to understand the mechanisms by which a given enzyme exerts high or low control on the metabolic flux and how the control of the pathway is shared between pathway enzymes and transporters. By applying MCA it is possible to identify the steps that could be modified to achieve a successful alteration of flux or metabolite concentration in pathways, in our case inhibit cancer cells energy metabolism. The results of our study indicate that the breast cancer cells have significant OXPHOS, in comparison of the control — normal breast tissue. The main regulative complexes of respiration in human BC cells are complex IV (flux control coefficient — FCC = 0.74), ATP synthase (FCC = 0.61), and inorganic phosphate carrier (FCC = 0.60), FCC(s) for other complexes were found to be lower, but still substantially high and close values (0.2–0.4). The reason of this high control coefficients are not diffusion restrictions, because the concentration range for inhibitors did not differ from those for isolated cardiomyocytes. The sum of the FCC is close to four indicating direct channelling between the complexes or formation of one supercomplex of the mitochondrial respiratory chain and OXPHOS system. In BC as well as in control breast and colorectal tissue there is no significant difference between the activities of respiratory chain complex I (CI) and CII, but in human colorectal cancer cells the oxygen consumption rate of complex II is significantly higher. The Km(ADP) is with close value in CC and control tissue, but in this cancer type, oxygen consumption rate is significantly higher in control tissue than cancer cells.

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Doxorubicin-induced cardiotoxicity — A key role of altered protein kinase signaling in the response to energetic, oxidative and genotoxic stress

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Doxorubicin is one of the most powerful drugs used in chemotherapy of a large number of cancers. However, its anti-tumor effects are associated with serious cardiotoxicity, which can lead to heart failure. So far, mechanisms responsible for cardiotoxicity are not fully understood [1–3]. Here we provide evidence that persistent alterations in protein kinase cell signaling may play a key role in the etiology of cardiotoxicity. In this study, we apply targeted analysis of key protein kinase pathways [4] as well as non-biased analysis of the entire cardiac phosphoproteome [5] in two different model systems: isolated perfused rat heart, and heart from doxorubicin-treated rats. Although doxorubicin induces energetic, oxidative and genotoxic stress in the heart, activity of the energy stress sensor AMP-activated protein kinase is paradoxically down-regulated [4]. Pro-survival MAPK and Akt pathways are activated, the latter via DNA damage sensed by DNA-PK. This is at least partially responsible for low AMPK activity, since Akt inhibition can restore AMPK activation. Combined inhibition of AMPK and activation of Akt and MAPKs also leads to activation of growth-stimulating mTOR signaling. Such signaling increases cellular energy deficits and, via active mTOR signaling, also contributes to the pathological cardiac phenotype. Cardiac phosphoproteomics based on 2D-gels and mass spectrometry revealed further alterations of phosphorylation and dephosphorylation events that are associated with the early response to doxorubicin [5]. Some candidate phosphoproteins with putative functions in cardiotoxicity are currently under investigation. This study emphasizes the importance of cell signaling for our understanding of doxorubicin cardiotoxicity.

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Availability of key metabolic substrates determines metabolic responses, local oxygenation and HIF-2a stabilization in pheochromocytoma PC12 cells

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Glycolysis, glutaminolysis, the Krebs cycle and oxidative phosphorylation are the main contributors to cell metabolism. Glycolysis and glutaminolysis are strongly elevated in cancer cells providing them with ATP and building materials for tumor expansion, although the mitochondria normally do not lose their capacity for energy production [1]. As a result, cancer cells can actively proliferate at deep hypoxia and often have elevated HIF-2 levels [2]. However, in energy stress conditions HIF pathways and viability of cancer cells may be suppressed by deficiencies in metabolic pathways, and this can be probed by depriving the cells in key metabolic substrates. We examined glycolysis, O_2 consumption rate (OCR), O_2 levels, metabolic responses and HIF-2 signaling in pheochromocytoma PC12 cells, maintained for up to 6 h on different combinations of key metabolic substrates (glucose, pyruvate or glutamine — 12 combinations in total). The cells maintained on one of the substrates did not lose viability, mitochondrial Ca^{2+}, membrane potential and respiration. Upon uncoupling with FCCP the mitochondria were depolarized similarly in all the cases, but strong increase in respiration was only seen in the cells fed on glutamine combined with either glucose or pyruvate. Surprisingly, the response to FCCP did not correlate with ATP levels, which rapidly dropped upon uncoupling in the absence of glucose. At reduced O_2 availability (4% and 0% of atmospheric O_2), cell bioenergetics and local oxygenation varied drastically depending on the substrate composition. Cellular ATP and O_2 levels, in turn, orchestrated HIF-2a stabilization. At 4% O_2, rapid and deep deoxygenation was observed in the cells lacking glycolytic ATP and maintained on glutamine and pyruvate. In these cells HIF-2a level reached maximum in 2 h and then gradually decreased. At the same atmospheric O_2, only minor HIF-2a stabilization was seen in the cells fed on glucose and/or