Lectures

L.1

Mitochondrial ion channels as pharmacological targets in cancer

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Mitochondria are hubs for cellular energetics and metabolism. In accordance, deregulation of mitochondria function might profoundly change cell fate via different mechanisms, including high ROS production and cytochrome c release. To date, a number of ion channels that show an altered expression in cancer cells were found to reside exclusively in mitochondria or both in the plasma membrane and mitochondria. Pharmacological modulation of such channels specifically in mitochondria can add a layer of specificity in modulating cancer cell behaviour with respect to drugs that affect mitochondrial metabolism or outer membrane permeabilization in general. The strategy of fusing a mitochondria-targeting moiety to specific channel inhibitors can be exploited to affect the behavior of different mitochondrial channels. Some of these agents were able to modulate cancer-cell specific functions *in vitro* and *in vivo*, leading to drastic reduction of tumor volume and migration, without toxicity.

L.2

Mitochondrial BK_{Ca} drives metabolic remodelling and promotes the Warburg effect in breast cancer cells

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Many cancer entities are associated with altered expression levels of K^+ channels. One of these channels, the voltage- and Ca^{2+} activated K^+ channel of large conductance, BK_{Ca}, was recently demonstrated to promote breast cancer development. Underlying molecular mechanisms remained, however, elusive. We demonstrate that a BK_{Ca} splicing variant localizes within the inner mitochondrial membrane of breast cancer cells, thereby stimulating the overall metabolic activity, promoting cancer cell proliferation and inducing a switch towards a metabolic phenotype frequently referred to as the "Warburg effect". Hence, cells possessing mitochondrial BK_{Ca} showed less dependency on molecular oxygen for maintaining their proliferative activity. Additionally, we found mRNA expression of mitoBK_{Ca} in primary human breast cancer biopsies, highlighting its clinical relevance. Our results emphasize that the pharmacologic modulation of BK_{Ca} in the inner mitochondrial membrane in combination with established anti-cancer approaches may represent a novel anti-cancer strategy, potentially for personalized anti-cancer treatments.

L.3

Keeping mitochondria in shape: role of FTSH4 and OMA1 proteases

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Mitochondrial proteases, including inner membrane metallopeptidases *i*-AAA and OMA1, are essential for maintaining a healthy mitochondrial population. We previously reported that in Arabidopsis, loss of the *i*-AAA protease FTSH4 leads to enlarged swollen mitochondria. The mechanism of how FTSH4 controls mitochondrial morphology is unknown, and no information on the impact of OMA1 on mitochondrial morphology in plants has been reported. In our current work, we applied co-immunoprecipitation and combined fractional diagonal chromatography (COFRADIC) to identify interaction partners and substrates of FTSH4 and OMA1. Among proteins co-precipitating with FTSH4 or OMA1, we identified proteins associated with the organization of intramitochondrial ultrastructure, namely subunits of the mitochondrial contact site and cristae organizing system (MICOS) and sorting and assembly machinery (SAM) complex, which form the intermembrane space bridging complex in mammals. Using COFRADIC, we identified the MIC60 subunit of MICOS and SAM50-1 as OMA1 and FTSH4 substrates, respectively. Transmission electron microscopy studies revealed the alteration of the inner mitochondrial membrane morphology with fewer cristae in both *ftsh4* and *oma1* mutants, similar to the plants lacking MIC60 protein. Our results indicate that in plants, FTSH4 and OMA1 proteases are required for the cristae organization and intermembrane contacts of mitochondria through their association with the MICOS and SAM machineries.

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L.4

Control of mitochondrial protein import

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Mitochondria fulfill a plethora of different tasks, which are essential for cell survival. In order to fulfill these functions mitochondria depend on the import of 99% of their protein content. These proteins are synthesized as precursors on cytosolic ribosomes and imported into the target organelle. The translocase of the outer membrane (TOM complex) forms the entry gate for the vast majority of the precursor proteins. Subsequently, dedicated protein machineries sort the precursor proteins into the different mitochondrial subcompartments: the outer and inner membrane, intermembrane space and matrix. Precursor proteins are imported in an unfolded state to pass the TOM channel. Prematurely folded or misfolded precursor proteins can arrest during translocation and cause clogging of the protein translocon. Impaired protein translocation via the TOM complex leads to massive proteotoxic stress. Therefore, the cell harbors molecular mechanisms that extract precursor proteins from the TOM channel and delivers them for proteasomal degradation. Thus, specific quality control factors monitor protein translocases to ensure proper protein import into mitochondria.

L.5

Chloride Intracellular Ion channel complex in the mitochondrion

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The discovery of mitochondrial chloride (Cl^-) channels has set the ground for understanding their role in cardioprotection and cardiac physiology. Anion channels are proteinaceous pores in biological membranes that allow the passive diffusion of negatively charged ions along their electrochemical gradient. Ionic homeostasis plays a major role in mitochondrial physiology, which is the key player in cardioprotection from ischemia-reperfusion injury. Supporting the cardioprotective role of mitochondrial ion channels, several ion channels (BK, KATP, and CLIC4), transporters (NCLX), and channel complexes (MCU and mPTP) have been identified and shown to be directly involved in mitochondrial function. However, the precise mechanism of Cl^- transport across both inner and outer mitochondrial membranes and their role in cardioprotection are not completely deciphered. We have identified the mitochondrial inner membrane Cl^- channel (CLIC5) and an associated/ outer membrane Cl^- channel (CLIC4) in cardiomyocytes. Pharmacological treatment of hearts with indanyloxyacetic acid 94 (IAA-94), a known chloride intracellular channel proteins (CLICs) blocker, before IR abolished the cardioprotective effect of ischemic-preconditioning (IPC), show cardio deleterious effect in *ex vivo* and *in vivo* models. IAA-94 also prevents cyclosporine A (CsA) from protecting the heart and increasing mitochondrial calcium capacity. We discovered that CLIC4 and CLIC5 are physically and functionally coupled to form a mitochondrial CLIC complex. The absence of both (but not individual CLICs) causes cardiac dysfunction and reduces calcium capacity. Taken together we have discovered that an anion channel complex exists in mitochondrial membranes which is responsible for maintaining its physiological function.

Oral communications

O.1

Does infrared light regulate activity of mitochondrial large conductance potassium channel?

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Mitochondria play a key role in ATP synthesis, ROS production as well as apoptosis and necrosis pathways. They are also involved in cytosolic Ca^{2+} buffering. These functions seem to be mediated, at some level, by mitochondrial potassium (mitoK) channels. Therefore, mitoK channels are seen as potential drugs targets. Unfortunately, for many mitoK channels modulators alternative targets, unrelated to their channel activity regulation, were described. Taking that into consideration we are exploring other possible non-chemical regulation mechanisms, in particular, photobiomodulation (PMB). The beneficial effects of PMB, especially in the infrared (IR) range, has been recognized in a lot of processes such as wound healing or reducing negative effects of ischemia/reperfusion injury. Described primary acceptor of infrared radiation within cell is a mitochondrion, precisely speaking a subunit of electron transport chain - cytochrome c oxidase (COX). This enzyme contains four active metal centres, two hems and two copper (Cu_A and Cu_B). The latter are of our particular interest. It is known that the Cu_A centre in the oxidized form absorbs radiation with a length of 820 nm, while the maximum absorption of the reduced Cu_B centre is 760 nm. Therefore during patch-clamp experiments we irradiated mitoBK$_{Ca}$ channel with this two wavelengths. In system oxidised by $K_3[Fe(CN)_6]$ the activity of the mitoBK$_{Ca}$ channel decreased and the channel activity was restored by irradiation with 820 nm wavelength. This effect has persisted over time of irradiation. Our results indicate that infrared light with 820 nm wavelength can activate the mitoBK$_{Ca}$ channel in oxidised state of proteins from the inner mitochondrial.

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O.2

Functionality of the tardigrade *Paramacrobiotus experimentalis* mitochondria under a hypomagnetic field

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Introduction: Tardigrades are known for their remarkable ability to withstand extreme conditions. An example of these conditions is a hypomagnetic field (HMF), which is known to influence metabolic processes. However, very few studies considering HMF impact were performed for organisms able to survive under extreme conditions and considered as suitable for outer space colonization. Therefore, we decided to test the impact of HMF on the eutardigrade *Paramacrobiotus experimentalis* focusing on the mitochondrial inner membrane potential ($\Delta\psi$).

Methods: Females and males from three different age classes (i.e., 30-60, 150-180 and >300 days) were isolated from laboratory culture and divided into experimental and control groups exposed to HMF and standard magnetic field (SMF), respectively, for three different durations, i.e., 7 days, 15 days and 30 days. The HMF treatment was performed in a special anti-magnetic chamber whereas SMF treatment was performed in a climate chamber. The TMRM (Tetramethylrhodamine, methyl ester) staining of intact animals was used to estimate $\Delta\psi$ by calculation of FI_{TMRM} (Fluorescence Index of TMRM).

Results & Discussion: The HMF-related changes in $\Delta\psi$ depended on age and sex. Accordingly, HMF effect was most pronounced for the oldest animals and males appeared to be more sensitive to HMF than females that correlated with the survival rate.

Conclusion: HMF affects tardigrade mitochondria and the effect may depend on the animals' age and sex.

O.3

Knockout of BK_{Ca} channel leads to dysfunction of human bronchial epithelial cells

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Bronchial epithelium as part of the respiratory airways form an external barrier and is constantly exposed to factors such as pathogens or urban dust.

In human bronchial epithelial (HBE) cells the large conductance calcium-activated potassium channel is present in plasma membrane (BK_{Ca}) and inner mitochondrial membrane (mitoBK_{Ca}). The *KCNMA1* gene encodes the pore-forming α subunit of the channel. The channel is regulated by auxiliary β and γ subunits. The mitoBK_{Ca} channel plays an important role in regulation of mitochondrial function such as membrane potential and reactive oxygen species synthesis. Activation of mitoBK_{Ca} induces cytoprotection.

We used CRISPR/Cas9 technique to develop HBE (16HBE14o-) cell line with *KCNMA1* gene knockout. The obtained cell lines showed no activity of the BK_{Ca}-type channels in the mitochondria and the plasma membrane. Loss of the α-subunit resulted in a decrease in mitochondrial respiration rate. We also checked the expression levels of mitochondrial genes including the respiratory chain components. Additionally, using blue native electrophoresis, the organization of the respiratory chain was studied.

We conclude that BK_{Ca} channel located in mitochondria and plasma membrane is essential for proper function of human bronchial epithelium including mitochondria. However, more research is needed to understand the observed changes in mitochondria.

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O.4

Are mitochondrial BK_{Ca} channels present and active in senescent vascular smooth muscle cells?

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Mitochondria typically comprise 3 – 5% of the smooth muscle cell (SMC) volume. They are thought to play a role in maintaining vascular tone, facilitating cellular transport and producing energy for vascular cell secretion. Moreover, mitochondria are important in many signaling pathways and ion homeostasis. Activation of cell membrane large-conductance calcium-activated potassium channels (BK channels) triggers a negative feed-back loop on depolarization-induced calcium influx and SMC contraction. In this work we identified, for the first time, the mitochondrial BK channels (mitoBK channels) in human smooth muscle mitochondria. The mitoBK channels are believed, together with the other mitochondrial potassium channels, to be engaged in cytoprotective phenomenon. Our study shows describe their abundance and activity in senescent SMC. Additionally, we characterized some features of mitochondrial functioning in senescent SMC.

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O.5

Melatonin - a versatile compound that could potentially alleviate mitochondrial dysfunction

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Introduction: In the last decade, several publications indicated a link between the progression of Non-Alcoholic Fatty Liver Disease (NAFLD) and mitochondrial dysfunction. One of said dysfunctions involves opening of the mitochondrial Permeability Transition Pore (mPTP). Although the mPTP functionality is known, still there is no consensus regarding its exact structure. The aim of our study was to investigate whether melatonin, a hormone with antioxidant properties, could have modulatory effect on the mPTP and thus alleviate mitochondrial dysfunction observed during NAFLD development and progression.
Materials and methods: Effect of melatonin on mitochondrial function and mPTP functionality has been tested on isolated mice liver mitochondria. Interestingly, C57BL/6J mice used by us have stable melatonin level regardless to circadian clock. Fluorometric, spectrophotometric and oximetric techniques tests have been performed to investigate modulatory effect of melatonin on the mPTP sensitivity to calcium, mitochondrial respiratory chain, focusing on ATPase/synthase activity of mitochondrial ATP synthase
Results: We have found that 100 μM melatonin shows modulatory effect on the mPTP when induced by calcium. Interestingly, in range of 10 nM - 100 μM melatonin does not have any significant effect on mitochondrial basal and maximal rate of oxygen consumption as well as on the mitochondrial membrane potential. This suggests that melatonin-mediated mPTP pore opening regulation is not related to the melatonin effect on mitochondrial bioenergetics. Moreover, we did not observe any effect of melatonin on ATPase/synthase activity of mitochondrial ATP synthase.
Conclusion: Based on the results obtained so far, melatonin is able to modulate the mPTP activity and, simultaneously, does not interfere with the mitochondrial bioenergetic parameters. Interestingly, mPTP melatonin-mediated effect is not related to ATPase/synthase activity of mitochondrial ATP synthase considered to form mPTP. Hence, it can be further investigated as a potential drug compound that might alleviate mitochondrial dysfunction observed during NAFLD development and progression.

O.6

Cardioprotective antibiotics as bitter taste receptor agonists and putative inducers of mitochondrial fusion in human arterial endothelium

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Mitochondria are one of the most dynamic organelles in the cardiovascular system, undergoing fusion and fission phenomena. Changes in mitochondrial dynamics in the cardiovascular system are crucial for its physiology. Previous studies have shown that chloramphenicol and erythromycin, clinically used antibiotics, promote mitochondrial fusion in mammalian cells. Moreover, it was proven that chloramphenicol also has cardioprotective effects against ischemia-reperfusion injury. These compounds, among many others, are agonists of human bitter taste receptors (TAS2Rs). Recently TAS2Rs were discovered in human pulmonary endothelium where they have an impact on endothelial barrier permeability. Our working hypothesis is that chloramphenicol and erythromycin-induced mitochondrial fusion may be due to TAS2Rs activation. In order to establish the expression of 25 functional TAS2Rs subtypes in the human primary aortic endothelial cell line (HAEC), and human primary coronary artery endothelial cell line (HCAEC), we performed a digital PCR reaction and analyzed an absolute quantification of the transcripts. For the first time, we discovered the expression of TAS2Rs mRNA in both human cell lines. Afterwards, using laser confocal microscopy, we examined chloramphenicol, erythromycin, and other TAS2Rs agonists' impact on mitochondria dynamics in HAEC and HCAEC.

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O.7

The link between vascular metabolism and age-related endothelial dysfunction and arterial stiffness

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Vascular ageing is associated with increased arterial stiffness and endothelial dysfunction, phenotypes that increase the risk of adverse cardiovascular events. This research aimed to find the relationship between age-dependent impairment in arterial phenotype and vascular metabolism, which has not yet been established. We adapted an approach of measurement of bioenergetics in isolated aortic rings *ex vivo* using the Seahorse XFe96 Analyzer to assess bioenergetics of the vascular wall on a tissue level.

Aged C57BL/6 mice displayed altered glycolytic and mitochondrial respiratory parameters compared to young mice. Vascular ageing was associated with impaired endothelial function evaluated with MRI *in vivo,* decreased NO production, and increased aortic stiffness measured with Doppler. Furthermore, inflammatory stimulation *ex vivo* induced an evident metabolic response of the aorta, which was altered in aged vessels. We also investigated the implications of ageing in E3L.CETP mice, a humanised dyslipidaemia model. The development of arterial stiffness was accelerated in E3L.CETP mice, but the effect of dyslipidaemia on vascular metabolism was less apparent.

Taken together, we demonstrated that age-dependent deterioration of the vascular phenotype is associated with altered vascular bioenergetics in healthy mice, but not in mice with dyslipidaemia and that inflammation influences metabolism of the vascular wall.

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O.8

Nitrogen-containing bisphosphonates induce adaptations of aerobic metabolism in endothelial cells

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Nitrogen-containing bisphosphonates (NBPs) are one of the first-line drugs used in osteoporosis treatment. They inhibit farnesyl diphosphate synthase (FPPS), a key enzyme of the mevalonate pathway impairing osteoclast activity and bone resorption. However this pathway is also responsible for biosynthesis of cholesterol, isoprenoid lipids or coenzyme Q. Coenzyme Q_{10} is important electron carrier in mitochondrial respiratory chain and cellular antioxidant. Endothelial cells are the first cells in contact with bloodtransported drugs. We investigated the chronic (6-day) exposure effects of alendronate and zoledronate on oxidative metabolism of cultured human endothelial cells (EA.hy926). NBPs lowered cell viability, induced oxidative stress and a pro-inflammatory state and downregulated the prenylation-dependent ERK1/2 signaling pathway in these cells. Additionally, through blocking the mevalonate pathway NBPs caused significant decrease in the level of coenzyme Q10 which led to increased formation of reactive oxygen species (ROS), upregulation of antioxidant enzymes, and impairment of mitochondrial respiratory function.

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O.9

Participation of putatively mitochondrial glycine-rich proteins (GRPs) in heat response and recovery in cauliflower plants

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Plant glycine-rich proteins (GRPs) with modular structure and complex classification are a newly analysed protein family governing gene expression. Little is known about the participation of GRPs in plant acclimation to stress. We investigated thus the expression pattern of cauliflower (*Brassica oleracea* var. *botrytis*) *grp* genes under heat stress (HS) and the subsequent heat recovery (HR) by *de novo* transcriptome sequencing, RT-qPCR and *non-label* LC-MS/MS. The transcriptomic response under HS (rather than HR) involved mainly downregulated cauliflower genes, while mitochondrial protein genes were preferentially upregulated. Functional classification of those genes indicated for the global reprogramming of mitochondrial metabolism under HS/HR switch.
By manual and *in-silico* searches, non-redundant 59 *grp* genes were extracted from the high throughput data, from which at least 11 genes encoded putatively mitochondrial GRPs (the mitochondrial localization of *Bo*GRP2-like has been experimentally verified). Diverse *grp* genes were involved in HS and HR responses. The level of transcripts encoding mitochondrial GRPs (e.g. *grpE*, *rbg3*, *rbg5*) was finely regulated in leaves. We showed also the decreased abundance of SINAL protein in floral heads and leaves after HS and HR and RZ1C protein in leaves after HR. GRP2A level decreased highly in floral heads than leaves in HS. We propose selected GRP proteins to be new candidates regulating cauliflower gene expression under temperature treatment.

O.10

Cardiac β-catenin regulates mitochondrial function in perinatal cardiomyocytes

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Mitochondrial ATP production is the main source of energy in the heart. Proper mitochondrial function is also crucial for the transition of the fetal heart from glycolytic to oxidative metabolism. β-Catenin is a key regulator of cardiogenesis, but little is known about its role in the regulation of mitochondrial function during the metabolic transition of the heart. To elucidate the regulatory role of β-catenin in mitochondrial regulation, we used mice with cardiac-specific deletion of β-catenin (KO) and neonatal rat ventricular myocytes (NRVMs) treated with the β-catenin transcription inhibitor XAV939. Loss of β-catenin led to the death of mice at late embryonic and perinatal stages. β-catenin KO mice had smaller hearts compared to wild-type (WT) mice. The amount of mitochondrial DNA was twofold lower in the hearts of β-catenin KO mice. The protein content of key subunits of the oxidative phosphorylation (OXPHOS) complex was reduced in β-catenin KO mice compared to WT mice. Treatment of NRVMs with XAV939 decreased mitochondrial content and activity as measured by Mitotracker Green and Rhodamine123, respectively. Mitochondrial morphology was significantly impaired in NRVMs with inhibited β-catenin. XAV939 treatment decreased the activity of OXPHOS complexes I, II, and IV and reduced ATP content. In conclusion, β-catenin in perinatal cardiomyocytes is required for mitochondrial biogenesis and maintains its activity

O.11

Unique profile of energy metabolism in liver sinusoidal endothelial cells (LSEC); reliance on oxidative phosphorylation

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Endothelial cells (ECs) are known to rely on glycolysis for ATP production, but they are also highly heterogeneous. Liver sinusoidal endothelial cells (LSECs) maintain the key functional characteristics of ECs from other vascular beds, however, they also display unique morphological and functional features, such as the presence of fenestrae and scavenging properties. Surprisingly, our results demonstrate that mitochondrial ATP production, not glycolysis, prevailed in LSEC energy metabolism. To further explore this peculiarity, we studied the dependence of primary murine LSEC energy metabolism on glucose, glutamine, and fatty acid oxidation. Investigations were carried out using the Seahorse XF technique for the evaluation of mitochondrial respiration and glycolysis, untargeted mass spectrometry–based proteomics for the analysis of proteins involved in energy metabolism pathways, and targeted spectrometry-based metabolomics for the analysis of acylcarnitine species. LSECs showed high metabolic plasticity, but the most effective in supporting mitochondrial respiration were glucose-derived pyruvate and short- and medium-chain fatty acids. In turn, long-chain fatty acids were not directly oxidized in mitochondria, but may have been preprocessed in catalase-rich peroxisomes before entering mitochondria for final oxidation. This was supported by: (1) the lack of etomoxir effect on mitochondrial respiration in LSECs exposed to palmitic acid, (2) the efficient mitochondrial respiration in the presence of short- and medium-chain fatty acids, and (3) the profiles of acylcarnitines and proteome in LSECs. In conclusion, our results suggest that in LSECs, representing a unique type of ECs, mitochondrial respiration based on the oxidation of glucose-derived pyruvate and short- and medium-chain fatty acids was the main source of ATP. This phenomenon might be instrumental for the harmless utilization of fatty acids in LSECs in the liver microenvironment.

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O.12

Vitamin D_3 regulates mitochondrial bioenergetics and morphology in malignant keratinocytes via VDR-dependent and partially RXRA-independent manner

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One of the proposed pharmacological targets to modify mitochondrial metabolism in cancer cells is the protein apparatus regulating the expression of nuclear-encoded mitochondrial proteins. Here we postulate that vitamin D_3 ($1,25(OH)_2D_3$) affects mitochondrial bioenergetics in A431 squamous cell carcinoma (SCC) and skin keratinocytes (HaCaT) via genomic manner. The effects of $1,25(OH)_2D_3$ on mitochondrial morphology and bioenergetics was studied in A431 cells with deletion of vitamin D_3 receptor VDR (A431Δ*VDR*) or its binding partner retinoid X receptor alpha RXRA (A431Δ*RXRA*), and compared to HaCaT keratinocytes. The genome-wide transcriptomic approach was applied to provide a comprehensive understanding of how $1,25(OH)_2D_3$ modulates mitochondrial functions.
Treatment of A431 cells with $1,25(OH)_2D_3$ resulted in changes in the morphology of mitochondria from networked to fragmented, reduction of the number of cristae and mitochondrial DNA copy number without changes in mitochondrial mass. Furthermore, mitochondrial membrane potential and basal respiration were decreased in A431 cells treated with $1,25(OH)_2D_3$ for 24h. Deletion of VDR resulted in changes in mitochondrial morphology, mitochondrial copy number, mitochondrial mass and fragmentation of mitochondria. In case of mitochondrial bioenergetics, A431 Δ*VDR* $1,25(OH)_2D_3$ treatment did not changed basal respiration and in addition, a slight increase in the mitochondrial potential was observed. Removal of the RXRA protein from A431 cells mildly affect mitochondrial response to $1,25(OH)_2D_3$ in comparison to A431 wild-type cells. Using transcriptomic approach, gene set enrichment and pathway analyses it was shown that $1,25(OH)_2D_3$ regulates expression of the genes responsible for mitochondrial depolarization and mitochondrial protein translation. Interestingly, most of the mitochondrial RXRA-dependent genes were downregulated and in contrast, RXRA-independent genes were upregulated after $1,25(OH)_2D_3$treatment.
Taken together, $1,25(OH)_2D_3$ regulate mitochondrial function via genomic manner, but partially RXRA-independently. The results open a new perspective on the effects of vitamin D_3 on mitochondria.

O.13

The role of the mitochondrial BK_{Ca} channel in bronchial epithelial cell damage induced by particulate matter

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The main place of deposition of inhaled urban particulate matter (PM) is the epithelium of the respiratory tract. In contact with cells, PM cause altered levels of reactive oxygen species (ROS), leading to inflammatory responses, and other harmful effects. Also, PM can damage the mitochondria inducing cell death. Recently, it has been shown that potassium (mitoK) channels located in the inner mitochondrial membrane are involved in the cytoprotection. In addition, activation of mitoK channels affects the synthesis of ROS, which may be a key mechanism of cytoprotection. Therefore, it seems that the protection of epithelial cells from PM-induced damage may be related to the activation of potassium channels present in the mitochondria.

To establish the involvement of mitoK channels in cytoprotection in response to PM-induced stress, we performed a series of studies using biophysical and biochemical techniques. Particulate matter samples < 4 mm (SRM-PM4.0) were used. In addition, a cell line (HBE $\Delta\alpha BK_{Ca}$) with a deletion of the gene encoding a calcium-activated large-conductance potassium (BK_{Ca}) channel was used as a model of damage to human bronchial epithelial cells (HBE wt).

Using the patch-clamp technique, it was shown that in the HBE $\Delta\alpha BK_{Ca}$ cells model, BK_{Ca}-type channel activity is not observed. In contrast, in the HBE wt model, the present mitoBK_{Ca} channel is activated by quercetin at a concentration of 10 mM, and the presence of an inhibitor (300 nM Penitrem A) abolishes this effect. In addition, to determine the role of mitoBK_{Ca} channel activation by quercetin in HBE wt cells, and using the HBE $\Delta\alpha BK_{Ca}$ deletion line, cellular respiration was measured using an oxygen electrode and changes in ROS levels using fluorescent probes. Using trypan blue staining, the survival of epithelial cells was determined in the presence of quercetin and Penitrem A. The study showed that PM damage HBE wt cells, both at the cellular and mitochondrial levels, and quercetin partially reverses this effect.

A better understanding of the relationship between mitochondrial metabolism and cell physiology may help in the search for effective cytoprotection strategies. Activators of mitochondrial BK_{Ca}-type channels, of natural origin, appear to be an effective way to support and induce mechanisms to counteract the consequences of PM-induced damage.

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O.14

In the quest to identify electron transfer paths in alternative complex III

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Alternative complex III **(**ACIII) catalyses quinol oxidation, the function of enzymes from cytochrome *bc* family (i. e. cytochrome bc_1). Interestingly, it is evolutionarily unrelated and structurally different from them. The molecular structure of ACIII revealed a supercomplex with aa_3 oxidase and highly intriguing architecture of cofactors.

Our recent work described the first genetic manipulations within ACIII of *Flavobacterium johnsoniae*, opening new possibilities for research on this enzyme. We used this system to delete the heme-containing subunits, ActA and ActE, as the ones involved in the catalytic mechanism.

The enzymatic activity assay showed lack of cytochrome aa_3 activity only in ActA deletion mutant, but not in ActE mutant. This indicates that ActE is not required for electron transfer between ACIII and cytochrome aa_3.

Another candidate that may act as a linker between ACIII and cytochrome aa_3 is a monoheme **m**obile **d**omain of ActA (mdA). To verify the proposed role of mdA, we obtained mutant that lacks mdA (ΔmdA) and examined whether and how the absence of this domain alters the activity of the supercomplex. The results showed that in ΔmdA mutant the electron transfer from ACIII to the cytochrome aa_3 does not occur. This confirmed that mdA heme is the sole donor of electrons to aa_3 oxidase.

The results of this work help us to define the electron transfer paths connecting ACIII with aa_3, and provide first insight into the functional organisation of the supercomplex.

O.15

Rearrangements of mitochondrial function in bronchial epithelial cells upon long-term exposure to carcinogenic metals

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Nickel and cadmium are carcinogens of respiratory tract, which can also induce malignant transformation *in vitro* in bronchal epithelial cells BEAS-2B. Using such model, we addressed the bioenergetic changes accompanying long-term exposure of bronchial epithelial cells to carcinogens. BEAS-2B cells were incubated with $NiCl_2$ and $CdCl_2$ for 8 weeks. Soft agar colony formation assay confirmed the development of malignant phenotype upon such treatment. Both carcinogens led to an increase in cytosolic superoxide levels, while mitochondrial superoxide was decreased. Characterization of mitochondrial physiology revealed different effects of Cd^{2+} and Ni^{2+}. In $NiCl_2$-treated cells respiration rates were decreased. In turn, upon treatment with $CdCl_2$, we observed reorganization of mitochondrial network and enhancement of mitochondrial turnover rates. The obtained results showed the rearrangements of mitochondrial function in BEAS-2B cells upon long-term exposure to sub-lethal doses of carcinogens. The profile of these changes varied depending on the carcinogen used.