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 between glycolysis and oxidative phosphorylation for ADP and Pi [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 there were 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 ∆i-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 electrochemical 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

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