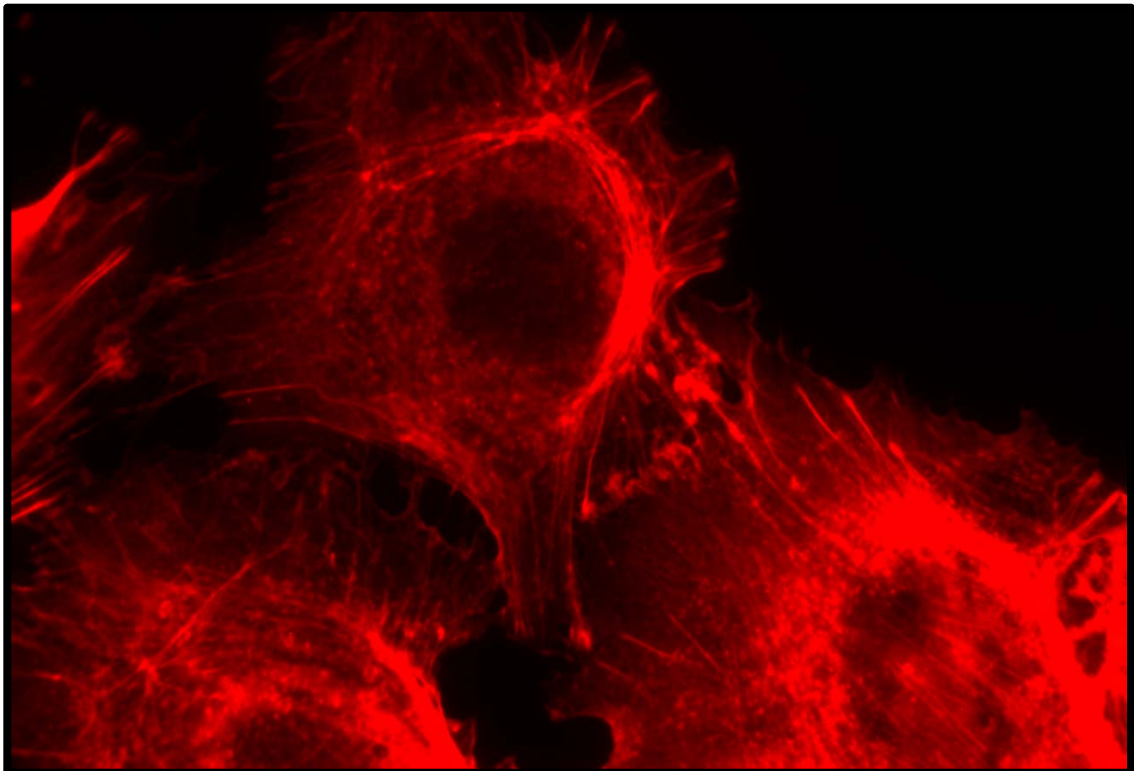


**IX MEETING of the  
SPANISH GROUP of RESEARCH on FREE RADICALS  
(GEIRLI)**

**3<sup>rd</sup>-5<sup>th</sup> June 2013**

**Aula Magna –School of Medicine- Valencia**



## ORGANIZING COMMITTEE

JUAN SASTRE (PRESIDENT)

M<sup>a</sup> TERESA MITJAVILA

M<sup>a</sup> BEGOÑA RUIZ-LARREA

JORDI MUNTANÉ

JOSÉ VIÑA

## SPONSORS



VNIVERSITAT [Q大]  
E VALÈNCIA



## PROGRAMME

3<sup>rd</sup> June, 2013

Morning: OXIDATIVE STRESS IN BIOMEDICINE (I)

- 9:00 *Opening remarks:* **M<sup>a</sup> Begoña Ruiz-Larrea and Juan Sastre**
- 9:05-10:00 **Young investigator session**  
*Chair: Carmen Gómez*
  
- **O1** CELLULAR OXIDATIVE STRESS AND ITS RELATIONSHIP WITH IDIOPATHIC FEMORAL OSTEONECROSIS  
**Pastor S<sup>1</sup>**; Silvestre A<sup>1,2</sup>; Carrasco J<sup>2</sup>; Dasí F<sup>1,3</sup>; Gomar-Sancho F<sup>1,2</sup>  
<sup>1</sup>FIHCUV/INCLIVA; <sup>2</sup>UVEG Facultad de Medicina, Departamento de Cirugía Universidad de Valencia; <sup>3</sup>UVEG Facultad de Medicina, Departamento de Fisiología, Universidad de Valencia.
  
- **O2** OXIDATIVE STRESS IS INCREASED IN SERUM OF PATIENTS WITH DEFICIT IN ALPHA-1 ANTITRYPSIN  
**Contreras S<sup>1</sup>**, Escribano A<sup>1,2,3</sup>, Amor M<sup>1,2,3</sup>, Codoñer-Franch P<sup>2,4</sup>, Sanz F<sup>5</sup>, Navarro-García MM<sup>6</sup>, Dasí F<sup>1,7</sup>  
<sup>1</sup>FIHCUV/INCLIVA; <sup>2</sup>UVEG Facultad de Medicina, Departamento de Pediatría, Obstetricia y Ginecología; Universidad de Valencia; <sup>3</sup>HCUV, Servicio de Pediatría; <sup>4</sup>Hospital Dr. Peset Valencia, Servicio de Pediatría; <sup>5</sup>Hospital General Valencia, Servicio de Neumología; <sup>6</sup>UVEG Facultad de periodismo, Universidad de Valencia; <sup>7</sup>UVEG Facultad de Medicina, Departamento de Fisiología. Universidad de Valencia.
  
- **O3** LAFORA DISEASE FIBROBLASTS EXEMPLIFY THE MOLECULAR INTERDEPENDENCE BETWEEN THIOREDOXIN 1 AND THE PROTEASOME IN MAMMALIAN CELLS  
**Marta Seco-Cervera**  
CIBERER. Centro de Investigación Biomédica en Red de Enfermedades Raras, FIHCUV-INCLIVA, Valencia, Spain. Dept. Physiology, Medicine School, University of Valencia, Valencia, Spain.

- **O4** LONG LIFE SPONTANEOUS EXERCISE DOES NOT PROLONG LIFESPAN BUT PREVENTS FRAILITY IN C57BL/6J. HEALTHSPAN VS LIFESPAN  
**García-Valles R<sup>1</sup>**, Sanchis-Gomar F<sup>1</sup>, Martínez-Bello VE<sup>1</sup>, Pareja-Galeano H<sup>1</sup>, Brioché T<sup>1</sup>, Ferrando B<sup>1</sup>, Cabo H<sup>1</sup>, Salvador-Pascual A<sup>1</sup>, Monleon D<sup>2</sup>, Morales JM<sup>2</sup>, Díaz A<sup>3</sup>, Noguera I<sup>3</sup>, Gómez-Cabrera MC<sup>1</sup> and Viña J<sup>1</sup>  
<sup>1</sup>Department of Physiology, University of Valencia. <sup>2</sup>Fundación Investigación Hospital Clínico Universitario/INCLIVA, Valencia, Spain. <sup>3</sup>UCIM, University of Valencia.
- **O5** ROLE OF GLYCINE:ARGININE AMIDINOTRANSFERASE IN GLUTATHIONE DEPLETION IN PANCREATIC ACINAR CELLS.  
**M<sup>a</sup> Luz Moreno**, Javier Escobar, Javier Pereda, Juan Sastre  
Departamento de Fisiología, Facultad de Farmacia, Universidad de Valencia

10:00 *Break*

#### **Lectures**

*Chair: Francisco Dasí*

- 10:30 **Nadezda Apostolova** (*Departamento de Farmacología, Universidad de Valencia*)  
Major role of mitochondria in the hepatotoxicity induced by the antiretroviral drug Efavirenz
- 11:00 **Víctor Víctor** (*FISABIO- Hospital Dr Peset-Servicio de Endocrinología, Valencia*)  
Oxidative stress, mitochondrial dysfunction and type 2 diabetes: physiological and clinical implications
- 11:30 **Elena Ruiz García-Trevijano** (*Departamento de Bioquímica y Biología Molecular, Universidad de Valencia*)  
Papel del GSH sobre la apertura de la cromatina
- 12:00 **Carmen Gómez** (*Departamento de Fisiología, Universidad de Valencia*)  
Frailty, physical exercise, and oxidative stress.

12:30 *Break*

**Lectures**

Chair: **M<sup>a</sup> Begoña Ruiz-Larrea**

- 13:00 **José Ignacio Ruiz-Sanz** (*Departamento de Fisiología, Universidad del País Vasco*)  
Follicular fluid analysis: looking for a biomarker of oocyte functionality
- 13:30 **M<sup>a</sup> Teresa Mitjavila** (*Departamento de Fisiología, Universidad de Barcelona*)  
Blood pressure modulation by components of the Mediterranean diet in Metabolic Syndrome.

Afternoon: **OXIDATIVE STRESS IN BIOMEDICINE (II)**

**Lectures**

Chair: **M<sup>a</sup> Teresa Mitjavila**

- 16:00 **Guillermo Sáez** (*Departamento de Bioquímica y Biología Molecular, Universidad de Valencia*)  
*Oxidative stress in human carcinogenesis. A model of translational research*
- 16:30 **Isabel Fabregat** (Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet, Barcelona).  
Role of the NADPH oxidase NOX4 in liver pathology
- 17:00 **Lisardo Boscá** (*Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid*)  
Use of non-invasive technology to evaluate active atheromatous lesions

17:30 *Break*

### **Lectures**

Chair: **Lisardo Boscá**

- 18:00 **M<sup>a</sup> Begoña Ruiz-Larrea** (*Departamento de Fisiología, Universidad del País Vasco*)  
Natural polyphenols: Antioxidants, anti-tumor agents, pro-oxidants or what?
- 18:30 **Antonio Martínez-Ruiz** (*Hospital de la Princesa, Madrid*)  
Hypoxia signaling mediated by ROS: new insights from superoxide measurement and thiol redox proteomics.
- 19:00 **Juan Sastre** (*Departamento de Fisiología, Universidad de Valencia*)  
Protein phosphatases modulate redox signaling and histone acetylation in acute inflammation.

**4<sup>th</sup> June, 2013**

Morning: **REDOX SIGNALING IN BIOMEDICINE (I)**

- 9:00-10:00 **Young Investigator Session**  
Chair: **Consuelo Borrás**
- **O6** ROS PRODUCTION IN HYPOXIA, A MATTER OF MITOCHONDRIA, SUPEROXIDE AND MINUTES  
**Hernansanz-Agustín P<sup>1</sup>**, Izquierdo-Álvarez A<sup>1</sup>, Sánchez-Gómez FJ<sup>2</sup>, Lamas S<sup>2</sup>, Bogdanova A<sup>3</sup> and Martínez-Ruiz A<sup>1</sup>  
<sup>1</sup>Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain.  
<sup>2</sup>Laboratorio Mixto CSIC-FRIAT de Fisiopatología Vascular y Renal, Centro de Biología Molecular Severo Ochoa, Campus Universidad Autónoma, 28049 Madrid, Spain. <sup>3</sup>Institute of Veterinary Physiology, Vetsuisse Department and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland.

- **O7** ESTRÉS OXIDATIVO Y ALTERACIÓN DE LA REGULACIÓN DEL CICLO CELULAR EN HEPATOCITOS.  
**Ana M. Tormos** , Raquel Talens-Visconti, Ana Bonora-Centelles, and Juan Sastre (Departamento de Fisiología, Universidad de Valencia)
- **O8** HISTONE H3 GLUTATHIONYLATION IS INVOLVED IN THE REGULATION OF NUCLEOSOME. THE REDOX CONTROL OF CHROMATIN  
**José Luis García-Giménez**  
Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Investigación Sanitaria INCLIVA, Dept. Fisiología, Facultat de Medicina i Odontologia, Universitat de València.
- **O9** ROLE OF miR-433 IN THE REGULATION OF BOTH  $\gamma$ -GLUTAMATE-CYSTEINE-LIGASE SUBUNITS IN REDOX RESPONSES.  
**Cristina Espinosa**, Marta Fierro, Francisco Sánchez and Santiago Lamas  
Centro de Biología Molecular Severo Ochoa, Madrid.
- **O10** BIOMECHANICAL FORCES AND ENDOTHELIAL MITOCHONDRIAL FUNCTION.  
**Rosa Bretón-Romero**<sup>1</sup>, Fernando Rodriguez-Pascual<sup>1</sup>, E. Rial<sup>2</sup>, J.A Enriquez<sup>3</sup> and Santiago Lamas<sup>1</sup>.  
<sup>1</sup>Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM), Madrid, Spain. <sup>2</sup>Centro de Investigaciones Biológicas (CSIC), Madrid, Spain. <sup>3</sup>Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.
- **O11** NOX4 IS A TUMOR SUPPRESSOR FACTOR IN THE LIVER  
**Eva Crosas-Molist**<sup>1</sup>, Esther Bertran<sup>1</sup>, Patricia Sancho<sup>1#</sup>, Judit López-Luque<sup>1</sup>, Joan Fernando<sup>1</sup>, Aránzazu Sánchez<sup>2</sup>, Margarita Fernández<sup>2</sup>, Estanis Navarro<sup>1</sup>, Isabel Fabregat<sup>1\*</sup>  
<sup>1</sup>Bellvitge Biomedical Research Institute (IDIBELL), L’Hospitalet de Llobregat, Barcelona, Spain. <sup>2</sup>Depto Bioquímica y Biología Molecular II, Facultad de Farmacia, Universidad Complutense, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos, IdISSC, Madrid, Spain.  
<sup>#</sup>Present address: Spanish National Cancer Research Center (CNIO), Madrid, Spain.

10:00 Break

### **Lectures**

Chair: **Juan Sastre**

- 10:30 **Antonio Miranda-Vizuet** (*Instituto de Biomedicina de Sevilla, CSIC, Sevilla*)  
Protective role of DNJ-27/ERdj5 in *Caenorhabditis elegans* models of human neurodegenerative diseases
- 11:00 **Consuelo Borrás** (*Department of Physiology, School of Medicine, University of Valencia, Spain*)  
Oxidative stress and proliferation rate differences in human dental pulp stem cells cultured under environmental against physiological oxygen conditions

11:30 Break

### **Lectures**

Chair: **José Viña**

- 12:00 **María Monsalve** (*Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid*)  
Aging and metabolic control come to terms
- 12:30 **Elena Hidalgo** (*Universidad Pompeu Fabra, Barcelona*)  
Control of redox homeostasis: regulation of oxidative protein damage in fission yeast
- 13:00 **Juan Pedro Bolaños** (*Institute of Functional Biology and Genomics (IBFG), University of Salamanca-CSIC*)  
Bioenergetic and redox coupling in the nervous system

### **Memorial Lecture “Ana Navarro”**

Chair: **Guillermo Sáez**

- 13:30 **José Viña** (*Departamento de Fisiología, Universidad de Valencia*)  
microRNAs: Regulación excepcional de la transcripción génica en centenarios



Afternoon: **REDOX SIGNALING IN BIOMEDICINE (II)**

*Lectures*

**Chair: José Ignacio Ruiz-Sanz**

- 16:30 **Susana Cadenas** (*Departamento de Biología Molecular, Universidad Autónoma de Madrid*)  
Regulation of mitochondrial uncoupling protein 3 expression and function under oxidative stress
- 17:00 **José Antonio Enríquez** (*Centro Nacional de Investigaciones Cardiovasculares, CNIC, Madrid*)  
Redox control of mitochondrial Complex I levels
- 17:30 **Santiago Lamas** (*Centro de Investigación Severo Ochoa, Madrid*)  
Regulation of redox signaling by hydrogen peroxide

18:00 *Break*

*Lectures*

**Chair: Federico Pallardó**

- 18:30 **Jordi Muntané** (*Instituto de Biomedicina de Sevilla, Hospital Universitario “Virgen del Rocío”, Sevilla*)  
Regulation of cell death signaling by sorafenib in hepatocellular carcinoma. Role of p53 gene family members
- 19:00 **Joaquim Ros** (*Departamento de Bioquímica y Biología Molecular, Universidad de Lleida*)  
Cellular consequences of frataxin depletion

19:30 **General Assembly of the Spanish Group of Research in Free Radicals (GEIRLI)**

5<sup>th</sup> June, 2013

**OXIDATIVE STRESS AND REDOX SIGNALLING IN PLANTS**

- 9:00-9:30 *Young Investigator Session*

*Chairs: Francisca Sevilla and José M<sup>a</sup> Palma*

- **O12** PROTEIN NITRATION IN PEPPER FRUITS AND ITS INVOLVEMENT IN RIPENING  
Paz Álvarez de Morales<sup>1</sup>, Mounira Chaki<sup>1</sup>, Juan B. Barroso<sup>2</sup>, Francisco J. Corpas<sup>1</sup>, José M. Palma<sup>1</sup>  
<sup>1</sup>Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Granada. <sup>2</sup>Unidad Asociada de Señalización Molecular y Sistemas Antioxidantes en Plantas, Universidad de Jaén, Campus Las Lagunillas, Jaén
- **O13** LICHEN PHYCOBIONTS DEPEND ON MYCOBIONT-DERIVED NO TO PROTECT THEIR CHLOROPHYLL FROM PHOTOOXIDATION. ECOLOGICAL BASIS FOR SYMBIOSIS EVOLUTION?  
Martín San Román S<sup>1</sup>, Catalá M<sup>1</sup>, Martínez-Alberola F<sup>2</sup>, Gasulla F<sup>2</sup>, García-Breijo F<sup>3</sup>, Reig-Armiñana J<sup>2</sup>, Barreno E<sup>2</sup>  
<sup>1</sup>Universidad Rey Juan Carlos, Biología Celular, Dpto. Biología y Geología, (ESCET), Madrid. <sup>2</sup>Universitat de València, Dpto. Botánica, ICBIBE-Jardí Botànic, Burjassot, Valencia. <sup>3</sup>Universidad Politécnica de Valencia, Dpto. Ecosistemas Agroforestales, Valencia
- **O14** MITOCHONDRIAL AtTRXo1: ITS TRANSCRIPTIONAL REGULATION AND ROLE UNDER SALT-STRESS.  
Ortiz-Espín A<sup>1\*</sup>, Iglesias-Fernández R<sup>2\*</sup>, Calderón A<sup>1</sup>, Sevilla F<sup>1</sup>, Carbonero P<sup>2</sup>, Jiménez A<sup>1</sup>  
<sup>1</sup>Departamento de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, Murcia, Spain. <sup>2</sup>Departamento de Biotecnología-UPM, E.T.S. Ingenieros Agrónomos, Madrid, Spain.

9:30 *Break*

## **Lectures**

**Chairs: Eva Barreno and Luis Alfonso del Río**

- 10:00 **Myriam Catalá** (*Universidad Rey Juan Carlos, Biología Celular, Dpto. Biología y Geología, ESCET, Madrid*)  
Lichens, skilled to survive outer space abiotic stress, succumb in our cities. Is the role of nitric oxide in the symbiosis determinant in this paradox?
- 10:30 **Luisa M<sup>a</sup> Sandalio** (*Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Granada*)  
Role of peroxisomes as sensors of cellular stress
- 11:00 **José M. Palma** (*Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Granada*)  
Antioxidants, ROS and RNS metabolism in pepper fruits
- 11:30 **Francisca Sevilla** (*Departamento de Biología del Estrés y patología Vegetal CEBAS-CSIC, Murcia*)  
The importance of mitochondrial redox systems and antioxidants under salt stress: regulation by S-nitrosylation

12:00 *Break*

## **Redox signalling in ageing and rare diseases**

**Chair: Jordi Muntané**

- 12:30 **Reinald Pamplona** (*Department of Physiology, University of Lleida*)  
Lipids and redox signaling in aging and longevity
- 13:00 **Daniel Ramón** (*Biópolis S.L., Valencia*)  
*Caenorhabditis elegans* as a model to study in vivo antioxidant effect of functional food ingredients
- 13:30 **Mónica De la Fuente** (*Department of Physiology. Faculty of Biology, Complutense University of Madrid, Spain*)  
Oxidative stress in immunosenescence and its involvement in the rate of ageing
- 14:00 **Federico Pallardó** (*Department of Physiology, School of Medicine, University of Valencia, Spain*)  
Oxidative stress and antioxidant defence in cells from segmental progeria diseases.

14:30 Concluding remarks: **Teresa Mitjavila and Juan Sastre**

**BOOK  
OF  
ABSTRACTS**

# LECTURES

## MAJOR ROLE OF MITOCHONDRIA IN THE HEPATOTOXICITY INDUCED BY THE ANTIRETROVIRAL DRUG EFAVIRENZ

Ana Blas-García<sup>1,2</sup>, Leysa J. Gomez-Sucerquia<sup>1</sup>, Haryes A. Funes<sup>1,2</sup>, Fernando Alegre<sup>1,2</sup>, Miriam Polo<sup>1,2</sup>, Juan V. Esplugues<sup>1,2</sup> and Nadezda Apostolova<sup>1\*</sup>

<sup>1</sup> *Facultad de Medicina, Universitat de València- CIBERehd, Valencia, Spain;* <sup>2</sup> *FISABIO- Hospital Universitario Dr. Peset, Valencia, Spain.*

\* *corresponding author: nadezda.apostolova@uv.es*

Efavirenz (EFV) is the most widely used Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) within the current multidrug therapy of HIV-1 infection. A substantial group of patients receiving EFV report hepatotoxicity whose cellular mechanisms are still not elucidated. Using the human hepatoma cell line Hep3B and primary human hepatocytes, we have described a specific mitotoxic effect with short-term exposures (up to 24h) to clinically relevant concentrations of EFV (10 and 25 $\mu$ M). EFV-treated cells displayed compromised mitochondrial respiration, reduced mitochondrial membrane potential and incremented mitochondrial reactive oxygen species generation. Transmission electron microscopy and fluorescence microscopy revealed a severe mitochondrial morphology derangement together with autophagic degradation of these organelles (mitophagy), confirmed by Western blot studies of specific marker proteins and colocalization assessments by confocal microscopy. The mitochondrial alterations were paralleled by changes in the ER (Endoplasmic Reticulum) function revealing the presence of ER stress and UPR. Namely, CHOP (CCAAT/enhancer binding protein) and GRP78 (Glucose-regulated protein 78) expression was up-regulated as well as the phosphorylation of eIF2 $\alpha$  (Eukaryotic initiation factor 2). In addition, we also detected an enhanced ER signal and a significant increase in the cytosolic calcium content (fluorescence microscopy employing LysoTracker and FLUO-4AM respectively). The effect of ER stress/UPR induced by EFV was greatly reduced in cells lacking functional mitochondria (Hep3B rho<sup>0</sup>) pointing to a pivotal role of this organelle in the EFV-induced cellular response. In conclusion, short-term treatment of human hepatic cells with clinically relevant concentrations of Efavirenz, produces a complex concentration-dependent effect involving both alterations of the mitochondrial integrity and function as well as presence of ER stress. These cellular modifications may be relevant for better understanding of the clinical manifestations regarding liver hepatotoxicity in patients undergoing EFV-containing therapy.

## **OXIDATIVE STRESS, MITOCHONDRIAL DYSFUNCTION AND TYPE 2 DIABETES: PHYSIOLOGICAL AND CLINICAL IMPLICATIONS**

**Victor M Victor**

*Fundación para la Investigación Sanitaria y Biomédica de la Comunidad Valenciana FISABIO, University Hospital Doctor Peset, Endocrinology Service, Avda Gaspar Aguilar 90, 46017, Valencia, Spain.*

Type 2 diabetes (T2D) promotes oxidative stress and, consequently, endothelial damage.

We evaluated metabolic and anthropometric parameters, presence of inflammatory and adhesion molecules, myeloperoxidase (MPO) concentration, and rates of oxidative stress and mitochondrial impairment in leukocytes and their interaction with the endothelium in T2D patients. In addition, we assessed if these parameters were related to any chronic macro- or microvascular complication of diabetes.

Our population consisted of 200 T2D patients and controls. Waist circumference, triglycerides, IL-6, TNF- $\alpha$ , glucose, insulin, and HOMA were elevated in diabetic patients versus controls. Mitochondrial function was impaired in T2D patients, which was evident in a decreased mitochondrial O<sub>2</sub> consumption, membrane potential and GSH/GSSG ratio and a higher mitochondrial ROS production. Rolling flux and adhesion of leukocytes to the endothelium were more pronounced in T2D patients than in controls, while rolling velocity was diminished. Leukocyte-endothelium interactions were enhanced in patients with silent myocardial ischemia (SMI; macrovascular complication) and were accompanied by an increase in VCAM-1 and E-selectin molecules. These interactions were also enhanced in patients with diabetic nephropathy (microvascular complication) and were accompanied by higher levels of E-selectin. MPO levels were higher in the diabetic group, specifically nephropathic patients, in which levels of MPO correlated positively with the albumin/creatinine ratio and with adhesion and rolling flux of leukocytes to the endothelium.

Our results suggest that T2D induces mitochondrial dysfunction and the inflammatory state and promotes leukocyte-endothelium interactions, which may contribute to the pathogenesis of microvascular complications such as nephropathy and macrovascular alterations such as SMI.

## **ID2 IN THE REGULATION OF CHROMATIN STRUCTURE AND GENE EXPRESSION. ROLE OF GSH ON ID2 MODULATION**

**Elena R. García-Trevijano, Iván Ferrer-Vicens, Luis Torres, Rosa Zaragoza and Juan R. Viña**

*Departamento de Bioquímica y Biología Molecular, Universidad de Valencia*

Deregulated expression of Id2, a Helix-loop-Helix protein, maintains a highly proliferative state. Since Id2 seems to facilitate and not to support cell cycle progression, we studied its possible role in the process of sensitization to proliferative stimuli. In quiescent liver Id2 was part of a repressor complex on c-myc promoter that affects chromatin structure. During experimental liver regeneration Id2 was released from c-myc promoter inducing its transcription initiation. Fluctuations in GSH content are involved in many of the processes, including liver regeneration, where c-myc is over-expressed. *In vivo* GSH depletion by BSO resembled the effects observed on Id2 during liver regeneration. Nevertheless, we show in a pathological model of acetaminophen-induced toxicity that the effect of GSH on Id2 is dependent on the cell microenvironment. We explored whether the mechanisms proposed here might be extended to the modulation of other genes involved in proliferation. Genome-wide ChIP/chip experiments in quiescent mice liver showed 442 E2F4/Id2-target genes mostly involved in tissue development and chromatin remodeling. A common E2F4/Id2-binding sequence was identified in 95% of genes at +200bp from TSS. E2F4/Id2 was part of a complex bound to paused RNAPol II in quiescent liver when Id2 concentration is low. When Id2 levels were increased either, in fetal liver or liver regeneration, Id2 was released from gene promoters inducing the expression of target genes. We conclude that several genes that share common E2F4/Id2-target sequences could be simultaneously activated by a common sensitization mechanism, although only additional and specific factors will render the final pattern of gene expression.

(Supported by FIS PS09-02360, BFU2010-18253, PROMETEO 2010-075).



## **FRAILITY, EXERCISE AND OXIDATIVE STRESS**

**Mari Carmen Gómez-Cabrera, Rebeca García-Valles, Marta Ingles, Juan Gambini, Mar Dromant y José Viña**

*Department of Physiology. Faculty of Medicine. University of Valencia*

The increase in life expectancy has resulted in an increase in age-related diseases and a larger number of dependent people. Frailty is a syndrome highly prevalent with increasing age characterized by weakness, declines in activity, weight loss, and vulnerability to adverse health outcomes. Frailty confers a high risk of mortality, institutionalization, falls, and hospitalization. Although the frailty phenotype has been well characterized a consensus on specific biomarkers useful for diagnosis is lacking.

We have studied the role of spontaneous wheel running in longevity and in the prevention of frailty, as well as the molecular mechanisms involved. Our major problem was the lack of a frailty test in experimental animals. Thus, we had to develop a new test to determine frailty in mice that we have termed the "Valencia Test".

Our results show that life-long spontaneous exercise does not increase longevity but prevents frailty. An active lifestyle maintains the integrity of the mitochondriogenic cell signaling pathway in skeletal muscle as the animals grew older.

Finally we have studied the oxidative stress as a biomarker of frailty both in humans (Toledo study) and in animal models.

## **FOLLICULAR FLUID ANALYSIS: LOOKING FOR A BIOMARKER OF OOCYTE FUNCTIONALITY**

**José Ignacio Ruiz-Sanz, Susana Meijide, Rosaura Navarro, M. Luisa Hernández, M. Begoña Ruiz-Larrea**

*Department of Physiology, Medicine and Dentistry School, University of the Basque Country UPV/EHU, Leioa 48940, Spain*

The assessment of oocyte quality in human *in vitro* fertilization (IVF) is a matter of major attention for embryologists. Oocyte selection and the identification of the best oocytes demonstrating high developmental competence to be transferred or cryopreserved would help to limit embryo overproduction and to improve the results in assisted reproduction. The ultimate goal in IVF is to achieve the transfer of high-quality embryos to the uterine cavity, thereby providing the infertile couple with maximal chances of conception. Follicular fluid is not a simple transudate of blood, but the resultant of both a complex, restricted component of serum that crosses the blood follicular barrier and the secretory activity of granulosa and thecal cells. Follicular fluid plays a major role in the nuclear and cytoplasmic maturation of the oocyte and the process of ovulation. Follicular fluid is easily available during oocyte retrieve and would represent an optimal source of non-invasive biochemical predictors of oocyte quality. Characteristics of the intrafollicular environment in which the pre-ovulatory oocyte grows and matures may be one of the major factors determining subsequent fertility. Intense research has been conducted around the study of follicular fluid content by assaying one or more substances in the fluid derived from individual follicles and by relating it (them) to the fate of the egg coming from that specific follicle. Our group has focused on the description of parameters related to the antioxidant status of the intrafollicular milieu and their relevance in the success of *in vitro* fertilization.

Funds: Research grants from the Ministry of Health and Consumption (FIS/FEDER, ref. PI11/02559), the Basque Country Government -Department of Education, Universities and Research [Refs. IT514-10, IT687-13] and DCIT [Ref. S-PE11UN036]-, and UPV/EHU (CLUMBER UFI11/20).

---

## **BLOOD PRESSURE MODULATION BY COMPONENTS OF THE MEDITERRANEAN DIET IN METABOLIC SYNDROME**

**M.T. Mitjavila<sup>a</sup>, C. Storniolo<sup>a</sup>, R. Casillas<sup>a</sup>, G. Sáez<sup>b</sup>, M.I. Covas<sup>c</sup>, J. Salas-Salvadó<sup>d</sup>, J.J. Moreno<sup>a</sup>**

<sup>a</sup> *Department of Physiology, INSA, University of Barcelona, Barcelona 08028, Spain*

<sup>b</sup> *Department of Biochemistry and Molecular Biology, University of Valencia, Valencia, Spain*

<sup>c</sup> *Cardiovascular Risk and Nutrition Research Group, IMIM-Institut de Recerca Hospital del Mar, Barcelona, Spain*

<sup>d</sup> *Human Nutrition Unit, IISPV, University of Rovira i Virgili, Reus, Spain*

Randomized clinical trials show that the traditional Mediterranean diet (TMD) reduces blood pressure. However, the mechanisms involved in this effect have not yet been elucidated. The reduction in serum nitric oxide (NO) and/or increased endothelin-1 (ET-1) in various pathophysiological circumstances such as endothelial dysfunction and hypertension development have been described. We hypothesized that the beneficial effect of the TMD might involve these two vascular mediators.

The study was performed by administering two TMD (one supplemented with extra virgin olive oil or EVOO and the other with nuts) and also a control low-fat diet for 1 year to non-smoking women with metabolic syndrome and essential hypertension. Serum stables NO metabolites and ET-1 levels and blood pressure were measured.

We observed a reduction of systolic and diastolic blood pressure after 1-year intervention with both TMD compared to their respective baseline values. An inverse association between changes in serum stables NO metabolites concentration (64% increase) and blood pressure after TMD plus EVOO was detected. Additionally, the blood pressure reduction (16%) was related with an impairment of serum ET-1 concentrations after TMD plus nuts. In concordance with the changes in NO and ET-1, we observed in a nutrigenomic study an up-regulation of *eNOS3* and a down-regulation of *CAV2* after TMD plus EVOO, as well as a down-regulation of *EDNRA* and *EDNRB* after TMD plus nuts.

The present data suggest that changes in serum NO and ET-1 can explain, at least partially, the effect of EVOO and nuts on lowering blood pressure among hypertensive individuals.

Supported by RD06/0045, FIS PI10/0082, CP06/00100, 61727/BFI and 2009SGR00438

## **OXIDATIVE STRESS IN HUMAN CARCINOGENESIS. AN EXPERIMENTAL MODEL OF TRANSLATIONAL RESEARCH.**

**Silvia Borrego<sup>1</sup>, Concha Cerdá<sup>1</sup>, Antonio Vazquez<sup>2</sup>, Cristóbal Zaragoza<sup>3</sup>, Carlos Sánchez<sup>4</sup>, Javier Boix<sup>5</sup>, Antonio Iradi<sup>6</sup>, Francisco Dasi<sup>7</sup>, Benjamín Climent<sup>8</sup>, Carmen Tormos<sup>9</sup>, Fatima Ben Allal El Amrani<sup>9</sup>, Julia Sánchez-Tormo<sup>9</sup>, Guillermo T. Sáez<sup>1,9</sup>**

*<sup>1</sup>Servicio de Análisis Clínicos-CDBI, HGUV. <sup>2</sup>Servicio de Cirugía, HGUV. <sup>3</sup>Servicio de Medicina General Mayor Ambulatoria, HGUV. <sup>4</sup>Unidad de Endocrinología y Nutrición, HGUV. <sup>5</sup>Depto. De Patología, Facultad de Medicina de Valencia. <sup>6</sup>Depto. de Fisiología, Facultad de Medicina de Valencia. <sup>7</sup>Fundación de Investigación (INCLIVA) HCV. <sup>8</sup>Servicio de Medicina Interna, HGUV. <sup>9</sup>Dto. de Bioquímica y Biología Molecular-CIBEROBN. Facultad de Medicina de Valencia. Universidad de Valencia.*

Oxidative stress has been implicated in the pathophysiology of different kind of human tumors. The role of reactive oxygen species (ROS) in the regulation of signal transduction pathways in normal and cancer cells has received great attention during the past decade. Indeed, redox modulation in tumor cells is a critical mechanism in the induction of apoptosis induced by different antitumor agents.

During malignant transformation, a common oxidative stress pattern seems to characterise the evolution of tumor cells. With no exception we found a decrease of SOD and Catalase activities and an increase of GSH levels and GPx activity in all analysed tumor specimens. Antioxidant enzyme impairment is accompanied by an increase of lipid peroxidation products and the oxidised base 8-oxo-dG. Oxidative modification of guanine formed in situ results in GC to TA transversions which have been observed in vivo in the *ras* oncogene and the p53 tumor suppressor gene in different human cancers.

It has been proposed that in human colon carcinoma, the increase of 8-oxo-dG may induce DNA instability and explain the accumulation of gene mutations during its clinical progression. On the other hand, in gastric carcinoma tumors, a significant increase of the damaged base together with an enhanced RNA expression of DNA repair enzymes such as hOGG1, MUYTH, MTH1 and RAD51 is observed. In the urine and circulating mononuclear cells of gastric cancer patients, 8-oxo-dG is also increased as compared with the control healthy group. After tumor resection, 8-oxo-dG decreased progressively to those values found in the healthy population. The potential use of 8-oxo-dG as a tumor marker could contribute to better diagnosis and follow-up of cancer patients.

## ROLE OF THE NADPH OXIDASE NOX4 IN LIVER PATHOLOGIES

**Eva Crosas-Molist<sup>1</sup>, Patricia Sancho<sup>1</sup>, Jèssica Mainez<sup>1</sup>, Esther Bertran<sup>1</sup>, Judit López-Luque<sup>1</sup>, Joan Fernando<sup>1</sup>, Estanis Navarro<sup>1</sup>, César Roncero<sup>2</sup>, Conrado M. Fernández-Rodríguez<sup>3</sup>, Fernando Pinedo<sup>4</sup>, Heidemarie Huber<sup>5</sup>, Robert Eferl<sup>5,6</sup>, Wolfgang Mikulits<sup>5</sup> and Isabel Fabregat<sup>1,7</sup>**

<sup>1</sup>*Bellvitge Biomedical Research Institute (IDIBELL) , L'Hospitalet, Barcelona, Spain;* <sup>2</sup>*Dep. Bioquímica y Biología Molecular II, Facultad de Farmacia, Universidad Complutense, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), Madrid, Spain;* <sup>3</sup>*Service of Gastroenterology, Hospital Universitario Fundación Alcorcón, Universidad Rey Juan Carlos, Alcorcón, Madrid, Spain;* <sup>4</sup>*Unit of Pathology, Hospital Universitario Fundación Alcorcón;* <sup>5</sup>*Department of Medicine I, Division: Institute of Cancer Research, Medical University of Vienna, Vienna, Austria;* <sup>6</sup>*Ludwig Boltzmann Institute for Cancer Research (LBI-CR), Vienna, Austria;* <sup>7</sup>*Dep. Physiological Sciences II, University of Barcelona, Spain.*

The NADPH oxidase (NOX) family has emerged in the last years as an important source of reactive oxygen species (ROS) in signal transduction. In our laboratory we have recently focused on NOX4 role in liver chronic diseases, particularly fibrosis, cirrhosis and cancer. We found that NOX4 plays a relevant role in mediating TGF- $\beta$ -induced apoptosis in hepatocytes, as well as in activating stellate cells to myofibroblasts, both processes contributing to liver fibrogenesis. NOX4 expression was increased in the livers of different experimental animal models of hepatic fibrosis, concomitantly with TGF- $\beta$  pathway activation, and in patients with hepatitis C virus (HCV)-derived fibrosis, increasing along the fibrosis degree. Indeed, inhibitors of NOX4 could prevent the progression to liver cirrhosis and, eventually, the initiation of a pretumorigenic event. However, silencing NOX4 expression in human liver tumor cells conferred them advantage to form earlier and bigger tumors in *in vivo* experiments in athymic mice. *In vitro* assays proved a crucial role for NOX4 in regulating liver tumor cell proliferation and migration. Interestingly, immunohistochemical analyses of NOX4 expression in human liver tumor tissues revealed decreased NOX4 protein levels in a great percentage of them. Overall, results suggest that NOX4 would play an essential suppressor role in pre-neoplastic situations of the liver. Indeed, it will be necessary to be cautious in the use of NOX4 inhibitors in chronic liver pathologies.

Grants to I.F. from the Ministerio de Economía y Competitividad, Spain (BFU2009-07219 and ISCIII-RTICC RD06/0020) and AGAUR-Generalitat de Catalunya (2009SGR-312). J.M. and E.C.-M. were recipient of pre-doctoral fellowships from the Spanish Government (FPI and FPU programs, respectively). J.F. and J.L.-L. were recipient of predoctoral contracts from IDIBELL.

## **USE OF NON-INVASIVE TECHNOLOGY TO EVALUATE ACTIVE ATHEROMATOUS LESIONS**

**Lisardo Boscá**

*Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid*

During development of atherosclerosis, ROS are key components in the outcome and nature of the constituents of the atheromatous lesion, defining a specific microenvironment (e.g. oxidized lipids, cytokines, hypoxia, et. ) that mediate monocyte/macrophage recruitment and a defined pro/anti-inflammatory phenotype. We have analyzed how macrophage proinflammatory activation and energetics are tightly linked under conditions relevant to atheroma, resulting in close titration of glycolysis relative to the degree of macrophage activation. Multicomponent analysis including hypoxia and cytokine/chemokine determination define specific patterns of macrophage activation that are involved in the generation of the atheromatous lesion. In this way, we have analyzed the synergistic actions with oxidized lipids and cytokines, via a mechanism that depends on hypoxia inducible factor (HIF) and ubiquitous phosphofructokinase 2 (uPFK2). Further, we have demonstrated that selective impairment of uPFK2 or hypoxia sensing leads to decreased macrophage viability, and therefore an inappropriate resolution of the inflammatory response with cells filled with lipidic particles without an efficient export activity, typical of systems that avoid atheromatous development. These observations provide new fundamental mechanistic insights into the interrelationship between macrophage activation and energetics, and offer opportunities to develop novel therapeutic targets to alleviate atherosclerotic inflammation and its clinical complications.

## **NATURAL POLYPHENOLS: ANTIOXIDANTS, ANTI-TUMOR AGENTS, PRO-OXIDANTS OR WHAT?**

**M. Begoña Ruiz-Larrea, Leandro J. Lizcano, Maite Siles, Jenifer Trepiana, Oihana López de Vergara, M. Luisa Hernández, Rosaura Navarro, José Ignacio Ruiz-Sanz**

*Department of Physiology, Medicine and Dentistry School, University of the Basque Country UPV/EHU, Leioa 48940, Spain*

The natural products based medicine has been used for centuries, and even today is the basis of phytotherapy and traditional medicine in indigenous communities. The beneficial actions of the natural healing have been attributed to the polyphenolic components, which show potent antioxidant activities. There is an increasing interest to find plant-derived natural products, which can be used in therapies and avoid the side effects of synthetic drugs. Screening of antioxidant properties of extracts from botanical sources is often studied to find bioactive compounds, among them chemopreventive agents against cancer. We have studied the cytotoxic actions of aqueous extracts from leaves of Colombian Amazonian plants of the *Vismia* and *Piper* genus on human and rat hepatocarcinoma cell lines, and the possible mechanisms involved. The extracts used contained polyphenols and exhibited high antioxidant activities *in vitro*. Our results showed that depending on the cell type, the extracts induced selective killing of human hepatocarcinoma cells. The extracts also induced cytotoxicity to rat McA-RH7777 hepatoma cells, but were innocuous in primary cultures of rat hepatocytes. The cytotoxic effects were accompanied by a significant increase of intracellular ROS, a general increase in superoxide dismutase activity and a reduced catalase activity, suggesting that hydrogen peroxide is responsible of the apoptotic cell response. Our results provide evidence for the anti-cancer activities of the studied plants, and suggest that the cell killing is mediated by ROS, thus involving mechanisms independent of the plant free radical scavenging activity. Again, the biological system and the complex molecular interactions of the components present in the system contribute to the final antioxidant or pro-oxidant actions exhibited by natural polyphenols.

Funds: Research grants from the Basque Country Government - Department of Education, Universities and Research [Refs. IT514-10, IT687-13] and DCIT [Refs. SA-2010/00125, S-PE12UN056]-, and UPV/EHU (CLUMBER UFI11/20). Technical and human support provided by SGIKER (UPV/EHU, MICINN, GV/EJ, ESF) is gratefully acknowledged.

## **HYPOXIA SIGNALLING MEDIATED BY ROS: NEW INSIGHTS FROM SUPEROXIDE MEASUREMENT AND THIOL REDOX PROTEOMICS**

**Izquierdo-Álvarez A<sup>1</sup>, Hernansanz-Agustín P<sup>1</sup>, Ramos E<sup>1</sup>, Sánchez-Gómez FJ<sup>2,3</sup>, Martínez-Acedo P<sup>3</sup>, Núñez E<sup>3,4</sup>, Lamas S<sup>2,3</sup>, Vázquez J<sup>3,4</sup>, Bogdanova A<sup>5</sup>, Martínez-Ruiz, A<sup>1</sup>**

<sup>1</sup>*Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain.*

<sup>2</sup>*Laboratorio Mixto Consejo Superior de Investigaciones Científicas (CSIC)/Fundación Renal “Iñigo Alvarez de Toledo” (FRIAT), Madrid, Spain*

<sup>3</sup>*Centro de Biología Molecular “Severo Ochoa”, CSIC-UAM, Madrid, Spain.*

<sup>4</sup>*Centro Nacional de Investigaciones Cardiovasculares, Melchor Fernández Almagro. 3, 28029 Madrid;*

<sup>5</sup>*Institute of Veterinary Physiology, Vetsuisse Department and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland.*

The adaptation to decreased oxygen availability (hypoxia) is crucial for proper cell function and survival. In metazoans, this is partly achieved through gene transcriptional responses mediated by the hypoxia-inducible factors (HIF). There has been a long-standing controversy around the paradoxical increase in the production of reactive oxygen species (ROS) in hypoxia, and its role in the activation of the HIF pathway.

We have developed new methodologies for measuring superoxide in short times of hypoxia, using specific probes. We have observed a superoxide burst in the first minutes of hypoxia, without observing an increased superoxide production after one hour. This superoxide burst is common to different cell types, and most probably comes from the mitochondria. Our results can explain the controversy among the few previous results that have measured superoxide in hypoxic cells, as they were taken at different times.

With complementary thiol redox proteomics techniques (redox fluorescence switch –RFS- and GELSILOX), we have identified several proteins that are specifically oxidized in cysteine residues in endothelial cells subjected to two hours of hypoxia. These cysteine oxidation signals may mediate different adaptations to hypoxia, before the HIF pathway is fully activated.

We hypothesize that ROS signals can be initiated by increased superoxide production in mitochondria, which can be converted in the oxidation of sensitive cysteine residues in cells subjected to acute hypoxia, mediating further functional adaptations.



## **PROTEIN PHOSPHATASES MODULATE REDOX SIGNALING AND HISTONE ACETYLATION IN ACUTE INFLAMMATION**

**Juan Sastre**

*Department of Physiology, School of Pharmacy, University of Valencia.  
juan.sastre@uv.es*

Histone acetylation via CBP/p300 coordinates the expression of pro-inflammatory cytokines in the activation phase of inflammation, particularly through mitogen-activated protein kinases (MAPK), nuclear factor-kappa B (NF- $\kappa$ B) and signal transducers and activators of transcription (STAT) pathways. In contrast, histone deacetylases (HDACs) and protein phosphatases are mainly involved in the attenuation phase of inflammation. The role of reactive oxygen species (ROS) in the inflammatory cascade is much more important than expected. Mitochondrial ROS act as signal-transducing molecules that trigger pro-inflammatory cytokine production via inflammasome-independent and inflammasome-dependent pathways. The major source of ROS in acute inflammation appears to be NADPH oxidases, whereas NF- $\kappa$ B, protein phosphatases, and HDACs are the major targets of ROS and redox signaling in this process.

There is a cross-talk between oxidative stress and pro-inflammatory cytokines through serine/trreonine protein phosphatases, tyrosine protein phosphatases, and MAPK that greatly contributes to amplification of the uncontrolled inflammatory cascade and tissue injury in acute pancreatitis. Chromatin remodeling during induction of pro-inflammatory genes would depend primarily on phosphorylation of transcription factors and their binding to gene promoters together with recruitment of histone acetyl transferases. PP2A should be considered a key modulator of the inflammatory cascade in acute pancreatitis through the ERK/NF- $\kappa$ B pathway and histone acetylation.

---

## PROTECTIVE ROLE OF DNJ-27/ERDJ5 IN *CAENORHABDITIS ELEGANS* MODELS OF HUMAN NEURODEGENERATIVE DISEASES

Fernando Muñoz-Lobato<sup>1</sup>, María Jesús Rodríguez-Palero<sup>1</sup>, Francisco José Naranjo-Galindo<sup>1,2</sup>, Freya Shephard<sup>3</sup>, Christopher J. Gaffney<sup>3</sup>, Nathaniel J. Szewczyk<sup>3</sup>, Shusei Hamamichi<sup>4</sup>, Kim A. Caldwell<sup>4</sup>, Guy A. Caldwell<sup>4</sup>, Chris D. Link<sup>5</sup>, Antonio Miranda-Vizuete<sup>1,2,\*</sup>

<sup>1</sup>Centro Andaluz de Biología del Desarrollo (CABD-CSIC), Depto. de Fisiología, Anatomía y Biología Celular, Universidad Pablo de Olavide, 41013 Sevilla, Spain. <sup>2</sup>Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, 41013 Sevilla, Spain. <sup>3</sup>MRC/Arthritis Research UK Centre for Musculoskeletal Ageing Research, University of Nottingham, Royal Derby Hospital, Derby, DE22 3DT, United Kingdom. <sup>4</sup>Department of Biological Sciences, The University of Alabama, Tuscaloosa, AL 35487, USA. <sup>5</sup>Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309, USA. \* [amiranda-ibis@us.es](mailto:amiranda-ibis@us.es)

Cells have developed quality control systems for protection against proteotoxicity. Misfolded and aggregation-prone proteins, which are behind the initiation and progression of many neurodegenerative diseases, are known to challenge the proteostasis network of the cells. We aimed to explore the role of DNJ-27/ERdj5, an ER-resident protein required as a disulfide reductase for the degradation of misfolded proteins, in well-established *Caenorhabditis elegans* models of Alzheimer, Parkinson and Huntington diseases. We demonstrate that DNJ-27 is an ER luminal protein and that its expression is induced upon ER stress via IRE-1/XBP-1. When *dnj-27* expression is downregulated by RNAi we find an increase in the aggregation and associated pathological phenotypes (paralysis and motility impairment) caused by human  $\beta$ -amyloid peptide,  $\alpha$ -synuclein and polyglutamine proteins. In turn, DNJ-27 overexpression ameliorates these deleterious phenotypes. Surprisingly, despite being an ER-resident protein, we show that *dnj-27* downregulation alters cytoplasmic protein homeostasis and causes mitochondrial fragmentation. We further demonstrate that DNJ-27 overexpression substantially protects against the mitochondrial fragmentation caused by human  $\beta$ -amyloid and  $\alpha$ -synuclein peptides in these worm models. Together, we identify *C. elegans dnj-27* as a novel protective gene for the toxicity associated with the expression of human  $\beta$ -amyloid peptide,  $\alpha$ -synuclein and polyglutamine proteins, implying a protective role of ERdj5 in Alzheimer, Parkinson and Huntington diseases. Our data support a scenario where the levels of DNJ-27/ERdj5 in the endoplasmic reticulum impact cytoplasmic protein homeostasis and the integrity of the mitochondrial network which might underlie its protective effects in models of proteotoxicity associated to human neurodegenerative diseases.

**OXIDATIVE STRESS AND PROLIFERATION RATE DIFFERENCES IN HUMAN DENTAL PULP STEM CELLS CULTURED UNDER ENVIRONMENTAL AGAINST PHYSIOLOGICAL OXYGEN CONDITIONS.**

**Borrás C, El Alami M, Viña JA, Gambini J, Viña J**

*Department of Physiology, Faculty of Medicine, University of Valencia/INCLIVA*

One of the current challenges in the odontological surgeries is the bone regeneration, especially of the alveolar bone for the accomplishment of the dental implant. In the last years, the mesenchymal stem cells derived from the dental pulp (DPSCs) have become one of the most promising candidates for the bone regeneration mostly due to their differentiate potential to odontoblasts and osteoblasts and their immunologic properties. Nevertheless, their clinical application is still limited due to the high cells number required and the time of its obtention. Therefore, we focused our study on the optimization of DPSCs obtention at physiological oxygen tension, and we studied the effect of environmental oxygen tension, widely used *in vitro*, compared to the physiological on the parameters of oxidative stress and proliferation. The obtained results were that the adhesion and the proliferation rate of the cells were significantly lower under the environmental oxygen tension compared to the physiological and the observed differences are oxidative stress dependent. Moreover, we proposed a p38/p21/Nrf2 pathway by which the DPSCs may regulate the cell cycle and the antioxidant defense in response to the environmental oxygen tension.

**Acknowledgements:** SAF2010-19498, ISCIII2006-RED13-027 and ISCIII2012-RED-43-029, PROMETEO2010/074, 35NEURO GentxGent and EU Funded CM1001 and FRAILOMIC-HEALTH.2012.2.1.1-2. This study has been co-financed by FEDER funds from the European Union.

## **AGING AND METABOLIC CONTROL COME TO TERMS**

**María Monsalve**

*Instituto de Investigación Biomédica Alberto Sols, CSIC, Madrid*

Sirt1 is a class III histone deacetylase that has been implicated in metabolic and ROS control. In the vasculature it has been shown to decrease endothelial superoxide production, prevent endothelial dysfunction and atherosclerosis. However, the mechanisms that mediate Sirt1 antioxidant functions remain to be characterized. The transcription factor FoxO3a and the transcriptional coactivator peroxisome proliferator activated receptor  $\gamma$ -coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) have been shown to induce the expression of antioxidant genes and to be deacetylated by Sirt1. Here we investigated Sirt1 regulation of antioxidant genes and the roles played by FoxO3a and PGC-1 $\alpha$  in this regulation. **Results:** We found that Sirt1 regulates the expression of several antioxidant genes in BAEC, including Mn superoxide dismutase (MnSOD), catalase, peroxiredoxins 3 and 5 (Prx3, Prx5), thioredoxin 2 (Trx2), thioredoxin reductase 2 (TR2) and uncoupling protein 2 (UCP-2) and can be localized in the regulatory regions of these genes. We also found that knockdown of either FoxO3a or PGC-1 $\alpha$  prevented the induction of antioxidant genes by Sirt1 over-expression. Furthermore, Sirt1 increased the formation of a FoxO3a/PGC-1 $\alpha$  complex as determined by co-immunoprecipitation (IP) assays, concomitantly reducing H<sub>2</sub>O<sub>2</sub> dependent FoxO3a and PGC-1 $\alpha$  acetylation. Data showing that FoxO3a knockdown increases PGC-1 $\alpha$  acetylation levels and /vice versa/, suggests that Sirt1 activity on FoxO3a and PGC-1 $\alpha$  may be dependent of the formation of a FoxO3a/PGC-1 $\alpha$  complex. **Conclusion:** We show that Sirt1 regulation of antioxidant genes in vascular endothelial cells depends on the formation of a FoxO3a/PGC-1 $\alpha$  complex.

## **CONTROL OF REDOX HOMEOSTASIS: REGULATION OF OXIDATIVE PROTEIN DAMAGE IN FISSION YEAST**

**Elena Hidalgo**

*Universitat Pompeu Fabra, C/ Dr. Aiguader 88, 08003 Barcelona*

Proteins are a major target of damage by reactive oxygen species (ROS). Reversible oxidation of cysteine and methionine residues in proteins by hydrogen peroxide has been considered a switch-on mechanism for the activation of signalling cascades, but it can also lead to higher and irreversible oxidation states with concomitant protein loss of function. ROS can also induce the formation of protein carbonyls, which constitute a classical mark of oxidative damage. We will present here experimental evidences showing that: (i) the reversible thiol proteome can be oxidized by extracellular peroxides and by oxidized thioredoxins; (ii) free methionine is a major target of peroxide reactivity; (iii) the only fate of carbonylated proteins is their degradation by the proteasome, with some specific chaperones contributing to their turnover.

## **BIOENERGETIC AND REDOX COUPLING IN THE NERVOUS SYSTEM**

**Juan P. Bolaños**

*Institute of Functional Biology and Genomics (IBFG),*

*University of Salamanca-CSIC*

Our laboratory is interested in understanding the molecular mechanisms that regulate the energetic and redox homeostasis in the cells of the central nervous system. We have observed that, in spite of the enormous energy demand that the neurotransmission process requires, neurons modestly utilize glucose as an energetic fuel –in contrast to the vast majority of other cells– due to ubiquitylation and proteasomal degradation of PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3), which catalyzes the biosynthesis of fructose-2,6-bisphosphate (F26BP), a potent positive allosteric effector of 6-phosphofructo-1-kinase (PFK1). We identified the E3 ubiquitin ligase responsible for PFKFB3 ubiquitylation, which is anaphase-promoting complex/cyclosome (APC/C)-Cdh1. Constant degradation of PFKFB3 by APC/C-Cdh1 activity in neurons wholly accounts for the low glycolytic rate in these cells. However, glucose metabolism is very active towards the pentose-phosphate pathway, which neurons use to conserve the redox energy of glucose as NADPH(H<sup>+</sup>), an essential cofactor in the regeneration of antioxidants, such as glutathione or thioredoxin. Here, it will be discussed the molecular mechanisms responsible for the metabolic adaptation of neurons to this status, including proteins (and other factors) that i) regulate the energetic homeostasis, ii) regulate the antioxidant homeostasis, and iii) responsible for the adequate coordination of both processes.

## **CENTENARIANS UP REGULATE THE EXPRESSION OF microRNAs**

**José Viña<sup>1</sup>, Consuelo Borras<sup>1</sup>, Eva Serna<sup>1</sup>, Juan Gambini<sup>1,2</sup>, Kheira Mohammed<sup>1</sup>, Juan A. Avellana<sup>2</sup>, Angel Belenguer<sup>2</sup>**

<sup>1</sup>*Departament de Fisiologia, Universitat de Valencia and INCLIVA, Valencia, Spain*

<sup>2</sup>*Servicio de Geriátría. Hospital de la Ribera. Alzira, Valencia, Spain*

Centenarians not only have an extraordinary longevity, but also show a compression of morbidity. They preserve the capacity of maintaining homeostasis, and this is the reason for them to reach such a long life. We hypothesized that centenarians should be extremely well regulated at molecular level, and studied their microRNA expression profile, since microRNAs are directly involved in the regulation of gene expression.

We compared microRNA expression profiles of centenarians, octogenarians and young individuals, by analysing the expression of 15,644 mature microRNAs and, 2,334 snoRNAs and scaRNAs in peripheral blood mononuclear cells.

Principal component analysis showed that centenarian microRNA expression profile was similar to that of the young individuals, but different from octogenarians. Moreover, centenarians show an up-regulation of the expression of 102 microRNAs when compared to octogenarians and only one down-regulated, and even when compared to young individuals, 7 microRNAs are up-regulated and none down-regulated. Of these seven only one is also up-regulated in octogenarians. Thus the remaining six that are specific for centenarians are miR21, miR130a, miR494, miR1975, miR1979 and SCARNA17

We conclude that centenarians up-regulate the expression of small non-coding RNAs like microRNAs and scaRNAs.. This may explain their exceptional ability to maintain homeostasis even in extreme aging..

For full description of these ideas see [www.nature.com/scientific-reports](http://www.nature.com/scientific-reports) 12-02982-T

## REGULATION OF MITOCHONDRIAL UNCOUPLING PROTEIN 3 EXPRESSION AND FUNCTION UNDER OXIDATIVE STRESS

Anedda A.<sup>1</sup>, López-Bernardo E.<sup>1</sup>, Acosta-Iborra B.<sup>1</sup>, Suleiman M.S.<sup>2</sup>,  
Landázuri M.O.<sup>1</sup>, Cadenas S.<sup>1,3</sup>

<sup>1</sup>*Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IP), 28006 Madrid, Spain;* <sup>2</sup>*Bristol Heart Institute, University of Bristol, Bristol BS2 8HW, United Kingdom;* <sup>3</sup>*Departamento de Biología Molecular, Facultad de Ciencias, Universidad Autónoma de Madrid (UAM), 28049 Madrid, Spain*

Uncoupling proteins (UCPs) belong to the mitochondrial anion carrier family. UCP3 is ~73% homologous to UCP2 and ~58% homologous to the highly thermogenic uncoupling protein, UCP1. Whereas UCP2 is expressed in a wide variety of tissues, UCP3 is expressed in skeletal muscle, and to a lesser extent in brown adipose tissue and heart. The physiological functions of UCP2 and UCP3 are still not established. Superoxide and the lipid peroxidation product 4-hydroxynonenal have been shown to induce proton leak through UCPs thus providing a negative feedback loop for mitochondrial ROS production. We studied the effect of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) on UCP3 expression levels in differentiated C2C12 (from mouse skeletal muscle) and in HL-1 (from mouse heart). H<sub>2</sub>O<sub>2</sub> treatment increased both UCP3 mRNA and protein. The transcription factor Nrf2 (NF-E2-related factor 2), an essential regulator of the cellular redox homeostasis, increased its nuclear accumulation after H<sub>2</sub>O<sub>2</sub> addition. Nrf2 interference by siRNA prevented H<sub>2</sub>O<sub>2</sub>-mediated UCP3 induction, increasing oxidative stress and cell death. ChIP assays identified an antioxidant response element (ARE) within the UCP3 promoter that bound Nrf2 following exposure to H<sub>2</sub>O<sub>2</sub>. Luciferase reporter experiments confirmed increased ARE activity in H<sub>2</sub>O<sub>2</sub>-treated cells. Importantly, H<sub>2</sub>O<sub>2</sub> increased the UCP3-mediated proton leak, suggesting a role for this protein in attenuating ROS-induced damage. Nrf2 nuclear accumulation and increased UCP3 protein were also detected in intact mouse heart subject to a condition known to increase ROS generation. Our results suggest that UCP3 functions as a member of the cellular antioxidant defence system that protects against oxidative stress.



## REDOX CONTROL OF MITOCHONDRIAL COMPLEX I LEVELS

**José Antonio Enríquez**

CNIC, Madrid

The relative proportions of mitochondrial respiratory chain components are exquisitely regulated to ensure efficient participation in metabolism and minimize production of reactive oxygen species. The molecular mechanism of this regulation is unknown. Respiratory chain complexes show remarkable asymmetry in their sensitivity to loss of other chain components. Thus while loss of complex I does not affect the levels of complexes III and IV or cytochrome c, loss of any of these components results in the degradation of complex I, and complex IV is degraded in the absence of cytochrome c. Dependence of complex I stability on other complexes is strongly modulated by oxygen availability. We described that accumulation of reduced ubiquinone (CoQH<sub>2</sub>) promotes retrograde electron flux and, in the presence of oxygen, a burst of superoxide formation at complex I that induces its degradation, explaining the requirement for other complexes for complex I stability.

## **REDOX SIGNALING RESPONSES IN VASCULAR ENDOTHELIAL CELLS**

**Santiago Lamas**

Centro de Biología Molecular “Severo Ochoa”, Madrid

For many years ROS were described as unwanted toxic products of cellular metabolism, assuming that the faster the elimination of them, the better for the cell. However, substantial evidences in the last decades, have established ROS as important signaling molecules in different cells. In endothelial cells (EC) an excess of ROS is pathological due to their cytotoxic and mutagenic properties, resulting in endothelial dysfunction. However, EC maintain an equilibrium between oxidizing agents and antioxidant responses and generate ROS in low concentrations which exert physiological roles. Our studies explored the mechanisms whereby LSS-mediated ROS generation activates key endothelial signaling pathways. We found that LSS rapidly promotes a transient production of H<sub>2</sub>O<sub>2</sub> which mediates the sequential activation of p38 MAPK, an increase in the activity of eNOS and the subsequent nitric oxide production. LSS is able to regulate the expression of antioxidant defence systems; however the early response to SS in the vascular endothelium is still unclear. We focused our attention on peroxiredoxins (PRXs), which are highly sensitive enzymatic sensors of H<sub>2</sub>O<sub>2</sub> and demonstrated that PRX3 (located in the mitochondria) is potentially regulated by flow. Our data suggested that mitochondriae behave as mechanosensor organelles in EC since LSS is able to induce mitochondrial fission mediated by Drp1, which correlates with an increase in mitochondrial membrane potential and a decrease in mitochondrial respiration. This work adds new insights into the response to fluid flow, providing data for undescribed pathways on the regulation of NO<sup>•</sup> signaling and mitochondrial function in the vascular endothelium.

## **REGULATION OF CELL DEATH SIGNALING BY SORAFENIB IN HEPATOCELLULAR CARCINOMA. ROLE OF p53 GENE FAMILY MEMBERS**

**Jordi Muntané**

*Department of General Surgery, Hospital Universitario “Virgen del Rocío”/IBiS/CSIC/ Universidad de Seville, Seville, Spain*

*National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd), Spain*

**Background:** Sorafenib, a multi-tyrosine kinase inhibitor, induces cell death in hepatoma cells. Different p53 gene family members have been shown to regulate the expression of cell death receptors during oxidative/nitrosative stress, chemo- and radio-therapy. **Objectives:** The aim of the study was the identification of the role of p53 during the regulation of cell death signaling in sorafenib-treated hepatoma cells. **Methods:** Liver tumor sections from patients suffering of hepatocarcinoma (Alcohol, VHB and VHC), and hepatoma cell lines with different p53 genetic profile (HepG2, Huh7 and Hep3B) were included. Different cell death parameters, and the expression of p53/p63/p73 isoforms, CD95, TNF-R1 and TRAIL-R1 were assessed. **Results:** The survival of patients with hepatocarcinoma was related to increased expression of cell death receptors, and reduced expression of p63 $\Delta$ N and p73 $\Delta$ N in tumors. Sorafenib increases caspase-8 activation in Huh7>HepG2>Hep3B cells. Sorafenib increased p63TA, p73TA and all cell death receptor expression, as well as decreased p63 $\Delta$ N expression in Huh7 and Hep3B. Sorafenib increased p53 and TNF-R1, and reduced p63 $\Delta$ N expression in HepG2. The ligand-induced cell death susceptibility in HepG2 and Hep3B was related to TNF-R1, as well as to CD95, TNF-R1 and TRAIL-R1, respectively. However, TNF- $\alpha$  and Trail, but not CD95L, increased cell death in sorafenib-treated Huh7 cells. **Conclusions:** 1) The survival of patients with hepatocarcinoma was associated with reduced p63 $\Delta$ N and p73 $\Delta$ N, and increased cell death receptor expression in tumor. 2) The induction of cell death receptor expression and apoptosis by sorafenib were related to increased p53/p63TA/p73TA, and reduced p63 $\Delta$ N expression in hepatoma cells.

## **CELLULAR CONSEQUENCES OF FRATAXIN DEPLETION**

**Jordi Tamarit, Stefka Mincheva, Elia Obis, David Alsina and Joaquim Ros.**

*Departament de Ciències Mèdiques Bàsiques, IRB-Lleida, Universitat de Lleida, Lleida, Spain*

Frataxin is a mitochondrial protein involved in cellular iron homeostasis whose deficiency in humans causes Friedreich ataxia. To understand the cellular consequences of frataxin deficiency we use yeasts, primary cultures of cardiac myocytes and dorsal root ganglia neurons as cell models. In yeasts, we have observed that in conditional Yeast Frataxin Homologue (Yfh1) mutants (tetO<sub>7</sub>-YFH1), Yfh1 depletion leads to down-regulation of many glucose-repressed genes. Most of them were Adr1 targets, a key transcription factor required for growth in non-fermentable carbon sources. Using a GFP-tagged Adr1, we observed that Yfh1 depletion promotes the export of Adr1 from the nucleus to the cytosol. We also observed that CTH2, a gene involved in the mRNA degradation of several iron-containing enzymes, was induced upon Yfh1 depletion. Accordingly, decreased levels of two Cth2 targets (aconitase and succinate dehydrogenase) were observed. Nevertheless, their levels were maintained in a  $\Delta$ cth2 mutant even in the absence of Yfh1, indicating that such downregulation is promoted by Cth2. In primary cultures from rat cardiomyocytes and DRG neurons frataxin depletion has been achieved by RNA interference using lentiviral particles. In cardiomyocytes, frataxin depletion triggers mitochondrial rearrangements and disturbs lipid metabolism, suggesting impairment of mitochondrial functions. Dorsal root ganglia neurons show, after frataxin depletion, markers of apoptotic cell death, increased levels of intracellular calcium, neurite degeneration and altered mitochondrial membrane potential. All these features can be reversed with the addition of a cell-penetrant TAT peptide coupled to the BH4 anti-apoptotic domain of Bcl-x<sub>L</sub> protein. As a conclusion, the use of these three models provide precise clues to understand the physiological events taking place after frataxin depletion and the rationale for new therapies.

**LICHENS, SKILLED TO SURVIVE OUTER SPACE ABIOTIC STRESS, SUCCUMB IN OUR CITIES. IS THE ROLE OF NITRIC OXIDE IN THE SYMBIOSIS DETERMINANT IN THIS PARADOX?**

**Catalá M<sup>1</sup>, Gasulla F<sup>2</sup>, García-Breijo F<sup>3</sup>, Reig-Armiñana J<sup>2</sup>, Rodríguez-Díaz C<sup>1</sup>, Barreno E<sup>2</sup>**

<sup>1</sup>*Universidad Rey Juan Carlos, Biología Celular, Dpto. Biología y Geología, (ESCET), Madrid*

<sup>2</sup>*Universitat de València, Dpto. Botánica, ICBIBE-Jardí Botànic, Dr. Moliner 50, 46100-Burjassot, Valencia*

<sup>3</sup>*Universidad Politécnica de Valencia, Dpto. Ecosistemas Agroforestales, Camino de Vera s/n, 46020-Valencia*

Lichens can be considered as ecosystems where symbiotic interaction results in qualities not found in isolated partners, allowing them to live in habitats previously unavailable. Adapted to extreme environments, they are the organisms that have survived the longest in outer space thanks to extraordinary abiotic stress defence systems. The ability of lichens to tolerate and even bioaccumulate heavy metals combined with their extraordinary capability to grow in a large geographical range, rank them among the best bioindicators. However, air pollution impairs lichen colonization and survival. SO<sub>2</sub>, O<sub>3</sub> and NO<sub>x</sub> have been related with lichen damage but the toxicity mechanisms are virtually unknown. The toxic effects of volatile organic pollutants (VOPs) on lichens have not been addressed yet. NO was regarded by plant biologists and lichenologists as a pollutant causing damage. Recent advances have demonstrated that endogenous NO is involved in signal transduction, cell death, ROS production and degradation among others. We demonstrated that NO plays a role in the regulation of lichen oxidative stress during rehydration, especially relevant when the VOP cumene hydroperoxide is present. Similar experiments with Pb show NO is not involved. Lichen's lack of dermal structures allows the entry of all the substances carried by water during rehydration, including pollutants that could interfere with the highly reactive NO. Pollution is a complex mixture, the combined effects of VOPs and ozone, nitrogen or sulphur oxides could be especially deleterious. Given that NO regulates response to VOPs, NO dysregulation could be devastating in polluted environments.

## **ROLE OF PEROXISOMES AS SENSORS OF CELLULAR STRESS**

**Luisa M. Sandalio, María Rodríguez, Luis A. del Río, María C. Romero**

*Department of Biochemistry and Cellular and Molecular Biology of Plants,  
Estación Experimental del Zaidín, CSIC, Apdo. 419, E-18008 Granada, Spain*

Peroxisomes are very dynamic and metabolically active cell organelles and are a very important source of reactive oxygen species (ROS) mainly produced in different endogenous metabolic pathways, such as fatty acid  $\beta$ -oxidation, photorespiration, nucleic acid catabolism, ureide metabolism, etc [1].

ROS were originally associated to oxygen toxicity but today it is known that these reactive species also play a central role in signaling networks regulating essential processes in the cell [1]. Peroxisomes have the capacity to rapidly produce and scavenge  $H_2O_2$  and  $O_2^-$  radicals which allows regulating dynamic changes in ROS levels [1] and are able to adjust their metabolism depending on developmental stage and environmental cues, which make peroxisomes a key switch of cellular signal transduction. Peroxisomes can perceive different signals and regulate their metabolism, morphology and proliferation, although the mechanisms involved and the role of ROS and post-translational modification of peroxisomal proteins in these processes is still unknown [1].

The use of catalase loss-of-function mutants has allowed to study the consequences of changes in the level of endogenous  $H_2O_2$  in peroxisomes and has improved our knowledge of the transcriptomic profile of genes regulated by peroxisomal ROS. Using *Arabidopsis thaliana* mutants deficient in glycolate oxidases and acyl-CoA oxidases, transcriptomic studies in plants under stress conditions have shown differential responses in various sources of peroxisomal ROS. It is now known that peroxisomal ROS participate in more complex signaling networks involving calcium, hormones, and redox homeostasis which finally determine the response of plants to their environment.

[1] Sandalio LM, Rodríguez-Serrano M, Romero-Puertas MC, del Río LA (2013) Role of peroxisomes as a source of ROS signaling molecules. In: *Peroxisomes and their Key Role in Cellular Signalling and Metabolism*. Springer ISBN 978-94-007-6888-8.

[Supported by ERDF-cofinanced grants from the MEC BIO2008-04067 and BIO2012-36742, and Junta de Andalucía BIO-337]

## **ANTIOXIDANTS, ROS AND RNS METABOLISM IN PEPPER FRUITS**

**José M. Palma, Francisco J. Corpas, Luis A. del Río**

*Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Apdo. 419, 18080 Granada, Spain*

*e-mail: josemanuel.palma@eez.csic.es*

Pepper (*Capsicum annuum* L.) is the second-most consumed vegetable worldwide and contains high levels of vitamin C and pro-vitamin A. Much of the information available on the antioxidants features of pepper fruits has been obtained from investigations addressed to elucidate their influence on the human health due to the fruit quality [1]. However, little is still known on the role of antioxidants on the fruit metabolism and under stress conditions. In this work, an overview of antioxidants and the metabolism of ROS and RNS in pepper fruits is given.

Fruit ripening is associated to changes in the antioxidant status where glutathione, NADPH and ascorbate play important roles. Thus, the involvement of NADPH-dehydrogenases has been proved in this physiological process [2], where ascorbate seems to participate as a redox buffer contributing to the stability of fruits [3].

Besides, results on the ROS metabolism and proteomics of fruit peroxisomes suggest that these organelles could have a relevant function in maturation. On the other hand, data on pepper fruits developed *in plants* under different environmental conditions reveal that the antioxidant systems participate in the response of fruits against temperature changes [3].

RNS appear to be also involved in ripening of fruits. Thus, the patterns of endogenous NO production, S-nitrosothiols content and nitro-tyrosine profile are influenced during maturation. Furthermore, the treatment with NO retards ripening with no negative effects on the physiology of fruits.

[1] Mateos et al (2013) *Int. J. Mol. Sci.* 14:9556-9580.

[2] Mateos et al (2009) *Physiol. Plant.* 135:130-139.

[3] Palma et al (2011) *Funct. Plant Sci. Biotechnol.* 5:56-61.

## THE IMPORTANCE OF MITOCHONDRIAL REDOX SYSTEMS AND ANTIOXIDANTS UNDER SALT STRESS: REGULATION BY S-NITROSYLATION

**Sevilla F<sup>1</sup>, Camejo D<sup>1</sup>, Ortiz-Espín A<sup>1</sup>, Romero-Puertas MC<sup>2</sup>, Lázaro JJ<sup>2</sup>, Jiménez A<sup>1</sup>**

<sup>1</sup>*Departamento de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, E-30100, Murcia, Spain*

<sup>2</sup>*Dpt. Biochemistry, Cellular and Molecular Biology of Plants, EEZ-CSIC, E-18080 Granada, Spain*

Increased reactive oxygen species (ROS) production is thought to be a common effect of all, abiotic stresses and higher ROS levels under salt stress has been reported. Together with ROS, nitric oxide (NO) has emerged as a key of the signal transduction induced by stress conditions. During the past few years, S-nitrosylation, the covalent and reversible binding of NO to the thiols of reduced reactive Cysteine residues, is considered as an important post-translational modification and a strong links between mitochondrial antioxidant defenses and plant salinity tolerance was reported. However, the S-nitrosylation pattern of mitochondrial proteins during progression of salt stress was not investigated. In this work we identified the protein targets of S-nitrosylation in leaf mitochondria of pea plants subjected to 150 mM NaCl for 5 and 14 days. The activities of mitochondrial Mn-SOD and Trxo1 together with the *in vivo* activities of the alternative pathway (AP) and the cytochrome pathway (CP) were also determined. A differential pattern of target proteins was found during plant development and salt stress, with a minor number of S-nitrosylated proteins at 14 days specifically some key enzymes related to respiration and photorespiration. An enhancement in NO measured by fluorimetry and confocal microscopy was observed in leaves, being part of the NO localized in mitochondria. An increase in mitochondrial GSNOR activity was produced in response to short and long-term NaCl treatment, where a higher number of nitrated proteins were also observed. The results indicated that posttranslational modifications seem to modulate respiratory and photorespiratory pathways, as well as some antioxidant enzymes, through differential S-nitrosylation/denitrosylation in control conditions and under salt stress.

(This work was supported by MICINN (BFU2011-28716) and Seneca Foundation, Murcia (04553/GERM/06), Spain



## **LIPIDS AND REDOX SIGNALING IN AGING AND LONGEVITY**

***Reinald Pamplona***

*Dept. of Experimental Medicine, University of Lleida-Biomedical Research Institute of Lleida, 25198 Lleida, Spain. E-mail: reinald.pamplona@mex.udl.cat*

This presentation begins with the premise that an organism's life span is determined by the balance between two countervailing forces: (i) the sum of destabilizing effects and (ii) the sum of protective longevity-assurance processes. Against this backdrop, the role of electrophiles is discussed, both as destabilizing factors and as signals that induce protective responses. Because most biological macromolecules contain nucleophilic centers, electrophiles are particularly reactive and toxic in a biological context. The majority of cellular electrophiles are generated from polyunsaturated fatty acids by a peroxidation chain reaction that is readily triggered by oxygen-centered radicals, but propagates without further input of reactive oxygen species (ROS). Thus, the formation of lipid-derived electrophiles is proposed to be relatively insensitive to the level of initiating ROS, but to depend mainly on the availability of peroxidation susceptible fatty acids. This is consistent with numerous observations that life span is inversely correlated to membrane peroxidizability, and with the hypothesis that electrophiles may constitute the mechanistic link between high susceptibility of membrane lipids to peroxidation and shortened life span. Experimental interventions that directly alter membrane composition (and thus their peroxidizability) or modulate electrophiles levels have the expected effects on life span, establishing that the connection is not only correlative but causal. Specific molecular mechanisms are considered, by which electrophiles could (i) destabilize biological systems via non-targeted reactions with cellular macromolecules and (ii) modulate signaling pathways that control longevity-assurance mechanisms.

## ***Caenorhabditis elegans* AS A MODEL TO STUDY *IN VIVO* ANTIOXIDANT EFFECT OF FUNCTIONAL FOOD INGREDIENTS**

**Daniel Ramón, Patricia Martorell, Silvia Llopis, Nuria González, Pepa Ortiz and Salvador Genovés**

*Biopolis SL*

*C. elegans* is considered an excellent model to study biological problems, not only because its genome and most of its corresponding genes are well-defined and have human orthologs, but also because this worm has been shown to have the same homeostatic control mechanisms as humans. Moreover, their short lifespan (21 days), together with body transparency and ease of culture in agar dishes, encourage their increasingly common use to evaluate potential therapeutical approaches.

In this communication we will describe the use of *C. elegans* as an *in vivo* model for the study of the antioxidant effect of several functional food ingredients, including polyphenols, peptides, fatty acids and probiotics. In these examples, transcriptomic and metabolic analysis combined with the use of several *C. elegans* mutants have been powerful tools for the search of the molecular pathways that are affected by the ingredient and the definition of the corresponding biomarkers.

## **OXIDATIVE STRESS IN IMMUNOSENESCENCE AND ITS INVOLVEMENT IN THE RATE OF AGEING**

**Mónica De la Fuente**

*Department of Physiology. Faculty of Biology. Complutense University of Madrid*

The principal cause of ageing is oxidative stress and we have suggested that this occurs in immune cells with age. Indeed, we have observed an age-related oxidative stress situation in peripheral blood leucocytes from men and women, as well as in peritoneal immune cells of mice (in a longitudinal study). Moreover, in mice we have observed that these redox changes in peritoneal leucocytes are shown by those in spleen and thymus as well as the redox state of other organs (liver, heart, brain,...). Due to the fact that the functional capacity of the immune system is a good marker of the health of each subject, the age-related changes of the immune system, which is called “immunosenescence”, have been proposed as “biological age” markers. Using several models of premature immunosenescence as well as long lived subjects we have shown that the life span of an individual is related to the functional capacity of its immune cells, and this depends on their redox state. Thus, in the recent oxidation-inflammation theory of ageing the oxidative and inflammatory stress situations of the immune cells seem to be involved in the rate of ageing and consequently in the life span of each individual. As life span depends on life style factors to a high degree, several strategies such as nutrition, physical exercise, control of emotional stress, social relations and other hormetic approaches, improve the function and redox state in immune cells from humans and mice, increasing the life span of the latter.

## **OXIDATIVE STRESS AND ANTIOXIDANT RESPONSE IN CELLS FROM SEGMENTAL PROGERIA DISEASES**

**Federico V. Pallardó**

*Departamento de Fisiología. Facultad de Medicina y Odontología de Valencia. Universidad de Valencia. Av. Blasco Ibañez 15. 46010 Valencia (ESPAÑA)*

Werner Syndrome (WS), Atypical Werner Syndrome (AWS) and X-linked Dyskeratosis Congenita (DKC), are segmental progerias in that they resemble some, but not all aspects of aging. All are uncommon, autosomal recessive human genetic disease that mimics premature aging in some organs or tissues. Here, we describe the oxidative stress profile and antioxidant defence system of different cell lines from patients with progeroid features. A cell line from an AWS patient with mutation in *LMNA* gene in heterozygosis, a second cell line from a WS patient with truncated *Wrn* protein and finally a third cell line with a polymorphism in heterozygosis in *WRN* gene although able to produce the *Wrn* protein with replacement (F1074L). In DKC telomerase activity is impaired due to the mutation in dyskerin, one of the proteins of the telomerase complex. Two cell lines from DKC patients are used, one of them has the telomerase activity restored. Here we present results showing a decrease in cell proliferation and the increase in oxidative stress determine by the GSSG/GSH ratio, and malondialdehyde and the nitrosylation of proteins in those cells from patients. The antioxidant response after determination of the mRNA level, expression of the protein and measurement of the enzymatic activity of SOD, catalase, GPx, and the glutaredoxin and thioredoxin expression was impaired in the different cell lines studied when compared to controls. We report changes in different epigenetic markers in DKC cells. The restoration of telomerase activity in DKC rescues the oxidative stress phenotype.

# **ORAL COMMUNICATIONS**

## **O1 CELLULAR OXIDATIVE STRESS AND ITS RELATIONSHIP WITH IDIOPATHIC FEMORAL OSTEONECROSIS**

**Pastor S<sup>1</sup>; Silvestre A<sup>1,2</sup>; Carrasco J<sup>2</sup>; Dasí F<sup>1,3</sup>; Gomar-Sancho F<sup>1,2</sup>**

<sup>1</sup>FIHCUV/INCLIVA; <sup>2</sup>UVEG Facultad de Medicina. Dept. Cirugía; <sup>3</sup>UVEG Facultad de Medicina. Dept. Fisiología.

**Background:** Non-traumatic idiopathic osteonecrosis of the femoral head (ONFH) results from the interruption of blood flow to the bone leading to the cell death of bone components. Chondral collapse occurs in 75-80% of the untreated-cases resulting in bone destruction, pain and loss of joint function. Recent studies in animal models suggest that oxidative stress may be involved in the development of the disease. There are no, however, studies in human subjects that have examined the correlation between oxidative stress and the development of ONFH.

**Objectives:** To evaluate the role of oxidative stress in the pathogenesis of human ONFH.

**Methods:** The oxidative stress status was determined in blood and urine of 60 patients with ONFH and 60 healthy subjects adjusted for sex and age. Oxidized vs. reduced (GSSG/GSH) glutathione ratio, 8-oxo-7, 8-dihydro-2 deoxyguanosine (8-OHdG), malondialdehyde (MDA), 15-isoprostane F2t levels and oxidized proteins (OP).

**Results:** A significant increase in the GSSG/GSH ratio, 8-OHdG, 15-isoprostane F2t, OP and MDA levels was observed in ONFH patients, although in the last case the differences were not statistically significant.

**Conclusions:** Oxidative stress parameters are increased in ONFH patients compared to controls, suggesting that oxidative stress may be involved in the pathogenesis of the disease.

**Funding:** FIS-ISCIPII PI10/02600

## O2 OXIDATIVE STRESS IS INCREASED IN SERUM OF PATIENTS WITH ALPHA-1 ANTITRYPSIN DEFICIENCY

**Contreras S<sup>1</sup>; Escribano A<sup>1,2,3</sup>; Amor M<sup>1,2,3</sup>; Codoñer-Franch P<sup>2,4</sup>; Sanz F<sup>5</sup>; Navarro-García MM<sup>6</sup>; Dasí F<sup>1,7</sup>**

*<sup>1</sup>FIHCUV/INCLIVA; <sup>2</sup>UVEG School of Medicine. Dept. Pediatrics, Obstetrics and Gynecology; <sup>3</sup>HCUV, Department of Pediatrics, <sup>4</sup>Hospital Dr. Peset of Valencia, Department of Pediatrics, <sup>5</sup>General Hospital of Valencia, Service of Pneumology; <sup>6</sup>UVEG School of Journalism; <sup>7</sup>UVEG School of Medicine. Dept. Physiology.*

**INTRODUCTION:** Despite the control of environmental factor such as smoking, there is a great variability in the severity of emphysema in patients with alpha-1 antitrypsin deficiency (AATD), indicating that there may be other mechanisms in addition to the classical protease-antiprotease imbalance that contribute to the development of emphysema. Results in animal models indicate that when AAT levels are low, the antioxidant defenses of the lung are very important in deciding the severity of the emphysema.

**OBJECTIVE:** To determine the profile of oxidative stress in serum of patients with AATD and correlate with their phenotype.

**MATERIAL AND METHODS:** Prospective, longitudinal study including children diagnosed with AATD at the children's pulmonology (HCUV) and Gastroenterology (Hospital Dr. Peset) units. Children were classified into three risk groups of lung and/or liver disease: low (phenotypes MM, MS, and SS), intermediate (MZ and SZ) and high (ZZ). Total glutathione, reduced vs. oxidized glutathione (GSSG/GSH), 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA), oxidized proteins (OP) and antioxidant capacity against oxygen radicals (ORAC) were determined in serum of AATD patients. Results are expressed as median and interquartile range.

**RESULTS:** 47 children with DAAT were included in the study. High-risk (ZZ) patients showed significantly higher GSSG/GSH, 8-OHdG, MDA, OP and lower total glutathione levels than low risk patients. Intermediate-risk patients showed intermediate oxidative stress values between high and low risk patients.

**CONCLUSIONS:** Oxidative stress parameters increase correspondingly in patients diagnosed with AATD, according to its phenotype (from lowest to highest risk), regardless of the clinical expression, indicating that oxidative stress can play an important role in the pathogenesis of the disease.

**FUNDING:** Sociedad Valenciana de Neumología, GVA AP-096/11 and FIS-ISCIPII P111/02884.

**O3 Lafora disease fibroblasts exemplify the molecular interdependence between thioredoxin 1 and the proteasome in mammalian cells**

**Marta Seco-Cervera**

*CIBERER. Centro de Investigación Biomédica en Red de Enfermedades Raras. Valencia. Spain.*

*FIHCUV-INCLIVA. Valencia. Spain*

*Dept. Physiology. Medicine School. University of Valencia. Valencia. Spain*

Thioredoxin 1, Trx1 is a key regulator of cellular redox balance and participates in cellular signaling events. Recent evidence in yeast have shown that members of the Trx family interact with the 20S proteasome, indicating redox regulation of proteasome activity. However, there is little information about the interrelationship of Trx proteins with the proteasome system in mammalian cells, especially in the nucleus.

Here, we show that Trx1 levels and its subcellular localization (cytosol, endoplasmic reticulum and nucleus) depend on the proteasome activity during cell cycle in NIH3T3 fibroblasts, endoplasmic reticulum stress conditions, when proteasome was inhibited. In addition, we also studied how the main cellular antioxidant systems and proteotoxic stress response are stimulated when proteasome activity is inhibited in NIH3T3 cells. Finally, we describe how Trx1 levels are decreased in Lafora fibroblasts. In addition, nuclear co-localization of Trx1 with 20S proteasome in laforin deficient cells was altered compared with control cells. Our results indicate a close relationship between Trx1 and 20S nuclear proteasome and give a new perspective to study diseases or physiopathological conditions in which defects in the proteasome system are associated to oxidative stress.



---

**O4 LONG LIFE SPONTANEOUS EXERCISE DOES NOT PROLONG LIFESPAN BUT PREVENTS FRAILITY IN C57BL/6J. HEALTHSPAN vs LIFESPAN.**

**García-Valles R<sup>1</sup>., Sanchis-Gomar F<sup>1</sup>., Martínez-Bello VE<sup>1</sup>, Pareja-Galeano H<sup>1</sup>., Brioché T<sup>1</sup>., Ferrando B<sup>1</sup>., Cabo H<sup>1</sup>., Salvador-Pascual A<sup>1</sup>., Monleon D<sup>2</sup>. Morales J.M<sup>2</sup>., Díaz A<sup>3</sup>., Noguera I<sup>3</sup>, Gómez-Cabrera MC.<sup>1</sup> and Viña J<sup>1</sup>.**

*<sup>1</sup>Department of Physiology. University of Valencia; <sup>2</sup>Fundacion Investigacion Hospital Clinico Universitario/ INCLIVA. Valencia. Spain; <sup>3</sup>UCIM. University of Valencia.*

The increase in life expectancy has resulted in an increase in age-related diseases and larger number of dependent people. The aim of our study was to determine whether life-long spontaneous exercise affects lifespan and the retardation of frailty in mice. Male C57Bl/6Jmice, were randomly assigned in two groups(n=72): sedentary or spontaneous wheel-runners. We compared the sedentary vs the active lifestyle in terms of longevity and developed a test to quantify frailty in our mice by evaluating unintentional weight loss, exhaustion and slowness, weakness, and low physical activity. Sedentary animals became frail as they aged. Although life-long spontaneous exercise didn't prolong longevity it prevented frailty. This improvement was accompanied by a significant increase in the mitochondrial biogenesis cell signaling pathway in skeletal muscle and the levels of BDNF. Free radicals have a role in causing aging. Thus, we aimed to study the induction of two well-known longevity genes, the antioxidant enzymes MnSOD and GPx. We used: RT-PCR and the determination of the enzymatic activities. We found differences in the activity of the antioxidant enzymes as mice grew older. The results support the idea that exercise training is an antioxidant because it induces the endogenous antioxidant defense. We studied the effect of spontaneous wheel-running on plasma metabolic profile in 20months old mice using nuclear magnetic resonance. We found a significant decrease in glucose, lactate and alanine, whilst a significant increase in glycerol, citrate and fatty-acids levels was observed in the spontaneous wheel-running mice. The differences in the circulating metabolome indicate better metabolic health in the exercised ones.

Acknowledgements: This work was supported by grants SAF2010-19498, ISCIII2006-RED13-027, PROMETEO2010/074, 35NEURO GentxGent and EU Funded COSTB35 and CM1001. This study has been co-financed by FEDER funds from the European Union.

---

**O6 ROS PRODUCTION IN HYPOXIA, A MATTER OF MITOCHONDRIA, SUPEROXIDE AND MINUTES**

**Hernansanz-Agustín P<sup>1</sup>, Izquierdo-Álvarez A<sup>1</sup>, Sánchez-Gómez FJ<sup>2</sup>, Lamas S<sup>2</sup>, Bogdanova A<sup>3</sup> and Martínez-Ruiz, A<sup>1</sup>.**

*<sup>1</sup>Servicio de Inmunología, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IP), Madrid, Spain*

*<sup>2</sup>Laboratorio Mixto CSIC-FRIAT de Fisiopatología Vascul ar y Renal, Centro de Biología Molecular Severo Ochoa, Campus Universidad Autónoma, 28049 Madrid, Spain*

*<sup>3</sup>Institute of Veterinary Physiology, Vetsuisse Department and Zurich Center for Integrative Human Physiology (ZIHP), University of Zurich, Zurich, Switzerland*

Eukaryotic cells can sense the reduction of oxygen availability, called hypoxia, and trigger a series of responses in order to adapt to it, including the limitation of oxygen consumption and switch to glycolytic metabolism. Most of these adaptations are mediated by the hypoxia-inducible factors (HIFs), which are a family of transcriptional factors that regulate gene expression after their stabilization. In parallel to the discovery of these proteins, a paradoxical increase in reactive oxygen species (ROS) was associated with their stabilization in hypoxia. Since then, many groups have focused into this and whereas experimental data by some support an increase in ROS in hypoxia, others data refuse it. We have observed that when different type of cells, ranging from tumour cells to primary endothelial cells and cardiomyocytes, are subjected to acute hypoxia, there is an increase in the superoxide production during the first 10 minutes, probably originated in mitochondria, and a subsequent decrease up to the basal level. These data were confirmed by different techniques, such as HPLC and Live imaging. Several probes were also used in order to confirm the mitochondrial localization. This could help to explain the paradoxical increase of ROS in hypoxia, since they are formed mainly in the first minutes when remaining oxygen could still be used to produce superoxide. Our results also could bring together the apparently divergent results found by different groups that have not taken into account the time scale of ROS detected. We propose that cells produce an initial superoxide burst when they are subjected to hypoxia, which may be translated into oxidative signals contributing to hypoxia adaptation.

## **O7 OXIDATIVE STRESS AND CELL CYCLE DYSREGULATION IN HEPATOCYTES**

**Ana M. Tormos**<sup>(1)</sup>, **Raquel Talens-Visconti**<sup>(2)</sup>, **Ana Bonora-Centelles**<sup>(1)</sup>,  
and **Juan Sastre**<sup>(1)</sup>.

<sup>1</sup>*Dpt. Physiology, Pharmacy. University of Valencia*

<sup>2</sup>*Dpt. Pharmacology and Pharmacotechnology, Pharmacy. University of Valencia.*

ROS signaling can activate or suppress cell cycle progression depending on the activation stimulus. Exposure of primary rat hepatocytes up to 25  $\mu\text{M}$   $\text{H}_2\text{O}_2$ , which is higher than the physiological levels of pro-oxidants achievable in hepatocytes, results in dose- and time-dependent cytotoxicity. Nowadays, the most common technique used for the isolation of primary hepatocytes is the collagenase digestion method. The difficulty of hepatocytes to proliferate in culture is probably due to the increase in the ploidy and binucleated cells. Isolation of with 5mM NAC reduced in a 14% the presence of polyploid cells, demonstrating that the oxidative stress generated during hepatocyte isolation acts as a proliferative agent. However, hepatocytes went through mitosis but not through cytokinesis. The blockage or alteration of the cytokinetic process ends with the appearance of polyploid primary hepatocytes.

We also tested the effect of oxidative stress in our model of liver specific p38 $\alpha$  knock-out mice. p38 $\alpha$  knock-out mice, which are characterized by higher levels of ROS than the control ones, underwent bile duct ligation and partial hepatectomy. Our experiments showed an increased rate of binucleated cells in the p38 $\alpha$  knockout livers and higher mitotic index. That was indicating that p38 $\alpha$  knockout mice had difficulties to complete mitosis due to cytokinesis impairment.

Thus, although p38 MAPK signalling has been mainly characterized as an inhibitor of cell proliferation and a potential tumor suppressor, the absence of p38 $\alpha$  may block or delay cell cycle progression at different stages, including cytokinesis, as observed by the high binucleation rate in liver specific p38 $\alpha$  knock-out mice. In addition, ROS may act downstream of p38 MAPK modifying pathways which control cell division.

**O8 HISTONE H3 GLUTATHIONYLATION IS INVOLVED IN THE REGULATION OF NUCLEOSOME. THE REDOX CONTROL OF CHROMATIN**

**José Luis García-Giménez**

Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER)

Instituto de Investigación Sanitaria INCLIVA

Dept. Fisiología. Facultat de Medicina i Odontologia. Universitat de València.

Chromatin is a dynamic structure formed by DNA and histones. Histone post-translational modifications (PTMs) are mechanistically involved in the regulation of chromatin compaction. However, little is known about the redox-related PTMs in histones. Histone H3, the only nucleosomal protein that possesses cysteine(s), can be modified by glutathione. Using Biotin labelled glutathione ethyl ester (BioGEE) treatment of nucleosomes *in vitro*, we show that glutathione, the most abundant antioxidant in mammals, binds to histone H3. Glutathionylation of H3 is maximal in fast proliferating cells, correlating well with enhanced levels of H3 glutathionylation in different tumor cell lines. We demonstrate biochemically and by mass spectrometry that histone variants H3.2/H3.3 are glutathionylated on their cysteine110. Furthermore, circular dichroism, thermal denaturation of reconstituted nucleosomes, and molecular modeling indicate that glutathionylation of histone H3 produces structural changes affecting nucleosomal stability. Additionally, we studied the glutathionylation of H3 *In Vivo*, using two different aging mice models. In conclusion, Histone H3 senses redox cellular changes. Its glutathionylation induces changes in nucleosome stability opening the chromatin during cell proliferation. Thus, the nucleosome is able to sense cellular redox changes.

**O9 ROLE OF miR-433 IN THE REGULATION OF BOTH  $\gamma$ -GLUTAMATE-CYSTEINE-LIGASE SUBUNITS IN REDOX RESPONSES.**

**Cristina Espinosa, Marta Fierro, Francisco Sánchez and Santiago Lamas**

*Centro de Biología Molecular Severo Ochoa. Madrid*

The microRNAs are small non coding RNA, that generally bind to the 3' untranslated region of different target mRNAs. Some microRNAs are capable to regulate proteins related with oxidative stress in the vascular endothelium and in other tissues that can be affected by reactive oxygen species. However, the regulation of glutathione synthesis related enzymes by microRNAs largely unknown.

One of the key enzymes in glutathione biosynthesis is  $\gamma$ -glutamate-cysteine-ligase (GCL), which carries out the first and limiting step in this synthesis. GCL is a heterodimeric protein with a catalytic subunit (GCLc) and a modifier one (GCLm). The transcriptional regulation of both of them has been previously described, but little is known about microRNAs and GCL expression. The "in silico study" of the 3' untranslated region of GCLc and GCLm provided a list of candidates that are conserved in mammals . Among them, we chose miR-433. Overexpression of this microRNA in endothelial cells showed that GCLc and GCLm expression were decreased. To study if this decreased of microRNAs was mediated by direct or indirect mechanism we cloned the GCLc and GCLm 3'-UTRs human sequences in the pSICHECK2 reporter vector. We observed a significant decrement in luciferase activity after transfection with miR- 433 in the GCLc and GCLm 3'-UTR. By contrast this effect was when these sites were mutated. Furthermore miR-433 was also capable of down-regulating the activity of GCL leading to reduced GSH levels and increased oxidative stress. Overall, our data provide new insight into the transcriptional regulation of GCLs by identifying miR-433 as a regulatory molecule in glutathione redox homeostasis.

## O10 BIOMECHANICAL FORCES AND ENDOTHELIAL MITOCHONDRIAL FUNCTION

**Rosa Bretón-Romero<sup>1</sup>, Fernando Rodríguez-Pascual<sup>1</sup>, E. Rial<sup>2</sup>, J.A. Enriquez<sup>3</sup>, and Santiago Lamas<sup>1</sup>**

<sup>1</sup>*Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM), Madrid, Spain.*

<sup>2</sup>*Centro de Investigaciones Biológicas (CSIC), Madrid, Spain.*

<sup>3</sup>*Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.*

Endothelial cells in the vascular system are constantly subjected to the frictional force of shear stress due to the pulsatile nature of blood flow. Laminar shear stress (LSS) is a protective hemodynamic regulator of endothelial function, and limits the development of different inflammatory diseases related to the redox state. Although several endothelial proteins or structures have been proposed as potential shear stress *mechano-sensors*, the identification of mechano-transducing molecules is still a matter of active research. We demonstrated that LSS activates intracellular signaling pathways that modulate not only mitochondrial dynamics but also mitochondrial function. At early time points of LSS, the fission-related protein DRP1 is activated by phosphorylation and recruited from the cell cytosol to mitochondriae increasing its activity, and thus, enhancing mitochondrial fission. Since alterations in mitochondrial dynamics have been related with changes in bioenergetic profiles, we studied the bioenergetic modulation of mitochondriae induced by LSS. We found that SS induced a decrease in  $O_2^-$  consumption rate which correlated with a transient increase in the mitochondrial membrane potential and an increment in mitochondrial reactive oxygen species. Although high levels of ROS have been described to be toxic, substantial evidence suggests that a transient generation of  $H_2O_2$  may act as an intracellular messenger regulating endothelial function. Our data support that LSS-mediated mitochondrial ROS production activates the highly specific peroxide sensor PRX3, which is located exclusively in the mitochondria. Our study provides insight into the importance of mitochondrial mechanosensing and proposes a role for mitochondrial ROS signaling in endothelial responses to LSS.

## O11 NOX4 IS A TUMOR SUPPRESSOR FACTOR IN THE LIVER

**Eva Crosas-Molist<sup>1</sup>, Esther Bertran<sup>1</sup>, Patricia Sancho<sup>1#</sup>, Judit López-Luque<sup>1</sup>, Joan Fernando<sup>1</sup>, Aránzazu Sánchez<sup>2</sup>, Margarita Fernández<sup>2</sup>, Estanis Navarro<sup>1</sup>, Isabel Fabregat<sup>1\*</sup>.**

<sup>1</sup>*Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.* <sup>2</sup>*Dep Bioquímica y Biología Molecular II, Facultad de Farmacia, Universidad Complutense, Instituto de Investigación Sanitaria del Hospital Clínico San Carlos, IdISSC, Madrid, Spain.* #. *Present address: Spanish National Cancer Research Center (CNIO), Madrid, Spain.*

The NADPH oxidase (NOX) family has emerged in the last years as an important source of reactive oxygen species (ROS) in signal transduction. NOX4 has been implicated in a variety of physiological processes, including cellular migration, differentiation and senescence, regulation of apoptosis or endoplasmic reticulum stress, as well as in pathological processes, particularly fibrosis. We previously found that in liver cells NOX4 up-regulation is necessary for TGF- $\beta$ -induced suppressor effects, in particular, for cell death. In order to analyze whether NOX4 may play a general tumor suppressor role in a TGF- $\beta$ -independent manner, we generated human liver tumor cells where NOX4 expression was attenuated, by means of stable expression of specific shRNAs. The analysis of their tumorigenic capacity in athymic mice suggested that NOX4 depletion conferred advantage to hepatocarcinoma cells to form earlier and bigger tumors *in vivo*. Here we show that NOX4 expression was also down-regulated under physiological proliferative situations of the liver, such as regeneration after partial hepatectomy in mice. *In vitro* assays proved a crucial role for NOX4 in inhibiting cell proliferation, through the control of p21(CIP1/WAF1) and Cyclin D1, as well as in mediating cell death. Moreover, it is worthy to note that lack of NOX4 decreased the capacity of cell-to-cell or cell-to-matrix adhesion and increased cell migration. Overall, results described here strongly suggest that NOX4 would play an essential suppressor role in the liver.

This work was supported by grants to I.F. from the Ministry of Economy and Competitiveness (MECC), Spain (BFU2009-07219 and ISCIII-RTICC RD06/0020) and AGAUR-Generalitat de Catalunya (2009SGR-312). E.C.-M. was recipient of a pre-doctoral fellowship from the FPU program, Ministry of Education, Culture and Sport, Spain. J.F. and J.L.-L. were recipient of a predoctoral contract from IDIBELL.

## O12 PROTEIN NITRATION IN PEPPER FRUITS AND ITS INVOLVEMENT IN RIPENING

Paz Álvarez de Morales<sup>1</sup>, Mounira Chaki<sup>1</sup>, Juan B. Barroso<sup>2</sup>,  
Francisco J. Corpas<sup>1</sup>, José M. Palma<sup>1</sup>

<sup>1</sup>*Departamento de Bioquímica, Biología Celular y Molecular de Plantas, Estación Experimental del Zaidín, CSIC, Granada*

<sup>2</sup>*Unidad Asociada de Señalización Molecular y Sistemas Antioxidantes en Plantas, Universidad de Jaén, Campus Las Lagunillas, Jaén*

E-mail: paz.alvarez@eez.csic.es

Nitration is a post-translational modification promoted by reactive nitrogen species which occurs in plant tissues under both physiological and stress conditions thus being an important process of protein regulation. Nevertheless, nitration alters the protein activity and, when it is overproduced under adverse circumstances, it has been associated to nitro-oxidative stress both in animals and plants [1-3]. In fact, nitrated proteins can participate in the modulation of cell signalling as an ROS intermediary in several processes [1].

Pepper (*Capsicum annuum* L.) fruits are the second worldwide consumable vegetables and excellent sources of many essential nutrients for humans, especially vitamin C,  $\beta$ -carotene and calcium. During ripening of pepper fruits a number of physiological/metabolic changes occur, the most visible one being the shift from green colour to red, yellow, and orange, depending on the variety [4].

In this work, using green and red mature pepper fruits from California type, the pattern of nitrated proteins was investigated. A notable protein tyrosine nitration always accompanied maturation of fruits. The characterization of nitrated proteins by proteomic analysis combining 2-D electrophoresis, western blotting with an antibody against nitro-tyrosine, and MALDI-TOF mass spectrometry allowed identifying a total of 12 proteins. The effect of nitration in the enzyme activity of some of them was studied.

[1] Ischiropoulos and Gow (2005) Toxicology 208:299-303.

[2] Corpas et al (2013) Front. Plant Sci. 4:29.

[3] Chaki et al (2009) J. Exp. Bot. 60:4221-4234.

[3] Mateos et al (2013) Int. J. Mol. Sci. 14:9556-9580.

[Supported by the ERDF-cofinanced grant AGL2011-26044, Ministry of Economy and Competitiveness, Spain]



**O13 LICHEN PHYCOBIONTS DEPEND ON MYCOBIONT-DERIVED NO TO PROTECT THEIR CHLOROPHYLL FROM PHOTOOXIDATION. ECOLOGICAL BASIS FOR SYMBIOSIS EVOLUTION?**

**Martín San Román S<sup>1</sup>, Catalá M<sup>1</sup>, Martínez-Alberola F<sup>2o</sup>, Gasulla F<sup>2</sup>, García-Breijo F<sup>3</sup>, Reig-Armiñana J<sup>2</sup>, Barreno E<sup>2</sup>**

<sup>1</sup>Universidad Rey Juan Carlos, Biología Celular, Dpto. Biología y Geología, (ESCET), Madrid

<sup>2</sup>Universitat de València, Dpto. Botánica, ICBIBE-Jardí Botànic, Dr. Moliner 50, 46100-Burjassot, Valencia  
Universidad Politécnica de Valencia, Dpto. Ecosistemas Agroforestales, Camino de Vera s/n, 46020-Valencia

Lichens are symbiotic organisms composed of at least a heterotrophic fungus (mycobiont) and a photosynthetic photobiont that can be either a cyanobacterium (cyanobiont) or unicellular green microalgae (phycobiont). This partnership endows lichens with a great tolerance to abiotic stress and allows them to colonize extreme environments such as deserts or the tundra. Despite the roles of NO in abiotic stress are especially relevant in eukaryotes, NO production in lichens has only been described recently and its roles are still unknown. Lichens are adapted to anhydrobiosis and repeated desiccation-rehydration cycles. Recent studies by our group show that NO seems to be related with contention of oxidative damage and chlorophyll stabilization during lichen rehydration. While genetic data show the presence of particular isoforms of NOS and NR in both mycobionts and phycobionts, inhibition assays have confirmed the involvement of NOS and NR activities in lipid peroxidation regulation during rehydration. Feelisch & Martin (1995, Trends in Ecology & Evolution, 10: 496-499) postulated that the ability of NO to neutralize O<sub>3</sub> could have an important role in the early evolution. Our results point towards that trend and an involvement of NO in the establishment of symbiotic relationships (mycorrhizae: Bonfante & Requena 2011, Current Opinion in Plant Biology 14: 451-457) has also been described. Could NO be one of the most important keys to the establishment of lichen symbiosis?

**O14 MITOCHONDRIAL *AtTRXo1*: ITS TRANSCRIPTIONAL REGULATION AND ROLE UNDER SALT-STRESS.**

**Ortiz-Espín A<sup>1\*</sup>, Iglesias-Fernández R<sup>2\*</sup>, Calderón A<sup>1</sup>, Sevilla F<sup>1</sup>, Carbonero P<sup>2</sup>, Jiménez A<sup>1</sup>**

\*These authors contributed equally

<sup>1</sup>*Departamento de Biología del Estrés y Patología Vegetal, CEBAS-CSIC, E-30100, Murcia, Spain*

<sup>2</sup>*Departamento de Biotecnología-UPM, E.T.S. Ingenieros Agrónomos, E-28040, Madrid, Spain.*

Thioredoxins (Trxs) are ubiquitous small proteins involved in the reduction of disulfide bonds of other proteins in different cell compartments. Among them, mitochondrial Trxo has been described to have a response in plants grown under salinity but there is scarce information about its functional role in abiotic stress or its gene regulation. In this work, the transcriptional regulation of the mitochondrial *AtTrxo1* gene has been studied for the first time, by producing several *AtTrxo1::GUS* transgenic *Arabidopsis* plants containing conserved domains chosen comparing Brassicacea *Trxo1* promoters. Three motifs were found as possible positive regulators and one as negative of *AtTrxo1* expression. Using an arrayed yeast library from *A. thaliana*, a set of transcription factors were shown as prey of *AtTrxo1* promoter, and among them, we underlined Bzip9 and AZF2, both strongly related with biotic and abiotic stress, respectively. We have also studied the response of mutant *AtTrxo1* plants to salinity, in order to gain insight on its functional role under stress. For it, a physiological characterization of the mutant and the study on its germination pattern was carried out. Results indicated that the lack of *AtTrxo1* produced phenotypic changes and influenced the time to 50% germination under salinity, without affecting the final germination percentage. Results are discussed in relation to *Trxo1* expression pattern and its regulation.