneration.

P10

Hsp60 levels in the skeletal muscle are fibre-type specific and increase after endurance training

C. Sangiorgi[1,2] R. Barone[1,2], F. Macaluso[1,2],D. Coletti[3], V. Moresi[3], S. Adamo[3], F. Cappello[1,2], F. Farina[1], G. Zummo[1], V. Di Felice[1]
[1]Dept of Experimental Biomedicine and Clinical Neurosciences (BioNeC), Univ. of Palermo, Palermo, Italy
[2]Euro-Mediterranean Institute of Science and Technology (IEMEST), Palermo, Italy
[3]Dept of Anatomical, Histological, Foresic & Orthopaedic Sciences, Sapienza Univ. of Rome, Rome, Italy

The purpose of this study was to investigate the Hsp60 protein level in the different fibre types of hindlimb muscles of trained and sedentary mice.
Forty-eight young healthy male mice were divided in two groups: sedentary and trained groups. A Rota-Rod was used to train mice for simulating an endurance training. Mice ran 5 days/week for 6 weeks with progressively increasing duration and intensity of training. Sedentary mice did not perform any type of supervised exercise training.
Eight mice of each group were sacrificed, by cervical dislocation, after 15, 30 and 45 days and posterior muscles group of hindlimb (gastrocnemius, soleus and plantaris) were dissected and preserved in liquid nitrogen or embedded in paraffin. Respectively, skeletal muscle lysate were blotted against Hsp60 and paraffin section were stained against Hsp60, MHC-I, MHC-IIa/x.
Sample analyses showed that Hsp60 was differently expressed in fibre-types: mainly in "MHC-IIa" and less in "MHC-IIb" (IIa>I>IIx>IIb). Moreover, endurance training increased significantly the level of Hsp60 in slow muscle fibres (MHC-I). Hsp60 levels are fibre type-specific, probably because of the differences in the mitochondrial content between slow and fast fibres.

P11

Abnormal keratinocyte proliferation in mice with a conditional deletion of rictor in the epidermis

B. Tassone, S. Saoncella, E. Calautti
Dept Biotechnology, Univ. of Turin

mTOR (mammalian target of rapamycin) is a serine-threonine protein kinase that regulates diverse cellular processes such as growth, metabolism, proliferation, survival and differentiation. mTOR nucleates two distinct protein complexes characterized by their different sensitivity to rapamycin, namely mTORC1 and mTORC2.
Rictor is an adaptor protein essential for mTORC2 assembly. mTORC2 phosphorylates both Akt and some PKC isoforms at their Hydrophobic Motifs (HM) and Turn Motifs (TM), thereby contributing to kinase activation and stability. Because both Akt and PKC signaling regulate epidermal cell biology, and*rictor* knockouts mice die before stratified epithelia formation, we generated mice with a conditional deletion of*rictor* in the epidermis to investigate the role of rictor/mTORC2 in epidermal homeostasis and disease. At the molecular level, *rictor*-deficient keratinocytes lack Akt phosphorylation at the HM (Ser473), have reduced Akt expression and diplay impaired phosphorylation/expression levels of both FoxOs and mTORC1 signaling components. Moreover *rictor*-deficient keratinocytes display abnormalities in the actin cytoskeleton dynamics. Interestingly, *rictor*-deficient keratinocytes show features consistent with impaired differentiation, both *in vitro* and *in vivo*. Notably, we found that *rictor*-deficient mice are less prone to epidermal hyperplasia induced by acute treatment with the TPA tumour promoter, indicating that *rictor* depletion impairs proliferative responses that favour neoplastic transformation. These findings identify mTORC2 as an important hub for the integration of signals at the switch between keratinocyte proliferation and differentiation.

P12

Mesoangioblast stem cell population is non-omogeneous as revealed by transcriptome analysis after a severe oxidative stress

G. Turturici[1], R. Tinnirello[1], F. Geraci[1,2], M. L. Alotta[1], F. Contino[1], S. Feo[1], G. Sconzo[1]
[1]Dept. STEBICEF, Univ., Palermo, Italy
[2]Euro-Mediterranean Institute of Science and Technology

The effect of exogenous oxidative stress on stem cell viability is not well understood, despite being a central issue in the development of cell-based therapies. We demonstrated that A6 mouse mesangioblast stem cells (Mabs) are not an omogeneous population since we were able to isolate, after a severe oxidative stress induced by H_2O_2, a cell clone (H2) that maintains all the characteristics of stemness (proliferation and differentiation capabilities), under normal growth conditions, but survives better than parental Mabs if subjected to a second oxidative stress and do not block in the G_2/M phase of cell cycle.
Microarray analysis showed differentially expressed genes between the two cell populations, thus allowing a more precise phenotypic analysis. About 800 significant ($q<0.05$ with FC ± 2) differentially expressed genes were found. Notably, in H2 cells a number of genes of the glutathione pathway (GSTa3, GSTM1, GST1-1, MGST1 and IDH1) were overexpressed. GST are enzymes that use the glutathione as reducing oxidized compounds, and they also convert H_2O_2 to H_2O, while IDH1 has a significant role in NADPH production, providing protection from ROS toxicity. ROS levels measured after a second oxidative stress indicated a decrease of 4-fold of ROS in H2 vs A6 cells, clearly confirming the increased ability of H2 cells to ROS detoxification. The GSTM1 acts sequestrating ASK1 protein-kinase, which in turn cannot activate p38 MAPK involved in the mitosis blockade of A6 treated cells. These results suggest that the overexpression of these genes correspond to an implemented capability of detoxification and of proliferation in H2 cell clone.