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Mitochondrial function and bioenergetics during malignant transformation and metastasis

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The present work aims to investigate several parameters associated to the metabolic reprogramming that occurs when cells progress from the normal stage to malignant transformation and metastasis. We used a murine model of melanoma in order to approach the matter. In this model a melanocyte cell line was subjected to several cycles of adhesion impediment, resulting in stable cell lines exhibiting phenotypes corresponding to different stages of malignization: non-tumorigenic cells melan-a (original murine melanocytes); non-tumorigenic cell line 4C (obtained after 4 cycles of adherence abrogation); non-metastatic 4C11− and metastatic 4C11+ melanoma cell lines, obtained by diluting the cells from the spheroids of 4C cell line [1]. The metabolic profile of each of these different cell lines was investigated by evaluation of the relevant parameters of glycolytic and oxidative metabolic pathways. Our results showed that metastatic cell line (4C11+) released the highest amounts of lactate and displayed increased pyruvate kinase (PK) and lactate dehydrogenase (LDH) activities. These results are compatible with the Warburg effect typical of tumor cells. In contrast, results obtained with high-resolution respirometry with 4C11+ intact cells indicated an increased oxidative metabolism, with increased rates of oxygen consumption coupled to ATP synthesis when compared to the other cellular stages. We believe this is a consequence of mitochondrial biogenesis, leading to rescue of mitochondrial function, a condition believed to be necessary to metastasis. We are currently conducting studies to confirm this hypothesis. We also observed an increase in hexokinase activity bound to mitochondria (mt-HK) in the non-tumorigenic 4C cells. When compared to normal cells (melan a), no increased activity was observed in other glycolytic enzymes such as LDH and PK, suggesting that the increase in the mt-HK activity is unrelated to the modulation of glycolytic flux. We believe this HK activity is part of an antioxidant mechanism [2]. A detailed characterization of these effects in a model of tumor progression might reveal new targets for the development of more specific therapies against cancer and help the understanding of the metastatic process.

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Mitochondrial characteristics of cells with glycogen branching enzyme deficiency

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Glycogen branching enzyme (GBE1) deficiency leads to a genetic disease: glycogen storage disease type IV (GSD IV). The effect of this deficiency is an accumulation of poorly branched glycogen molecules, which have lower intracellular solubility and may lead to mechanical cell damage. Another consequence is slower glucose release from such glycogen, which can in turn disturb the energy metabolism.

The symptoms of GSD IV include dysfunctions of liver, skeletal muscles and the nervous system. They often lead to early death or strongly decrease the quality of life in adult patients. It is not clear which of the possible mechanisms leads to the symptoms, whether it is an effect of energy metabolism disruption or mechanical damage.

In our study we characterize the following parameters of human skin fibroblasts (from GSD IV patients and healthy controls): respiration rate, mitochondrial morphology, respiratory complexes content and GBE1 level.

We find a difference in the expression levels of respiratory chain complexes III and IV between cells with mutated GBE1 and controls.

Our further studies are aimed at finding whether those differences are a result of the cell adaptation to glycogen metabolism malfunction or are secondary to mechanical cell damage.

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Mitochondrial involvement in regulation of apoptosis via redox state of external cytochrome c

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There is accumulating evidence that mitochondrial pathway of apoptosis can be regulated at post-cytochrome c level by the mechanism involving reduction of cytosolic cytochrome c. We and others have shown that oxidized cytochrome c is more potent in caspase activation than reduced form and that chemicals and enzymes that reduce cytochrome c may prevent caspase activation in several models of mitochondria-mediated cell death. In this study, we present our findings demonstrating that polyphenolic redox active plant compounds anthocyanins may prevent ischaemia-induced caspase activation in perfused rat heart and that this capacity of anthocyanins correlates with their cytochrome c reducing activity. We also demonstrate that the redox state of cytosolic cytochrome c may be regulated by mitochondria. In apoptotic cells with leaky outer membrane, cytosolic cytochrome c may be oxidized by cytochrome oxidase. Besides cytochrome c-oxidizing activity, mitochondria have NADH-dependent external cytochrome c-reducing activity. We found that mitochondria from different tissues have variable NADH-dependent external cytochrome c-reducing activity: the highest activity was found in liver mitochondria, the lowest — in brain mitochondria. External NADH-cytochrome c reductase activity was found to be insensitive to nitric oxide, but was inhibitable by S-nitrosothiols indicating involvement of -SH groups in activity of the enzyme. This NADH-dependent cytochrome c reducing activity is also suppressed in some pathological states such as during heart ischaemia. The activity is partially inhibited by DIDS, a selective inhibitor of anion channels. In liver mitochondria, cytochrome b_5 reductase is the main enzyme responsible for NADH-dependent reduction of external cytochrome c. In contrast, heart mitochondria have relatively low content of this enzyme, and when VDAC (porin) was purified from heart mitochondria it was found to possess NADH-dependent cytochrome c reducing activity. Altogether, our data suggest that reduction of cytosolic cytochrome c by redox active anthocyanins can prevent ischaemia-induced cell death in the heart and that mitochondria can regulate apoptosis by changing the redox state of cytochrome c released into cytosol.

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