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Object: Outline Application MC member-COST Action MITOEAGLE-Belgium UNamur
Profs : Thierry Arnould and Patricia Renard

Dear Prof Gnaiger,
Dear Erich,

As you kindly invited us to describe what could be our interest and contribution for the COST Action MITOEAGLE, here are few sentences to describe our current activities in the group of Organelle Dysfunction ran by Professors Patricia Renard and Thierry Arnould in the Laboratory of Biochemistry and Cell Biology, Namur Research Institute of Life Sciences (https://www.narilis.be/) at the University of Namur (UNamur), Belgium.
While we are interested in cell responses and cell signaling triggered by mitochondrial dysfunction in several pharmacological and genetic models, some of our research aspects deal directly with mitochondria respiration and the way it might be modulated by dynamics and morphology of mitochondria per se, in response to organelle crosstalks with ER or other organelles such as lysosomes when dealing with stress conditions/storage disorders, in response to infection by intracellular bacteria and during stem cell differentiation.

1) Organelle crosstalks : ER stress-Mito and Lysosomal storage disorders/alterations-Mito

ER stress leading to UPR (Unfolded Protein Response) is most likely encountered in many situations (antibody production by plasma B cells, in β-cells overproducing insulin in case of insulin resistance, … but also in more artificial situations such as transfected cells with expression plasmids to overproduce a protein of interest). In this context, we recently studied and reported the impact of a non-lethal and transient ER stress induced by Thapsigargin (TG) or Brefeldin A (BFA) on mitochondrial respiration. We examined the real-time oxymetric changes (mitochondrial oxygen consumption rate in time [OCR]) using a Seahorse XF96 bioenergetic analyzer. We found that the basal respiration was rapidly but transiently decreased in TG-treated cells when compared with control cells. Similarly, basal respiration was decreased in cells incubated for 1 h with 500 nM BFA. Moreover, the coupling efficiency, representing the fraction of the basal respiration that is used for ATP production presented the same pattern. We thus recently demonstrated that, rapidly after the initiation of a non-lethal and

sublethal ER stress, cells seem to reduce oxygen consumption while, when adapted to the ER stress or during recovery, cells have a more efficient OXPHOS system (Vannuvel et al., J. Cell Physiol. 2016). In addition, several molecular markers of mitochondrial alterations such as mitochondria fragmentation of the mitochondrial network can also be identified in tripeptidyl peptidase-1 (TPP-1)-deficient fibroblasts, a lysosomal hydrolase encoded by the gene mutated in the LINCL (late infantile NCL, CLN2 form). This morphological alteration is accompanied by an increase in the expression of the protein BNIP3 (Bcl2/adenovirus E1B 19 kDa interacting protein 3) as well as a decrease in the abundance of mitofusins 1 and 2, two proteins involved in mitochondrial fusion (Van Beersel et al., Biosci Rep. 2013).

In the future and fitting with the goal of WP4 (cultured cells) of MITOEAGLE, we would be interested to measure the respiration of mitochondria according standardized methods in cells with lysosomal or peroxisomal disorders as one axe of our research interests is devoted to the understanding of physical and functional interactions between different organelles and mitochondria in the context of their « normal biology » or in response to a dysregulation of their function/activity. In addition, and combined to our expertise in cell signalling and protein analysis, it would be the opportunity to systemically analyse the respiration and bioenergetics of mitochondria and confront the aspect of different morphologies of the organelle. Indeed, we have data showing that a comparable morphology (fragmented) might be reflected by differences in the OXPHOS activity.

2) Biogenesis of mitochondria and respiration during cell differentiation of stem cells

In the context of cell differentiation (known to be mainly associated with a biogenesis of mitochondria) and more particularly during the hepatogenic differentiation of bone marrow-derived mesenchymal stem cells (BM-MSCs), an increase in the abundance of several mitochondrial proteins, mitochondrial DNA and respiration is observed. Differentiation of these MSCs is thus accompanied by a mitochondrial biogenesis resulting in enhanced mitochondrial respiration (Wanet et al., 2014; Wanet et al., 2015). We also observed that BM-MSCs appear to display a more elongated and more developed mitochondrial network than the one reported in embryonic stem cells (ESCs). Unexpectedly, while ESCs mitochondrial network tends to favour mitochondrial fusion during differentiation, we observed a fragmentation of the network in BM-MSC hepatogenic differentiation.

We found that both quantitative and qualitative changes in mitochondrial composition, perhaps driven by mitochondrial biogenesis regulators and leading to a metabolic shift toward increased OXPHOS, may accompany the hepatogenic differentiation of BM-MSCs as suggested by the higher fraction of cytoplasmic area occupied by mitochondria in differentiated BM-MSCs accompanied by an increase in mtDNA copy number and in the abundance of several mitochondrial proteins during the differentiation process. The increased abundance of several OXPHOS complex subunits is reflected by changes in mitochondrial respiration at the end of the hepatogenic induction step. Indeed, the basal respiration was significantly increased in differentiated vs. undifferentiated cells, which suggests that differentiated cells rely more on OXPHOS for their ATP production. Moreover, the coupling efficiency, representing the fraction of the basal respiration that is used for ATP production, was significantly higher in differentiated cells. The spare respiratory capacity was also higher in differentiated cells. Altogether, these data strongly support a metabolic shift toward increased OXPHOS during the hepatogenic induction of BM-MSCs (Wanet et al., 2014).

In the future and fitting with the goal of WP4 of MITOEAGLE, we would be interested to measure the respiration of mitochondria according standardized methods in different types of stem cells undergoing various differentiation programmes. In addition, the mitochondrial morphology network modifications would be systematically analysed during stem cells differentiation.

3) Sirtuine 3 – differentiation of adipocytes and control of mitochondrial biology and bio-energetics

The last research outlined below builds upon previous work we have done on the mitochondrial Sirtuin 3 (Sirt3, the major mitochondrial NAD^+-dependent deacetylase) that allows resistance to high fat diet (HFD)-deleterious effects in transgenic mice that over-express the deacetylase specifically in adipose tissue. The transgenic (tg) animals have been generated by Dr. Eric Bell in the laboratory of Prof. Leonard Guarente (Glenn Laboratory for the Science of Aging and Koch Institute for Integrative Cancer Research, MIT, Cambridge, USA). Their characterization, as well as preliminary data, have been obtained during a long scientific mission of T. Arnould in the laboratory of Prof. L. Guarente (MIT). The goals of this research are: 1. To experimentally delineate the mechanism(s) by which overexpression of Sirt3 in the mitochondria of adipocytes in adipose tissues limits inflammation and fibrosis during a HFD-treatment of transgenic mice 2. To delineate the mitochondrial role of over-expressed Sirt3 in adipose tissues by analysing key mitochondria parameters (abundance, morphology, dynamics, respiratory activity) and the changes in cell signalling leading to modify the expression level of several adipokines, especially pro-inflammatory cytokines such as MCP-1, IL-6 and TNFα.

Sirt3 is the only deacetylase in the mitochondria as only Sirt3-KO mice showed significant hyperacetylation of mitochondrial proteins. Recent proteomics analyses ("mitoacetylome") have shown that Sirt3 allows the regulation of mitochondrial function/metabolism by deacetylating modifying the activity of many mitochondrial targets (between 35 and 50 % of mitochondrial proteins are lysine acetylated) that affect almost all functions of mitochondria, including electron transport chain, TCA cycle, apoptosis control, amino acid metabolism, and FFAs β-oxidation. However, the role of the enzyme in metabolic syndrome and obesity is still poorly characterized and has mainly been addressed with systemic KO mice but not transgenic mice. In addition, the role (if any) of Sirtuin 3 in the control of adipogenesis is unknown. In the context of obesity and in transgenic mice models (conditional adipose overexpression or germline), **we plan to study of the effect of Sirtuin 3 over-expression on key parameters of mitochondria, especially when mitochondria dysfunction is triggered by a high calorie and/or high fat diet.** The over-expression of Sirt3 could modulate several aspects of mitochondrial biology such as ROS production, mitochondrial mass, ATP production, mitochondrial dynamics known to be affected by HFD and clearance of defective mitochondria in WAT of transgenic mice exposed to HFD. We will thus first investigate how changes in mitochondrial protein acetylation (by establishing mitoacetylomes) from mitochondrial fraction prepared from adipocytes isolated from tg mice exposed to HFD and identification of differential acetylated target by mass spectrometry (MaSUN Technological Platform, UNamur). We will next delineate the role of Sirt3 in mitochondrial adaptive capacity (mitoAC). The role of Sirt3 in modulation of MitoAC will be studied *in vitro* using KO MEFs or transduced MEFs to bring back Sirt3 or catalytically inactive enzyme for relevant controls. Combined challenges of [glucose]/[lipid] and uncoupling [FCCP] ranges will be studied in both differentiated and undifferentiated cells to discriminate MitoAC response to Sirt3 KO or over-expression during differentiation. Moreover, an *in vitro* approach easily allows studying multiple parameters simultaneously, including mitochondrial dynamics, which is far more challenging in *in vivo* studies. We will determine ROS (using MitoSox fluorescent probe), membrane potential (JC-1 probe) and morphology to evaluate MitoAC using a functional cell-omics approach via high content confocal microscopy (using our in-house BD Pathway 855). If modifications of the mitochondrial morphology are observed, fusion (Mfn1/Mfn2) and fission (Drp-1, Fis-1, Mff, OPA-1) effectors will be analysed in terms of abundance, post-translational modifications, localisation and interactions. In addition to dynamic aspects, the abundance of mitochondria will be characterized by the quantitation of the abundance of mtDNA, Mitotracker Green labelling (FACS analysis), specific mitochondrial marker proteins to cover several mitochondrial functions as well as respirometry analysis (Seahorse, collaboration with Prof. P. Sonveaux, UCL) of adipocytes from Sirt3 transgenic mice exposed to a HFD will be

studied.

These studies fit perfectly with WP3 of MITOEAGLE data repository on fat tissues and other tissues (liver) as we will analyse the role/function and activity of Sirtuin 3, a mitochondria deacetylase dependent on NAD^+ in the context of adipocyte differentiation *in vitro* and *in vivo* in HFD-obesity model studied in transgenic mice that over-express the enzyme.