the flexible region of the protein appeared to modify drastically the enzymatic properties suggesting that peculiar residue might play a crucial role in regulation of the enzyme activity.

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S12.P1

In Saccharomyces cerevisiae fructose-1,6-bisphosphate contributes to the Crabtree effect through closure of the mitochondrial unspecific channel

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In Saccharomyces cerevisiae is a Crabtree-positive yeast. The “Crabtree effect” [1] is a decreased mitochondrial metabolism by adding glucose to the culture medium and is due to various metabolic conditions such as competition by glycolysis and oxidative phosphorylation for ADP and P₇ [2]. Moreover glucose-6-phosphate (G6P) and fructose-1,6-bisphosphate (F1,6BP) appears to be important for the induction of this effect. G6P activates mitochondrial complex III, while F1,6BP inhibits the activity of complexes III and IV [3]. In an effort to understand the mechanism underlying the Crabtree effect, F1,6BP and G6P were tested in isolated mitochondria for their effects on the S. cerevisiae mitochondrial unspecific channel (ScMUC). G6P promoted partial opening of the mitochondrial ScMUC. In contrast, fructose-1,6-bisphosphate (F1,6BP) closed ScMUC, increasing coupling and thus inhibiting the rate of oxygen consumption. When added together, F1,6P reverted the mild G6P effects. F1,6BP is proposed as an important modulator of the ScMUC that upon closing triggers the “Crabtree effect”. [1] H.G. Crabtree, Observations on the carbohydrate metabolism of tumours, Biochem J 23 (1929) 536–545. [2] D.H. Koobs, Phosphate mediation of the Crabtree and Pasteur effects, Science 178 (1972) 127–133. [3] R. Díaz-Ruiz, N. Averet, D. Araiza, B. Pinson, S. Uribe-Carvajal, A. Devin, M. Rigoulet, Mitochondrial oxidative phosphorylation is regulated by fructose 1,6-bisphosphate. A possible role in Crabtree effect induction?, J Biol Chem 283 (2008) 26948–26955.

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S12.P2

Studies of the role of CK and AK energy transfer pathway in human colorectal cancer

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In this study have been used saponin-treated post-operational tissue samples of colorectal cancer (CRC) in order to estimate alteration flux of main phosphor transfer systems creatine kinase (CK) and adenylate kinase (AK) in cancer formation. Coupling between OXPHOS and CK or AK was estimated by high resolution respirometry. Both, colon tissue and CRC are expressed mitochondrial and cytosolic isoforms of CK and AK. Mitochondrial respiration was activated with creatine (10 mM) in the presence of pyruvate kinase-phosphoenolpyruvate ADP trapping system which indicated functional coupling between OXPHOS and mitochondrial CK in colon cells. But, such functional coupling was absent in tumor cells. This shows that mitochondrial CK may be downregulated or alternative function in cancer compare with normal cells. Adenylate kinase phosphor transfer system main function is maintaining ATP/ADP ration in cells under stress condition. In addition, several studies were shown that AK system can compensate ATP turnover in cells where CK system was downregulated. In this study was shown that in CRC had two fold lower activity of CK than colon cells which indicated that in cancer CK expression was decreased. But, at the same time experiments suggested that in cancer cells AK activity was 40% higher than control tissue. Furthermore, in colon cells and CRC cells mitochondrial respiration was activated with AMP (2 mM) which indicated AK functional coupled with OXPHOS in both tissues. In addition, the AK coupling was calculated and as a result in cancer was observed 40% increase coupling compared with normal control tissue. These results are accordance with AK activity. In conclusion, both colon and CRC cells express the mitochondrial and cytosolic isoforms of CK and AK but during cancer formation phospho transfer enzymes gene profile as well as those enzymes functional coupling with OXPHOS are changed. Further work is needed to research in detail AK system function in cancer.

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S12.P3

Active oxidation of several NADH-linked substrates and high oxidative phosphorylation protein contents indicate fully functional mitochondrial metabolism in AS-30D and HeLa cancer cells

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Oxidative phosphorylation (OxPhos) protein contents, enzyme activities, NAD-linked substrate oxidation and electrical potential (∆Ψm) were evaluated in mitochondria isolated from rat AS-30D hepatoma and liver (RLM). High protein contents (2–4 times) as well as enzyme activities (1.7–13 times) of Krebs cycle (ICD, 2OGDH, PDH, ME, GA), respiratory chain (COX) and β-oxidation (CPT1 and acyl-CoA dehydrogenase) were determined in hepatoma, whereas others were similar (SDH, ATP synthase and ANT) or significantly lower (GDH), vs. RLM. Hepatoma mitochondria oxidized several NAD-linked substrates at rates 1.6–6.6 times faster than RLM, without apparent change in the mitochondrial electron transfer potential, although increased cholesterol content (9.3-times vs. RLM) was determined in the hepatoma inner and outer mitochondrial membranes. The contents of mitochondrial enzymes were also assessed in in situ mitochondria (i.e., in rat AS-30D, rat hepatocytes and human HeLa cells). The contents of mitochondrial enzymes in human and rat tumor cells were higher than those observed in isolated rat hepatocytes. The acyl-CoA dehydrogenase increased content (1.5 times higher) in both tumor mitochondria and cells compared with their normal counterparts correlated with an active
cellular consumption (2–15-times) of free fatty acids after prolonged 60-min incubation. These observations clearly demonstrated that functional mitochondria are operating in aggressive tumor cells. Thus, anti-mitochondrial therapeutics appears as an alternative promising approach to deter malignant tumor growth.

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S12.P4

Bioenergetic profiling of human colorectal cancer clinical material
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In our study we have analyzed quantitatively the cellular respiration in post-operational tissue samples taken from 42 patients diagnosed with colorectal cancer (CRC). High resolution respirometry, confocal microscopy and permeabilized cell techniques in combination with Metabolic Control Analysis (MCA) were applied to detect OXPHOS defects in energy conversion systems. We performed a comparative study of normal and malignant colon cell samples by using MCA to quantify the control exerted by different electron transport chain complexes and by ATP synthasome on the respiratory flux. Flux control coefficients were determined using the inhibitor titration method with direct stimulation of respiration by ADP. The distribution of the control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of control over this system seems to be similar in both tumor and control tissues, only minor changes observed in distribution of

S12.P5

The influence of palmitic acid on the mitochondrial respiratory functions of human endothelial EA.hy926 cells
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The endothelium is considered to be slightly dependent on the mitochondrial energy supply. An excess of free fatty acids in plasma contribute to the endothelial dysfunction inducing oxidative stress, apoptosis and inflammatory response. The aim of the study was to examine the aerobic metabolism, mainly mitochondrial respiratory functions in endothelial cells and to assess the influence of the free fatty acid exposure on their viability and function. Human umbilical vein endothelial hybrid cell line (EA.hy926) was cultured in a medium containing different concentrations (100–400 μM) of palmitic acid. The cell response was observed after a short (12 h, 24 h and 48 h) or a chronic (6 days) exposure to palmitic acid. To find an optimal concentration of the applied free fatty acid, which causes an inflammatory effect, a level of protein expression of intercellular adhesion molecule 1 (ICAM1) was determined immunologically. In general, the longer time the cells were treated with a high concentration of palmitic acid, the higher expression level of ICAM 1 was, indicating elevated inflammatory response. The cellular oxygen consumption rate (OCR) was measured using the Clark-type electrode. We estimated the maximal OCR that the cells can sustain, ATP-linked OCR and non-ATP-linked OCR components of the basal respiratory rate in control and palmitic acid-treated cells. The respiratory response to elevated palmitic acid was observed in cells grown in 100 or 150 μM palmitic acid for at least 6 day or longer. To examine how endothelial cells grown under control and high palmitic acid conditions respond to simply change in respiratory substrates, mitochondrial respiratory function was measured with the glycolytic (glucose, pyruvate), lipid (palmitic acid) and amino acid (glutamine) fuels. Protein expression of markers of different catabolism pathways was studied. Moreover, high palmitic acid conditions produced increased mitochondrial and non-mitochondrial ROS formation, leading to decreased cell viability. This work is supported by the European Union from the resources of the European Regional Developmental Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

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