## S7.P15

### Sirtuin 3 interacts with Lon protease and regulates its acetylation state

Marcello Pinti[a], Lara Gibellini[b], Francesca Beretti[b], Gianluca Carnevale[b], Sara De Biasi[b], Ciro Leonardo Pierri[c], Andrea Cossarizza[b]
[a]*Department of Life Sciences, University of Modena and Reggio Emilia, Italy*
[b]*Department of Surgical, Medical, Dental and Morphological Sciences, University of Modena and Reggio Emilia, Italy*
[c]*Department of Biosciences, Biotechnology and Biopharmaceutics*
*E-mail address:* marcello.pinti@unimore.it

Lon is a nuclear-encoded, mitochondrial ATP-dependent protease that degrades oxidized damaged proteins, assists protein folding and participates in maintaining mitochondrial DNA levels. Lon is upregulated at the mRNA level by several stressors, including (but not limited to) ROS, heat shock and hypoxia. However, we have observed that in many cases changes in mRNA levels, protein levels and activity are not directly correlated, suggesting the possibility that Lon activity could be regulated at post transcriptional and translational level. Lysine acetylation has emerged as an important post translational modification used to regulate mitochondrial proteins. While acetylation within mitochondria is basically a non enzymatic process, lysine deacetylation is carried out by sirtuins, a class of $NAD^+$-dependent class III histone deacetylases. Thus, we hypothesized that Lon activity could be regulated by Sirt3, the most important mitochondrial sirtuin. We first analysed the colocalization of Lon and Sirt3 in mitochondria by confocal microscopy, in three different human cell lines (MCF7, RKO, HepG2). The two proteins colocalize, with an estimation of 70% of colocalization signal. In hypoxic conditions, or under oxidative stress, the colocalization signal increased to 80%, at least in RKO cells. Coimmunoprecipitation experiments confirmed a direct interaction between Lon and Sirt3, both in basal conditions, and in the conditions of hypoxia or oxidative stress. While silencing of Lon did not alter Sirt3 levels, silencing of Sirt3 caused a marked increase of Lon acetylation, suggesting that Lon is a target of Sirt3 deacetylation activity. An in silico analysis of Lon primary sequence indicated K357, K361 and K917 as possible acetylation targets. We focused our attention on K917, a Lys on the surface of the catalytic domain of Lon. Protein-protein docking analysis suggested that acetylated K917 can enter the cavity of Sirt3 known to be involved in the deacetylation of the Ac-ACS peptide – a substrate proved to be deacetylated by Sirt3 – and that the same residues involved in the binding and deacetylation of this substrate are involved in interactions with Lon. In conclusion, Lon appeared to be a substrate of Sirt3, and its proteolytic activity could be regulated by the acetylation status of K917.

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## S7.P16

### Chemosensitization of tumor cells using drugs that affect the cellular energy metabolism

Eduardo Rial, Cristina Estañ, Eva Calviño, Carlos Sánchez-Martín, Beatriz Guillén-Guío, Cristina Villar-Martín, Patricio Aller
*Centro de Investigaciones Biologicas, CSIC, Spain*
*E-mail address:* rial@cib.csic.es

The cellular energy metabolism, and mitochondria in particular, is becoming a prominent target for the development of new anticancer strategies [1]. Agents acting on mitochondria, termed "mitocans", include compounds that affect a variety of mitochondrial functions [2]. Some of them are currently undergoing clinical trials. Among the drugs under evaluation are 2-deoxy-d-glucose, 3-bromopyruvate, etomoxir or metformin. However, most of these agents are not normally very effective when used in monotherapy and thus, their clinical use may be restricted to combined therapies as radio- and chemo-sensitizing drugs. Arsenic trioxide (ATO, Trisenox) is a clinically established drug for the treatment of acute promyelocytic leukemia [3] and also of possible interest against other neoplastic diseases [4]. However, its efficacy is frequently limited by the requirement of high doses to effectively induce apoptosis. We have analyzed the chemo-sensitizing efficacy of different mitocans to ATO in HL60 cells, a human acute myeloid leukemia cell line characterized as poorly sensitive to this drug. We show that the above-mentioned mitocans potentiate the lethality of ATO and we analyze the biochemical basis of their chemo-sensitizing action.

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## S7.P17

### Lipotoxic effect of free fatty acid in the in vitro model

Agnieszka Sliwa, Joanna Goralska, Beata Kiec-Wilk, Anna Gruca, Anna Polus, Aldona Dembinska-Kiec
*Jagiellonian University Medical College, Poland*
*E-mail address:* a.sliwa@uj.edu.pl

Western diet is characterized by a high content of fats and carbohydrates and a limited intake of plant products. Nutrient overload (increased free fatty acids and glucose intake) leads to metabolic disorders such as insulin resistance, type 2 diabetes and obesity. FFAs are more than substrates for oxidative metabolism playing a significant role in cell signaling; they influence enzymatic activities, gene expression, ion homeostasis, etc. Excess of fatty acids accompanied by triglyceride accumulation in multiple tissues including adipocytes and pancreatic beta cells results in chronic cellular injury and dysfunction. FFAs increase mitochondrial generation of ROS by depolarization of the mitochondrial inner membrane due to the uncoupling effect. The aim of the study was to investigate the effect of selected free fatty acids on mitochondrial function in murine pancreatic beta cells and human preadipocytes. As an experimental model mouse beta cells (BTC6) line and immortalized human preadipocyte cell line (Chub-S7) was selected. The cells were incubated for 24 h with different concentration of FFAs (PA, AA, EPA). Chub-S7 cells were incubated with lower