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Metabolic remodeling in human colorectal cancer and surrounding tissues: alterations in regulation of mitochondrial respiration and metabolic fluxes

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ABSTRACT

The aim of the work was to evaluate whether or not there is glycolytic reprogramming in the neighboring cells of colorectal cancer (CRC). Using postoperative material we have compared the functional capacity of oxidative phosphorylation (OXPHOS) in CRC cells, their glycolytic activity and their inclination to aerobic glycolysis, with those of the surrounding and healthy colon tissue cells. Experiments showed that human CRC cannot be considered a hypoxic tumor, since the malignancy itself and cells surrounding it exhibited even higher rates of OXPHOS than healthy large intestine. The absence of acute hypoxia in colorectal carcinomas was also confirmed by their practically equal glucose-phosphorylating capacity as compared with surrounding non-tumorous tissue and by upregulation of VEGF family and their ligands. Studies indicated that human CRC cells *in vivo* exert a strong distant effect on the energy metabolism of neighboring cells, so that they acquire the bioenergetic parameters specific to the tumor itself. The growth of colorectal carcinomas was associated with potent downregulation of the creatine kinase system. As compared with healthy colon tissue, the tumor surrounding cells display upregulation of OXPHOS and have high values of basal and ADP activated respiration rates. Strong differences between the normal and CRC cells in the affinity of their mitochondria for ADP were revealed; the corresponding K_m values were measured as 93.6 ± 7.7 μM for CRC cells and 84.9 ± 9.9 μM for nearby tissue; both these apparent K_m (ADP) values were considerably (by almost 3 times) lower in comparison with healthy colon tissue cells (256 ± 34 μM).

1. Introduction

Colorectal cancer (CRC) is a major cause of cancer death worldwide necessitating new strategies for treatment of this disease. Recent studies show that targeting cancer cell energy metabolism is possibly a new and very effective therapeutic approach for selective ablation of malignancies [39,61,70]. Intracellular ATP levels may be a key determinant of chemoresistance of human CRC cells [110]. There are some indications in literature that mitochondria (the main cell system for ATP generation) could play a supportive or possibly even a triggering role in metastasis of cancer cells [4].

Abbreviations: AK, adenylate kinase; ANT, adenine nucleotide translocator; AP5A, diadenosine pentaphosphate; BSA, bovine serum albumin; BB-CK, – brain type creatine kinase; CAT, carboxyatractyloside; CIMP, CpG island methylator phenotype; COX, cytochrome c oxidase; CK, creatine kinase; CRC, colorectal cancer; ETC, electron transport chain; FDG, 18-fluorodeoxyglucose; HK, hexokinase; K_m, Michaelis–Menten constant; uMtCK, ubiquitous mitochondrial creatine kinase; OXPHOS, oxidative phosphorylation; MI, Mitochondrial Interactosome; MOM, mitochondrial outer membrane; PCr, phosphocreatine; PET, positron emission tomography; PEP, phosphoenolpyruvate; PYK, pyruvate kinase; qPCR, real-time quantitative PCR; TMPD, N,N,N′,N′-tetramethyl-p-phenylenediamine; VEGF, vascular endothelial growth factor; VDAC, voltage dependent anion channel; V_0, basal respiration level; V_m, maximal respiration rate

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The first half of the 20th century led to substantial breakthroughs in bioenergetics and mitochondrial research. During that time, Otto Heinrich Warburg observed abnormally high glycolysis and lactate production in cancer cells even in the presence of oxygen (later named as "aerobic glycolysis"), leading him to suggest that defects in mitochondrial functions are at the heart of malignant cell transformation [117].

The exact mechanisms mediating the strong tendency of some cancers to aerobic glycolysis remain still unclear. Several different hypothesis have been proposed to explain the causes of the Warburg effect, such as: 1) poor tumor vascularization leading to hypoxia-induced dysfunction of mitochondria [95] and stabilization of HIF-1α-a master of regulation of glycolytic fluxes [103], 2) post-translational modifications, 3) glutamine metabolism [26,29]; 4) miRNA expression [42], 5) epigenetic changes [17], 6) nuclear and mitochondrial DNA mutations [10,18] leading to mitochondrial dysfunction in cancer cells [77], and 7) oncogene activation and loss of tumor suppressor genes function [115].

A very attractive hypothesis for explanation of the Warburg phenomenon was proposed by Pedersen and colleagues [75]. They have suggested that in highly glycolytic malignant cells the overexpression of hexokinase-2 (HK-2) associated with its binding to voltage-dependent anion channel (VDAC, located on the outer mitochondrial membrane) plays a crucial role in mediating their high rate of aerobic glycolysis. In tumor cells, the interaction of HK-2 with VDAC induces very rapid phosphorylation of glucose through the use of mitochondrially-generated ATP. Also, the binding of HK-2 to mitochondria strongly (almost 5-fold) increases its affinity for ATP [12]. It is important to note that binding of HK-2 to VDAC maintains this channel in the open state [98] which further facilities the transport of adenine nucleotides across mitochondrial membranes in malignant cells. In cancer tissues, the high glycolytic activity requires an up-regulation of the key glycolytic enzymes including HK(s). Interestingly, the percentage of hexokinase binding to the mitochondria is also significantly increased in some cancer cells. For instance, in AS-20D liver tumor cells, the hexokinase protein level (mainly HK-2) was found to be more than 500 times higher than in normal liver cells, which mainly express HK-IV instead. Furthermore, around 80% of HK-2 is found to be associated with mitochondria [7]. Due to the frequent up-regulation of HK-2 in cancer cells and its important role in glycolytic pathway, this enzyme seems to be an attractive target for anticancer drug development. In line with this, Chen et al. [19] have shown treatment of cancer cells with 3-bromopyruvate (an inhibitor of glycolysis) caused a covalent modification of HK-2 protein that triggered its dissociation from mitochondria, leading to a specific release of apoptosis-inducing factor from the mitochondria to cytosol and cell death.

The regulation of mitochondrial function is a central issue in the bioenergetics of cancer cell. Studies performed during the past decade showed that interaction between cytoskeletal proteins and mitochondria is deeply involved in the regulation of mitochondrial function. A lot of experimental data demonstrate the importance of the structural factors in the intracellular arrangement of mitochondria and in the control of outer mitochondrial membrane permeability [104,118,40,45,47,5,6]. Potential candidates for the key roles in this regulation are the cytoskeletal proteins such as plectin and tubulin [118,45,47,5,6,63]. It was hypothesized that in high-energy demand tissues there is a colocalization of β-tubulin isotype II with mitochondria (through VDAC) and it was suggested that it can be coupled with the adenine nucleotide translocase (ANT), mitochondrial creatine kinase (MtCK) and VDAC. This mitochondrial supercomplex (ANT-MtCK-VDAC) is responsible for the efficient intracellular energy transfer *via* the phosphocreatine (PCr) pathway. It is shown that the localization and function of β-tubulin isotypes varied in different muscle tissues and neoplastic cells [112,118,63,78].

Recent investigations have clarified the benefits and selective advantages of aerobic glycolysis. Although glycolysis yields a lower amount of ATP compared to mitochondrial OXPHOS, several key benefits inherent in aerobic glycolysis drive cancer cells to favor glycolysis over mitochondrial oxidation [28]. Firstly, it was proposed [91] that the high rates, but low yields of ATP production through glycolysis, may give selective advantage under rivalry for shared energy sources. Moreover, the rate of ATP generation may be 100 times faster with glycolysis as compared with OXPHOS [36]. The low efficiency of ATP generation by glycolysis is nevertheless sufficient to meet intracellular demand. Secondly, besides ATP, cancer cells need additional metabolic intermediates and precursors that are decisive for the biosynthesis of macromolecules, the ultimate building blocks necessary to expand the tumor mass during its growth and proliferation [116]. Currently, human CRC is considered as a neoplasm of the Warburg phenotype with deregulated OXPHOS system. Positron emission tomography (PET) with 18-fluorodeoxyglucose (FDG) showed that the malignancy exhibits, as compared to surrounding normal intestine tissue, higher rates of glucose consumption [22] that in turn was associated with increased intratumoral levels of lactic acid [54], overexpression of GLUT-1 [48] and genes encoding glycolytic enzymes such as pyruvate kinase M2 (PKM2) [1], glyceraldehyde-3-phosphate dehydrogenase and enolase-1α [3], LDH5 [62], and HK-2 [52].

It is becoming evident that the upregulation of glycolysis exhibited by some cancer cells does not necessarily imply a strict anaerobic phenotype or a dysfunctional OXPHOS system. Rather, it is believed that the normal interplay between the glycolysis in the cytosol and OXPHOS in the mitochondria becomes disturbed or reprogrammed in tumor cells; the Crabtree effect was observed in cancer cells that exemplifies the intimate connection between glycolysis and the oxidative metabolism [90]. Moreover, recent studies have shown that not all tumor mitochondria display OXPHOS deficiency [111,121,30,60,95]. The OXPHOS system may be the principal ATP producer ($>90\%$) for several malignant tumor cell types under normoxic conditions [111,96,97]. Therefore, drug therapy targeting OXPHOS has emerged as an important alternative for growth arrest of oxidative type tumors [39,82,96].

In our recent study, we clearly showed that CRC cannot be regarded as a tumor of purely Warburg phenotype and that in these cancer cells the OXPHOS system is the main energy source instead of aerobic glycolysis [58]. Although total glycolytic capacity of human CRC cells was found to be similar with normal cells, all their respiratory rates (both basal and ADP-activated) exceeded considerably those of healthy colon tissue samples. Furthermore, our studies indicated that the OXPHOS system may be even up-regulated in CRC cells; the content of mitochondria in human CRC cells was found to be at least 2-times higher than that in healthy colon tissue cells [58].

Recently, a new framework of "Reprogramming the of Tumor Stroma metabolism" or "Reverse Warburg effect" was introduced in experimental oncology [108,123,68]. According to the paradigm, there is metabolic coupling between mitochondria in cancer cells and catabolism in stromal cells that promotes tumor growth and metastasis. In another words, cancer cells can induce the reprogramming of tumor microenvironment (fibroblasts, macrophages and other tumor-associated cells) towards the Warburg phenotype, so they donate the necessary fuels (L-lactate, ketone bodies, glutamine and others) to anabolic cancer cells, which metabolize these *via* the tricarboxylic acid cycle (TCA) and OXPHOS. Pioneering studies showed that such metabolic symbiosis may occur between breast cancer cells and the tumor stromal fibroblasts [107,120,73], and now this paradigm has extended to other malignancies like osteosarcoma, ovarian cancer, head and neck