Posters

P.1

Deubiquitinase UCH-L1 and its link with mitochondrial dysfunction

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Mitochondria are double-membrane organelles essential for the energetics, metabolism, and regulation of eukaryotic cells. Ubiquitin carboxyl-terminal hydrolase isozyme L1(UCH-L1) is a part of Ubiquitin Proteasome System (UPS) and was originally described as deubiquitinating enzyme, however, it was also later shown to act in vitro as ligase/hydrolase. UCH-L1 is found in Lewy bodies present in Parkinson`s disease (PD) and neurofibrillary tangles formed in Alzheimer`s disease (AD) and therefore it was linked toneurodegeneration. Interestingly, UCH-L1 expression is highly decreased in the dysfunctional mitochondria which are pathological features considered one of the hallmarks of neurodegeneration. At the same time, we know very little about UCH-L1 function in the mitochondria, including its relation to neurodegeneration. My research is aimed at studying deubiquitinase UCH-L1 in the context of mitochondrial dysfunction. The presented preliminary results point to differential regulation of UCH-L1 in different cellular models of mitochondrial dysfunction. We found that the levels of UCH-L1 are increased in complex I deficient cells. On the other hand, the levels of UCH-L1 abundantly reduced in complex III and IV mutant cells. Based on the presented preliminary results, we conclude that the deubiquitinase UCH-L1 is differently expressed in mitochondrial deficiency cellular models and further detailed studies are under process to check its involvement in mitochondrial dysfunction.

P.2

The interaction of flavonoids with the paxilline binding site of the mitochondrial BK_{Ca} channel

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Large-conductance Ca^{2+}-regulated potassium (BK_{Ca}) channel is known to play an important role in physiological and pathological processes. This channel has been found to be located not only in the cell membrane but also in the membranes of intracellular compartments, such as in the inner mitochondrial membrane. With some differences, the mitoBK_{Ca} channel has been shown to be inhibited or activated by both synthetic and natural compounds. Paxilline, has been considered to be a canonical blocker of this channel. In the previous study, we showed that the natural origin substance quercetin activates the mitoBK_{Ca} channel at ten times lower the concentration compared to channel present in the plasma membrane. Here, we report that after inhibition of mitoBK_{Ca} channels by paxilline, quercetin activates these channels, indicating a paxilline and quercetin binding competition in the regulation of the mitoBK_{Ca} channel. To support our data, we used an analog of quercetin - isorhamnetin, a substance with one substituent changed. Isorhamnetin has no effect on the mitoBK_{Ca} channel activity, and after its application, paxilline fully inhibits the channel. Additionally, the molecular modeling studies were introduced. The results of docking quercetin and paxilline to the BK_{Ca} channel suggest that paxilline cannot bind after activation of the channel with quercetin. It seems that the likely mechanism of this phenomenon is the formation of spatial hindrance by quercetin. The results obtained shed a completely new, groundbreaking in the paxilline context, light on the current knowledge about mitochondrial potassium channel regulation.

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P.3

Distinct roles of the ubiquitin-proteasome system in the quality control of the arrested mitochondrial protein import intermediates

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Proteins that build mitochondria are first synthesized in the cytosol as protein precursors. These proteins are then selectively imported into the organelle using specific translocation channels. However, this process is not always successful. Translocation failure can result from misfolding of proteins in transit, causing them to stall in translocation channels. Such protein import blockade disturbs mitochondrial proteome turnover and causes protein mislocalization. Our research involved comparing the quality control of failed import in yeast and human cells using model proteins with stable folding domains. We found that yeast cells relied more on proteasome engagement, while human cells relied on mitochondrial factors. In yeast, the combination of ubiquitination, Cdc48, and proteasomes effectively removed stalled precursors. In contrast, in human cells, mitochondrial membrane depolarization was necessary to trigger proteolytic cleavage of the stalled protein, which was mediated by mitochondrial proteases like OMA1. The cleavage allowed for quick removal of the part of the protein blocking the translocase of the outer membrane, which was then further cleared by VCP and proteasome.

P.4

The role of CFTR in the impairment of human bronchial epithelial cells induced by particulate matter

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Cystic fibrosis (CF) is an autosomal genetic disease which is caused by various mutations in gene encoding the CFTR (cystic fibrosis transmembrane conductance regulator). The CFTR mutations result in malfunction of many secretory tissues and organs such as airway epithelia, sweet glands, the pancreas and the gastrointestinal tract. It was observed that CF patients are at a special risk from air pollution. Here we investigated the impairment of CF cell function induced by PM administration.
In our study, two cell lines were investigated: control HBE (human bronchial epithelial cell line) with functional CFTR channel and CFBE cell line (human cystic fibrosis bronchial epithelial cell line) with ΔF508 CFTR mutation. To assess the toxicity of particulate matter, PM <4μm diameter was used and MTT cell viability assay was conducted as well as trypan blue staining. The reactive oxygen species (ROS) level was determined using fluorescent probe- DCFDA. The results show the difference in cell viability of HBE and CFBE cells, upon treatment with different concentrations of PM. Lower values of absorbance in CFBE cells in MTT test indicate different metabolic state of the cells, as the MTT test is based on the activity of dehydrogenases in mitochondria. It was also discovered that basal ROS level of untreated with PM cells was higher in CF cell line compared to control HBE cells. Additionally, PM induced higher ROS production in CF cells.
In conclusion, we noticed higher susceptibility of CF cells to PM induced toxicity. The effect is correlated with ROS overproduction and may by associated with different metabolic state of the cells.

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P.5

Protein homeostasis and degradation in mitochondrial diseases models

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In yeast and nematodes dysfunction of mitochondria triggers the assembly of proteasome that results in an increased proteasomal activity. We have recently discovered that the genetic ablation of respiratory complex I in human cells activates the proteasome and triggers its rearrangement to incorporate the alternative catalytic subunits PSMB9 and PSMB8, referred as immunoproteasome subunits due to their expression in immune cells. In our studies, we further analyzed the relationship between the proteasome and mitochondria in pathological conditions. We identified the alternative proteasome assembly program in cells of patients suffering from mitochondrial diseases and found that cells overexpressing immunoproteasome subunits demonstrated an upregulated proteasomal activity. To investigate the role of the newly identified alternative proteasome in excessive degradation of mutated mitochondrial proteins, we specifically inhibited the activity of immunoproteasome subunits. In our studies we also aimed to analyze whether immunoproteasome-specific inhibitors reduce cytotoxic effects of the pan-proteasome inhibitor, Bortezomib. We hypothesize that the mitochondrial stress-induced alternative proteasome with overexpression of immunoproteasome-specific subunits is a potential novel molecular target for future therapies of mitochondrial diseases.

P.6

From ROMK2 to mitoK$_{ATP}$: Exploring the Pharmacology of Intracellular Channels

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Potassium channels in the inner mitochondrial membrane regulate vital cellular processes. One such channel, the ATP-regulated potassium channel (mitoK$_{ATP}$), is associated with cell survival and death. The present study investigates the influence of pharmacological agents on the activity of the ROMK2 potassium channel, a potential molecular constituent of mitoK$_{ATP}$. To accomplish this, ROMK2 was expressed in *Escherichia coli*, partially purified, and incorporated into planar lipid bilayers after solubilization in polymer nanodiscs. We examined the effects of established mitoK$_{ATP}$ channel modulators on ROMK2 activity. Our findings indicate that the ROMK2 channel is activated by the mitoK$_{ATP}$ channel opener diazoxide and inhibited by mitoK$_{ATP}$ blockers such as ATP/Mg^{2+}, 5-hydroxydecanoic acid, and the antidiabetic sulfonylurea glibenclamide. These results suggest that the ROMK2 potassium protein may serve as a pore-forming subunit of mitoK$_{ATP}$ and that the impact of channel modulators does not depend on the presence of accessory proteins.

P.7

ATP synthase new subunit *l* has a role in permeability transition pore in yeast

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In *S. cerevisiae*, the uncharacterized protein Mco10 was previously found to be associated with mitochondrial ATP synthase and referred to as a new 'subunit *l*'. However, recent cryo-EM structures of *S. cerevisiae* ATP synthase could not ascertain Mco10 as a structural subunit of the enzyme making questionable its role as a structural subunit. The N-terminal part of Mco10 is very similar to Atp19 (subunit *k*) of ATP synthase. The subunit *k*/Atp19, along with the subunits *g*/Atp20 and *e*/Atp21 plays a major role in stabilization of the ATP synthase dimers. We investigated the impact of Mco10 on ATP synthase functioning. Biochemical analysis revealed in spite of similarity in sequence and evolutionary lineage, that Mco10 and Atp19 differ significantly in function. The absence of Mco10 delays the induction of PTP, while the deletion of Atp19 does not. Conversely, lack of Atp19 reduces slightly ATP synthase activities, while deletion of Mco10 has no effect. This is the first work to show Mco10 is an auxiliary ATP synthase subunit that only functions in permeability transition.

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P.8

Loss of the BK_{Ca} channel causes an increase in mitochondrial reactive oxygen species in glioblastoma cells

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Mitochondrial potassium (mitoK) channels play an important role in cellular physiology. These channels are expressed in healthy tissues and cancer cells. Activation of mitoK channels can protect neurons and cardiac tissue against injury induced by ischemia/reperfusion. In cancer cells, inhibition of mitoK channels leads to an increase in mitochondrial reactive oxygen species, which leads to cell death. In glioma cells activity of the mitochondrial, large conductance calcium-activated potassium (mitoBK$_{Ca}$) channel is regulated by the mitochondrial respiratory chain. Additionally, it has been suggested that this channel may interact with complexes and supercomplexes of the respiratory chain of glioma cells. In our project, we used CRISPR/Cas9 technology in human glioblastoma U-87 MG cells to generate knockout cell lines lacking the α subunit of the BK$_{Ca}$ channel encoded by the *KCNMA1* gene. Mitochondrial patch-clamp experiments showed the absence of an active mitoBK$_{Ca}$ channel in knockout cells. Additionally, the absence of this channel resulted in increased levels of mitochondrial reactive oxygen species. However, analysis of the mitochondrial respiration rate did not show significant changes in oxygen consumption in the cell lines lacking BK$_{Ca}$ channels compared to the wild-type U-87 MG cell line. These observations were reflected in the expression levels of selected mitochondrial genes, organization of the respiratory chain, and mitochondrial morphology, which did not show significant differences between the analyzed cell lines. In conclusion, we show for the first time that the pore-forming subunit of the mitoBK$_{Ca}$ channel is encoded by the *KCNMA1* gene in U-87 MG cells. Additionally, the presence of this channel is important for the regulation of reactive oxygen species levels in mitochondria.

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P.9

Inhibition of inducible NO synthase restores endogenous H_2S, constitutive NO synthesis and reduces sensitivity of mPTP to Ca^{2+} in the heart of old rats

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Introduction. Nitric oxide (NO) and hydrogen sulphide (H_2S) are endogenously produced gaseous molecules that mediate an important signalling functions and regulate biochemical and physiological processes under normal conditions and pathology. It was shown that ageing is accompanied by a significant decrease in constitutive and increase in inducible NOS activity and enhanced of oxidative stress markers.

The aim of the current work was to study the protective effects of aminoguanidine (AG) as inducible NO-synthase (iNOS) inhibitor on coupling and activity of the mitochondrial NO-synthase (mtNOS), H_2S content and Ca^{2+}-dependent mitochondrial permeability transition pore (mPTP) opening in old rat heart. Old rats (22-24 months) were injected intraperitoneally with AG at a dose of 20 mg/kg per day for 10 days.

Results. The use of AG induced the increase of mtNOS (constitutive) activity in 2,7 times compared with those in old animals. An unexpected recovery of endogenous H_2S was also observed when AG was administered. In particular, its content in the mitochondria of old rat hearts was restored to the levels of adult rats and amounted to 4.32 ± 0.23 nmol/mg protein. The recovery of mtNOS activity and NO bioavailability upon administration of AG led to a decrease in mPTP sensitivity to Ca^{2+}, which was characterised by an increase of the threshold concentration of an inducer that causes swelling of heart mitochondria in old animals.

Conclusions. Thus, inhibition of inducible NO synthesis has a mitoprotective effect that partially prevents the development of mitochondrial dysfunction.

P.10

The association between mitochondrial large conductance potassium channel (BK_{Ca}) and mitochondrial respiration

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It is well known that potassium channels are present not only in plasma membrane, but also in many intracellular compartments, such as mitochondria. It is documented that mitochondrial large conductance potassium channel ($mitoBK_{Ca}$) plays crucial role in many protective events. However, the exact mechanism of action is still not elucidated. Here we show the potential association of $mitoBK_{Ca}$ with mitochondrial electron transport chain (ETC).

The experiments involved two cell lines: HBE wt (wild type human bronchial epithelial cell line) and HBE $\Delta\alpha BK_{Ca}$ (human bronchial epithelial with deletion of α subunit of $mitoBK_{Ca}$). The methods incorporated in the study involved: oxygen electrode to measure oxygen consumption rate (OCR), JC-1 fluorescent probe to establish changes in mitochondrial potential and BioTracker Green staining to assess mitochondrial content.

It was established that HBE $\Delta\alpha BK_{Ca}$ cells show reduced OCR and mitochondrial function. However, the mitochondrial content and mitochondrial membrane potential was similar in both cell lines. The reduced OCR results in HBE $\Delta\alpha BK_{Ca}$ cells may be linked to ETC, as in TMPD/ascorbic acid experiments, HBE $\Delta\alpha BK_{Ca}$ cells displayed lower activity of ETC complex IV.

In conlusion, we show that $mitoBK_{Ca}$ channel seems to be an important factor for mitochondrial fuction, linking the mitochondrial potassium channel to ETC activity.

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P.11

Is the BK_{Ca} channel important for bronchial epithelium barrier function?

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The bronchial epithelium forms a protective barrier that lines the airways and plays a crucial role in defending against inhaled pathogens, allergens, and other harmful substances. The proper functioning of this barrier is essential for maintaining lung health. Recently, a large-conductance calcium-activated potassium channels (mitoBK_{Ca}) has been identified in the inner mitochondrial membrane of the human epithelium. These proteins control potassium fluxes between mitochondrial intermembrane space and mitochondrial matrix which directly regulates mitochondrial and cell functions. It has been found that activation of mitochondrial potassium channels with pharmacological substances preserves mitochondria against damage induced by various factors including ischemia/reperfusion. Despite intensive research, the exact mechanism of proper cell function involving the influx of potassium still remains under investigation.

In this study, we used wild-type human bronchial epithelium cells (HBE wt) and cells with the deletion of the alpha subunit of the BK_{Ca} channel (HBE Δα BK_{Ca}). Using the patch-clamp technique, it was shown that in the HBE Δα BK_{Ca} cells model, BK_{Ca}-type channel activity is not observed. HBE Δα BK cells displayed mitochondrial dysfunction and lower transepithelial electrical resistance. Also, it has been observed that HBE Δα BK_{Ca} cells displayed reduced clone formation capabilities. This is a significant feature as it shows phenotypic effects that require time and quite a few cell divisions to develop, in comparison with short-term colorimetric cytotoxicity assay based on MTT. To determine whether reduced clone formation capabilities are associated with cell cycle phase distribution changes, we conducted a cell cycle analysis. The incorporation of 5-bromo-deoxyuridine by HBE cells showed G2/M DNA-damage cell cycle arrest in HBE ΔαBK cells. In summary, obtained results indicate that the BK_{Ca} channel is important for bronchial epithelium barrier function.

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P.12

PDIA3 gene knockout affects metabolic status of A431 squamous cell carcinoma

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An oxidoreductase family member, PDIA3 protein, has broad range of functions from promoting protein folding in ER to pro-apoptotic activities in mitochondria. Moreover, it was shown that PDIA3 can be localized in a mitochondria associated membranes region of endoplasmic reticulum. Several studies shown that PDIA3 functions as a chaperon to STAT3 protein which can suppress mitochondrial bioenergetics functions. Vitamin D is a steroid hormone that regulates calcium-phosphorus homeostasis along with various cellular processes. Canonically vitamin D acts through the complex of its receptors: VDR and RXR, regulating expression of many genes in human genome. However not all effects of $1,25(OH)_2D_3$ can be related to genomic action of VDR-RXR. Consequently, PDIA3 was identified D responsible to non-genomic response to hormone. Recent studies also shown that calcitriol can regulate functions and gene expression levels of elements of mitochondrial membrane such as potassium channels.

The aim of our research was to assess an impact of PDIA3 deletion on mitochondria morphology and bioenergetics in squamous cell carcinoma (A431).

It was observed that PDIA3 deletion resulted in changes of morphology of mitochondria. A decrease in percentage of mitochondrial section area, maximal diameter and perimeter was observed in *PDIA3*-deficient cells. $1,25(OH)_2D_3$ treatment of A431Δ*PDIA3* cells partially reversed the effect of *PDIA3* deletion increasing aforementioned parameters, but there was no visible effect on A431WT cells except an increase in mitochondrial section area. Moreover, PDIA3 knockout had impact on mitochondrial bioenergetics. Oxygen Consumption Rate (OCR) was significantly increased, with no visible effect of $1,25(OH)_2D_3$ treatment in A431Δ*PDIA3* cells. In contrast, in A431WT cells $1,25(OH)_2D_3$ treatment resulted in slight decreased in basal and ATP-linked respiration. In case of Extracellular Acidification Rate (ECAR) rate an increase was observed for glycolysis and glycolytic capacity parameters in case of non-treated A431WT cells versus A431Δ*PDIA3* cells. $1,25(OH)_2D_3$ treatment had no significant effect on glycolytic parameters. Taken together presented results suggests that PDIA3 is strongly involved in regulation of mitochondrial bioenergetics in cancerous cells and $1,25(OH)_2D_3$.

P.13

Effect of daidzein TPP+ on mitochondrial function in human fibroblasts

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Daidzein is a phytochemical that has estrogenic and antioxidant properties. Due to these features it can affect the functioning of the cell. TPP^+ cation allows to direct a given molecule to the mitochondrial matrix. It is well known that in the process of cellular aging mitochondria undergo functional remodeling to adapt to stress conditions. We examined the effects of daidzein and daidzein TPP^+ on the remodeling of mitochondrial function during early cellular aging. We compared effect of daidzein and daidzein TPP^+ on the primary human fibroblasts from early passages (<10) with the ones from higher passages (>18) and with fibroblasts with ROS – induced aging. We have shown that daidzein and daidzein TPP^+ affect the level of ROS, the reorganization of the mitochondrial network and the concentration of Ca^{2+} ions. Daidzein and daidzein TPP^+ have similar effects on mitochondria. Consequently, daidzein improves mitochondrial function in aging fibroblasts.

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P.14

Pyridoxal-5-phosphate improves mitochondrial function in heart of old rats through AKT/GSK3β/Sirt1/PGC-1α axis

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Aging of the myocardium is usually accompanied by disturbances in cardiac metabolism and mitochondrial function. Mitochondrial dysfunction, which includes disorders in oxidative phosphorylation, mitochondrial dynamics, and autophagy, is a critical event leading to reduced ATP production and oxidative stress. Consequently, correcting age-related metabolic changes in the heart is an intriguing task. In this study, we focused on the effect of pyridoxal-5-phosphate (PLP) on mitochondrial function and the key signaling cascades that regulate cardiac metabolism. After two weeks of PLP administration, PGC-1α and Sirt-1 levels were slightly increased in the hearts of old rats compared to control group. Analysis of key subunits of the OXPHOS complex revealed significantly lower expression of complex I in old rats treated with PLP compared to untreated animals. Protein levels of Complex III were 30% higher after the course of PLP treatment. We found an increase in β-oxidation in the hearts of old PLP-treated animals compared to control animals, as evidenced by significantly higher level of phosphorylated acetyl-CoA carboxylase former group. Higher levels of respiratory control ratio and efficiency of oxidative phosphorylation (ADP/O) indicators in the hearts of old rats treated with PLP indicate the restoration of oxidative phosphorylation processes in the mitochondria of these animals. Additionally, decreased content of CD36 suggests a reduction in fatty acid transport into the cell after two weeks of PLP consumption. Decreased levels of proteins involved in glucose transport is one of the markers of metabolic imbalance in the aging heart. We found that PLP administration increased GLUT4 level, apparently, glucose uptake. Increased content of pAKT (Ser 473) and phosphoinositide-dependent kinase-1 proteins also indicated an increase in glycolysis in the hearts of old rats. We did not observe significant changes in the level of phosphorylated AMPK. However, Western blot analysis of proteins involved in the regulation of canonical WNT (activated β-catenin, Axin) suggested an inhibition of its activity in the hearts of old rats treated with PLP. Overall, our data show that PLP may mitigate metabolic imbalance and mitochondrial dysfunction through the AKT/GSK3β/Sirt1/PGC-1α axis.

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P.15

Stress adaptation in mitochondria affected by plastic particles.

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Plastics play fundamental role in modern living, it is a useful material ubiquitously present in our everyday lives. At the same time, the amount of plastic waste in the environment poses a significant challenge. Plastic waste degrades and gets reduced to micro- or nano-particles (MP or NP). NP has been detected all around the world in atmospheres of urban and remote locations. It is clear that the air pollution with NP is a global problem. Inhaling MPs and NPs can cause asthma or allergies. Moreover, inhaled NP possess the ability to enter the cell. This study is innovative because so far the influence of inhaled plastic on human health was not broadly researched. We are investigating the effect of stress caused by the NPs on mitochondria, as mitochondria are the center of cellular metabolism, and play a pivotal role in adaptation to stress conditions. In this project we investigate how the NP pollution affects mitochondrial stress, how does it activate and modulate pathways of mitochondrial retrograde signaling and how mitochondria adapt to stress. In our research model we are using bronchial epithelial cells, healthy (BEAS-2B) and cancerous (A459). We investigated the effect of short term stress on ROS level and mitochondria morphology. The short term stress did not change the inner mitochondrial membrane potential, mitochondrial age or calcium uptake. In further studies we will be interested in the long-term stress caused by NPs as well.

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P.16

Stearoyl-CoA desaturase 1 deficient perivascular adipocytes are metabolically activated and resistant to palmitate-induced mitochondrial alterations

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Perivascular adipose tissue (PVAT) is particularly important in terms of vascular homeostasis, as it secretes vasodilating and anti-inflammatory factors. Obesity-induced decline in mitochondrial function in adipose tissue has been shown to be associated with a secretory profile directed at promoting inflammation, thereby increasing cardiovascular risk. Stearoyl-CoA desaturase 1 (SCD1) is an enzyme that converts palmitate and stearate into palmitoleate and oleate, respectively, and has been shown to be implicated in the regulation of metabolism, insulin sensitivity and inflammation. However, evidence regarding SCD1-dependent mitochondrial function in PVAT during obesity is currently lacking. Therefore, we treated primary wild-type (WT) and SCD1-deficient perivascular adipocytes with palmitate (16:0). The 16:0 treatment increased mitochondrial complex I and ATP synthase protein levels. Complex IV was upregulated only in SCD1-deficient cells treated with 16:0. Untreated SCD1-deficient adipocytes showed higher level of mitochondrial fragmentation accompanied by a higher oxygen consumption rate (OCR) than WT cells. Moreover, 16:0 treatment was able to increase fragmentation and OCR only in WT adipocytes. Mitochondrial membrane potential was reduced in both 16:0-treated groups, but overall mitochondrial mass remained unchanged. In conclusion, our data suggest that while loss of SCD1 by itself promotes mitochondrial remodeling, it is not further enhanced by 16:0.

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P.17

Establishing a Split GFP-based reporter to study the factors affecting membrane translocation of mitochondrial proteins

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Mitochondrial proteins are majorly encoded by nuclear genes, produced in the cytosol, and transported to the mitochondria. While in the cytosol, mitochondrial proteins are exposed to cytosolic modifications that can impact their transport or function. One such modification is ubiquitination, marking proteins to be degraded by the proteasome. Part of ubiquitinated proteins bypasses proteasomes. Preliminary observations indicated that ubiquitin attachment directly interferes with precursor protein mitochondrial import. This allowed us to propose a new role for ubiquitination as a direct regulator of mitochondrial precursor protein import.
We use the Split GFP-based to develop in vivo import reporter. We cloned split GFP fragments in fusion with mitochondrial proteins, with and without the addition of ubiquitin, to monitor their mitochondrial import. The individual expression and co-expression of the constructs were confirmed using a western blot. With cell fractionation experiments, split GFP-tagged mitochondrial proteins were found to be localized in the mitochondrial fraction. Fluorescent microscopy confirmed that split GFP fragments could assemble to produce fluorescence. The GFP fluorescent signal co-localized with that of the mitochondrial stain Mitotracker Deep Red FM. Together, our data supports the applicability of split GFP as a reporter to screen for factors that modulate the import of mitochondrial proteins in human cells.

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Nutraceutical Sulforaphane impact on mitochondrial morphology and ROS level in an *in vitro* model of normal tissue

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Sulforaphane is an isothiocyanate originating from *Brassicae* Family. It exhibits chemopreventive and also antiproliferative properties. It is an Nrf2 detoxification pathway inducer and affects the level of intracellular ROS. Apart of that it exhibits cytotoxicity – it induces the cell death via apotosis , mainly via mitochondrial pathway.
Mitochondrial impairment is critical in several human diseases like metabolic disorders and neurodegeneration. At the same time SFN was shown to exhibit protective properties in neurodegenerative diseases and aging and some researchers pointed that SFN may have an impact on mitochondria [1, 2]. However, the mechanism by which SFN may affect mitochondria remains still unclear.
Thus, to shed a light on the mechanism of SFN action, we have studied its effect on mitochondrial morphology and mitochondrial ROS level by means of confocal microscopy. The results, obtained in normal tissue *in vitro* model, indicate that high concentration of SFN induces mitochondria shrinkage and elevation of mitochondrial ROS level. Thus, the results obtained led a new light on supposed protective SFN effect indicating that further research on SFN impact on mitochondria should be conducted.

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P.19

The effects of atorvastatin and simvastatin on the bioenergetic activity of mitochondria isolated from the rat brain

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The aim of this study is to elucidate the direct effects of two commonly used blood cholesterol lowering drugs used to treat hypercholesterolemia, atorvastatin and simvastatin, on respiratory function, membrane potential and reactive oxygen species formation in mitochondria isolated from rat brain. Both hydrophobic statins, induced a loss of outer mitochondrial membrane integrity, an increase in hydrogen peroxide formation, and a decrease in maximum (phosphorylating and uncoupled) respiratory rate and membrane potential. In addition, both statins reduced the efficiency of oxidative phosphorylation (coupling parameters: ADP/O ratio and respiration control ratio) in isolated rat brain mitochondria. Changes induced by statins indicate impaired function of brain mitochondria at the level of ATP synthesis and at the level of the respiratory chain, presumably complexes I and III. The effects induced by ATOR appear to be more potent than those induced by simvastatin at a given concentration. The effect of calcium-containing atorvastatin on rat brain mitochondria was highly calcium-dependent and caused disruption of mitochondrial calcium homeostasis. The results indicate that hydrophobic statins that cross the blood-brain barrier, widely used as an antiatherosclerotic agent, have a direct negative effect on isolated rat brain mitochondria.

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Qualitative and quantitative analysis of serum and cerebro-spinal fluid circular cell free DNA of Parkinson's disease patients

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Parkinson's disease (PD) is a neurodegenerative disorder, which leads to an excessive loss of dopaminergic neurons in the *substantia nigra* of the brain. Despite intensive research there is still a need to optimize the diagnostic process and optimalisation of therapies of this neurodegenerative diseases. Circular cell free DNA (ccfDNA) are double-stranded fragments of DNA of different size found in various body fluids. Taking into account the content of ccfDNA it was distinguished two types; the nuclear derived (nu-ccfDNA) and mitochondrial (mt-ccfDNA). Interestingly, this different identity could be a consequence of many different cell processes as cell death, necrosis or apoptosis, and active release by viable cells, including exocytosis and NETosis.

The main aim of our study was to determine the copy number of mt-cfDNA and nu-ccfDNA ccfDNA of PD human serum and cerebro-spinal fluid (CSF) and healthy controls with the use of droplet digital PCR (ddPCR). CcfDNA from serum (29 PD patients and 15 healthy controls) and CSF (13 PD patients, 5 healthy controls) were purified according to Qiagen procedure. The copies number were calculated using Poisson equation based on positive and negative droplet numbers while the statistical analysis was calculated using Mann-Whitney U test.

Here we present the results of qualitative and quantitative analysis of serum and CSF ccfDNA. In case of serum our data reflects high level of mt-ccfDNA and nu-ccfDNA *versus* control while for mt-ccfDNA the increased was very significant. The studies on CSF present decreased level of mt-ccfDNA *versus* control what is opposite to the proportion observed for serum. Interestingly, in total analysis we observed significantly higher number of mt-ccfDNA than nu-ccfDNA. In summary, this is, for our knowledge the first report that revealed the characterisation of serum and CSF ccfDNA of PD patients. The presented results suggest pivotal role of the mitochondria in PD biogenesis what need deeper study. On the other hand the obtained results could be considered in future for optimalisation of diagnosis and therapy of PD patients.

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Steroyl-CoA desaturase 4 controls mitochondria quality in the heart of obese mice

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Stearoyl-CoA desaturase (SCD) is an endoplasmatic reticulum protein located in the mitochondria-associated membrane region. It is involved in *de novo* lipogenesis, i.e. it catalyzes the formation of monounsaturated fatty acids by introducing the first double bond in saturated fatty acids. There are four isoforms of the enzyme in mice, of which SCD4 has been shown to be cardiac-specific. Loss of SCD1, the most ubiquitous isoform, has many beneficial effects – reducing obesity and cardiac steatosis, decreasing cardiomyocytes apoptosis and improving cardiac function. However, the role of SCD4 in the heart, especially in the bioenergetics of cardiomyocytes, is still unknown. To clarify this issue, we used SCD4-deficient mice (SCD4-/-) fed a high-fat diet (HFD) to induce obesity. The results showed that SCD4 deficiency prevented the enlargement of cardiac mitochondria induced by HFD feeding. Moreover, we found reduced level of ROS and NADH dehydrogenase activity in the myocardium of SCD4-/- mice fed HFD, compared to the corresponding wild-type group. This was supported by reduced *Ndufv2* expression and NADH dehydrogenase protein level. The levels of cardiac proteins involved in mitophagy (Parkin, LAMP1, LC3B) were increased in HFD-fed SCD4-/- mice, in contrast to wild-type mice. In addition, mitochondria biogenesis proteins (NRF1, TFAM) were also up-regulated in SCD4-/- mice. Our data suggest that SCD4 deficiency has beneficial effects on cardiac mitochondria, preventing obesity-induced mitochondria enlargement, hyperactivity and ROS production.

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Decrease of mitochondrial BK_{Ca} channel activity caused by H_2O_2 induced senescence in human vascular smooth muscle cells

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Senescence is a cellular response to the endogenous or exogenous stress factors, characterized by a stable growth arrest and other phenotypic alterations that include a pro-inflammatory secretome and significant changes in the functioning of mitochondria. The presence of senescent smooth muscle and senescent endothelial cells was confirmed in the vascular plaque. Unfortunately, little is known about involvement of mitochondrial potassium channels in these processes. Therefore, we decided to estimate potential changes in mitochondrial large conductance calcium-activated (mitoBK_{Ca}) channels in vascular smooth muscle cells undergoing cellular senescence. Human aortic smooth muscle cells were treated with a single dose of H_2O_2, what induced visible senescence within seven days after addition. Basic markers of senescence processes were confirmed, changes in the mitochondrial network determined and transcription differences in genes encoding proteins involved in mitochondrial function and biogenesis were described. Additionally, overexpression of SOD2 protein was preliminary confirmed with Western blot. Regardless of the unchanged specific mRNA expression, decrease in the level of BK_{Ca} channels protein was observed. The typical electrophysiological activity of mitoBK_{Ca} was identified in the control cells, but was no detected in senescent smooth muscle cells.

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