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The role of hexokinase in cancer

Rola heksokinazy w nowotworach

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Summary

A thorough understanding of the processes occurring in cancer cells is necessary to make cancer treatment as effective as possible. Changes in cellular metabolism in relation to normal cells are considered particularly important. One of the most interesting and promising areas is glucose metabolism and the factors affecting this process, with special emphasis on the potential role of hexokinases, especially the isoform II of this enzyme. Hexokinases (HK) are transferase enzymes involved in the process of glycolysis. Hexokinase II (HK II) plays an important role in initiating and maintaining the glycolysis process at a high level of efficiency, which is crucial for the growth and proliferation of cancer cells. An increase in the number of copies of the *HK II* gene and increased transcription of this enzyme resulting in the suppression of apoptosis and the enhancement of cell proliferation have been found in tumor cells. Hexokinase II also participates in the Crabtree effect by affecting the amount of ATP and thus the efficiency of the Ca2+ removal process outside the cell membrane by Ca2+ ATPase. Overexpression of HK II has thus far been found in pancreatic cancer, gastric cancer, breast cancer, squamous cell carcinoma of the larynx, glioblastoma multiforme, ovarian cancer and biliary tract cancer, indicating the possible key role of this enzyme in their formation and progression and providing the basis for seeking potential benefits of cancer treatment using HK II as a target of new drugs.

Keywords: **hexokinase II • tumors • oncogenesis**

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Abbreviations: **3-BrOP** – 3-bromo-2-oxopropionate-1-propyl ester, **acetyl-CoA** – acetyl coenzyme A, **AIG** – anchorage-independent growth, **ATP** - adenosine 5′-triphosphate, **Bcl-2** – B-cell lymphoma-2, **FDG** – fludeoxyglucose, **GLUT** – glucose transporter, **GSK3β** – glycogen synthase kinase 3β, **HCV** – hepatitis C virus, **HeLa** – cell line derived from cervical cancer cells, **HIF-1α** – hypoxia-inducible factor-1 alfa, **HK** – heksokinase, **HR** – hazard ratio, **LoVo** – colon carcinoma cell line, **NADH** – reduced form of nicotinamide adenine dinucleotide, **PI3K-Akt** – phosphatidyl-inositol 3-kinase-serine/threonine kinase, **U-87 MG** – human glioblastoma cell line, **VDAC** – voltage-dependent anion channel, **VEGF** – vascular endothelial growth factor

INTRODUCTION

The complex biology of cancer cells has been the subject of research for decades. The development of molecular biology and cell biochemistry has contributed to the discovery of differences in the metabolism and the transmission of intracellular signals in cancerous and normal cells. It is these differences that determine their ability to expand, grow and survive. A thorough understanding of the processes occurring in these cells is necessary to make cancer treatment as effective as possible. Changes in cellular metabolism are considered particularly important. One of the most interesting and promising areas is glucose metabolism and factors affecting this process, with special emphasis on the potential role of hexokinases, especially the isoform II of this enzyme.

GLYCOLISIS

The initial glucose metabolism in the cytoplasm, called glycolysis, is common to both anaerobic and aerobic respiration. In the case of normal tissues and normoxia conditions, pyruvate formed in the glycolysis process is converted to acetyl-CoA, which then enters into the citric acid cycle and oxidative phosphorylation. The process of complete oxidation of glucose takes place in the mitochondria and results in the formation of 32 ATP molecules (adenosine-5'-triphosphate) [46]. Anaerobic respiration is dominant in cancer cells. As a result of an uncontrolled increase in the tumor mass, the rate of angiogenesis is not sufficient and, hence, the cells have limited access to oxygen. This encourages the process of anaerobic intracellular respiration to meet the energy needs of the cancer cell [18]. In consequence, glycolysis is used as the main source of ATP, and the resulting pyruvate is converted into lactic acid in the process of lactic fermentation. The predominance of glycolysis and respiration deficits in cancer cells and rapidly dividing cells was noticed by Otto Warburg already at the beginning of the 20th century. He was the first to suggest that cancer cells, unlike normal cells, prefer anaerobic respiration even in the presence of sufficient oxygen, using mainly glucose as a substrate for the lactic fermentation process. This phenomenon is called the Warburg effect or aerobic glycolysis [47, 48]. Over the years, it has been repeatedly confirmed that increased glucose uptake and reduction of oxidative phosphorylation are typical for many proliferating cells, including cancer cells [17, 46]. Interestingly, the process of aerobic glycolysis is not characteristic of non-proliferating cells, which could indicate higher efficiency of this process and greater benefits for intensely dividing cells, although fewer ATP molecules are formed in its course than in the oxidative phosphorylation process (2 ATP molecules and 32 ATP molecules, respectively) [25].

In recent years, a new hypothesis has emerged highlighting the role of activated stromal fibroblasts. This theory, called the "reverse Warburg effect" has so far been confirmed for breast cancer [5]. It assumes that the process of aerobic glycolysis takes place in fibroblasts, which are in close cooperation with aerobic tumor cells. Hydrogen peroxide secreted by tumor cells leads to oxidative stress and aerobic glycolysis in tumor stroma [53]. Oxidative stress in stromal fibroblasts leads, in turn, to catabolic processes, such as autophagy, mitochondrial degradation or lactic fermentation. The compounds formed in these processes (lactate, ketones and glutamine) are then used in anabolic processes and aerobic respiration. Oxidative phosphorylation is assumed to take place in epithelial cancer cells, where enzymatic complexes NADH (nicotinamide adenine dinucleotide dehydrogenase) were detected [50, 53]. Currently, it is suggested that the predominance of the process of aerobic glycolysis is not the result of mitochondrial damage, as Warburg initially assumed [4]. The metabolites formed in the cell are believed to perform a role similar to oncogenes by changing signaling pathways and inhibiting cell differentiation [49]. Under the influence of lactic acidosis, cancer cells can switch from aerobic glycolysis to oxidative phosphorylation. This may contribute to targeting cancer treatment to metabolic processes occurring in mitochondria [55]. Not only long-term processes such as the Warburg effect would be important, but also short-term adaptations, such as the Crabtree effect. The Crabtree effect involves the inhibition of aerobic metabolism in cells undergoing intense proliferation under the influence of glucose, allowing them to adapt to the heterogeneous conditions characteristic of tumors. Inhibition of aerobic respiration by glucose is accompanied by the activation of the glycolysis process [4]. Although factors promoting the Crabtree effect have not yet been precisely defined, most likely glycolytic enzymes compete with mitochondria for access to cytoplasmic ADP (adenosine-5'-diphosphate) and inorganic phosphate necessary for the production of ATP [14]. Calcium ions are not insignificant, the concentration of which in the cytosol of cancer cells increases under the influence of glucose, inhibiting the enzymes involved in oxygen metabolism, especially the F_1F_0 com-

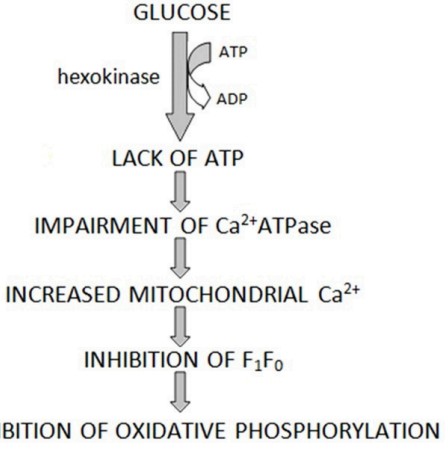

Fig. 1. The role of hexokinase and Ca2+ in the Crabtree effect. Own elaboration based on [4]

plex (ATP synthase) [4]. This is probably due to increased activity of hexokinase which leads to a decrease in the amount of ATP by phosphorylating glucose, which in turn has an impact on reducing the efficiency of Ca^{2+} removal process outside the cell membrane by Ca^{2+} ATPase [4]. It indicates a direct relationship between the Crabtree effect and HK activity in cancer cells. The understanding of this process was enhanced by research on *Saccharomyces cerevisiae* yeast cells, whose metabolism resembles the metabolism of cancer cells [14].

Although the efficiency of the glycolysis process in tumor cells can be regulated by many enzymes, most studies indicate the key importance of the increased activity of glucose transporters and hexokinases, in particular hexokinase II [27, 28, 38].

HEXOKINASES

Hexokinases (HK) are transferase enzymes involved in the process of glycolysis. In human cells, there are 4 isoforms of hexokinases – I, II, III and IV, which is also called glucokinase [22]. HK I is an isoform commonly found in many cells, with the highest concentration in brain cells; HK II is typical for insulin-sensitive cells (adipose tissue, cardiac muscle and skeletal muscle), as well as many cancerous tumor cells [40]. HK III is not predominantly present in any of the tissues, whereas HK IV is an enzyme typical of the liver and pancreas [52]. Glucokinase is a hexokinase that has the least affinity with glucose and is the only one that is not regulated through feedback by glucose-6-phosphate [52]. In contrast, hexokinase II, unlike the other enzymes, has two ends, one of which has catalytic functions and the other one has regulatory functions [3, 45].

Due to the presence of a hydrophobic N-terminal sequence, hexokinase isoforms I and II have the ability to bind to the mitochondrion, which is why they are sometimes referred to as mitochondrial hexokinases [44, 51]. Such binding provides the enzyme molecules with better access to ATP of mitochondrial origin, which, alongside glucose, is its second substrate and, at the same time, makes it resistant to the inhibitory effect of glucose-6-phosphate. Since the activity of mitochondrial hexokinases was found to be even 200 times higher in tumor cells, it is considered to be the driving force of the glycolysis process, which is extremely efficient in tumor cells [8]. It has been shown that 70% of hexokinase activity in cancer cells is associated with mitochondria, and their removal leads to a significant decrease in the efficiency of the glycolysis process [9]. Major importance in this process is attributed to hexokinase II.

HEXOKINASE II

Hexokinase II (HK II) catalyzes the reaction of converting glucose transported into the cell by glucose transporters (GLUT) to glucose-6-phosphate [13]. The resulting glucose-6-phosphate can be used by the cell as a substrate for different pathways.

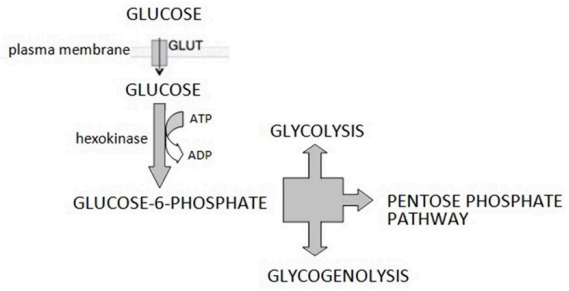

Fig. 2. The role of hexokinase in the glucose metabolism. Own elaboration based on [46]

HK II plays an important role in initiating and maintaining the process of glycolysis at a high level of efficiency, which is crucial for the growth and proliferation of cancer cells [26]. An increase in the number of copies of the HK II gene and increased transcription of this enzyme have been found in tumor cells [20]. Subcellular studies have demonstrated an association between HK II and the inhibition of apoptosis, as well as the proliferation of tumor cells. This is directly influenced by HK II binding to anionic channels dependent on membrane potential (VDAC – voltage-dependent anion channel) located on the outer membrane of the mitochondrion, which results in blocking the mitochondrial pathway of apoptosis [41]. At the same time, this binding activates the catalytic functions of this enzyme [9, 23, 30]. Further studies have shown that the 5th amino acid of the N-terminal end of HKII – histidine – is key for this binding. Its conversion to proline completely prevented the binding of hexokinase II to the outer mitochondrial membrane [7]. Therefore, studies have been carried out to determine whether blocking the binding of HK II to the mitochondrion may sensitize tumor cells to cytostatic agents. One of the studies conducted on a cell line derived from cervical cancer cells (HeLa) showed a synergistic increase in cytotoxicity induced by chemotherapeutics when the binding of HK II to the mitochondrion was disrupted. This effect was obtained by inhibiting the activity of the PI3K-Akt signaling pathway (phosphatidylinositol 3-kinases/serine/threonine kinase) responsible for the regulation of the processes of apoptosis, angiogenesis and proliferation, more precisely of the Akt kinase. This led to the activation of the β-isoform of glycogen synthase 3 (GSK3β – glycogen synthase kinase 3β), which after activation led to phosphorylation of VDAC, preventing its binding to HK II [31]. Studies have shown that negative regulation of HK II sensitized colon carcinoma cell line (LoVo) to 5-fluorouracil [33] and increased the cytotoxicity of cisplatin when HK II was removed from the mitochondria [42].

Similar studies have been carried out in relation to the sensitivity of cancer cells to radiotherapy. In human glioblastoma cell line (U-87 MG), HK II was translocated from the outer membrane of the mitochondrion into the cytoplasm by means of clotrimazole, which resulted in cell arrest at the late G_1 phase of mitosis and sensitized

them to ionizing radiation [24]. This provides the basis for seeking potential benefits of cancer treatment using HK II as a target of new drugs.

HEXOKINASE II AND NEOPLASTIC TUMORS

Increased activity of hexokinase II and its role in intensified tumor glycolysis has become the subject of many studies. In the last decade, numerous studies have reported its potential prognostic value in various cancers. Overexpression of HK II has so far been found in pancreatic cancer, gastric cancer, breast cancer, squamous cell carcinoma of the larynx, glioblastoma multiforme, ovarian cancer and biliary tract cancer, among others, indicating the possible key role of this enzyme in their formation and progression [2, 32, 34, 37]. Most reports are concerned with gastrointestinal tumors. In the case of pancreatic ductal adenocarcinoma, increased activity of HK II was found to be particularly evident in metastatic changes, indicating a possible association between HK II expression and aggressive tumor biology [2]. There was a relationship established between increased activity of the enzyme and a shorter overall survival time of patients undergoing surgery. An increase in HK II activity led to increased lactate production, cell proliferation and anchorage-independent growth (AIG) and influenced the ability to metastasize, resulting in rapid progression of the tumor. Pharmacological blockage of lactate production reduced the ability of the tumor to invade, while increasing lactate concentration promoted invasion. It confirmed the relationship between glycolysis activity and metastatic potential of the tumor [2]. In a study of stomach cancer, 257 samples taken from patients were subjected to immunohistochemical analysis. Increased activity of HK II was found in 43 of them, where it correlated with a worse prognosis, advanced stage of the disease and low differentiation of tumor cells. Moreover, in hypoxic conditions, the HK II values increased. In addition, it was shown that patients with increased HK II activity concurrent with reduced activity of Bcl-2 (proteins located on the outer mitochondrial membrane responsible for the regulation of apoptosis) were characterized by the lowest survival rates [37]. In another study, there was an increase in HK II activity found in 40 of the 188 subjects examined. What is more, this increase was shown to be correlated with increased levels of HIF-1α (hypoxia-inducible factor 1-alpha) responsible for the body's response to reduced oxygen concentrations and the elimination of their harmful effects. The study determined both factors to be independent factors affecting survival [34]. There are also studies on biliary tract tumors. In one of them, HK II levels were tested in 26 untreated patients by means of positron emission tomography using a glucose derivative in which the hydroxyl group was replaced with a radioactive atom (FDG – fludeoxyglucose) [32]. Hexokinase II was shown to be associated with increased FDG transport to neoplastic tumor cells and increased glucose metabolism, especially in moderate- and low-differentiated tumors [32]. In a colon cancer study, the amount of HK II was related to tumor size, depth of infiltration, presence of liver metastases, increased the risk of relapse and shorter survival. HK II was determined to be an independent prognostic factor for both disease-free survival and overall survival [21]. Studies on hepatocellular carcinoma arrived at similar conclusions (increase in HK II activity in 54 out of 97 specimens related to shorter overall survival – the mean survival in these patients was 33 months and was significantly shorter than in patients with a normal level of HK II activity). What is more, increased HK II activity was associated with a relatively higher risk of a worse prognosis (HR = 2.049) [16].

As in the case of gastrointestinal tumors, an increase in HK II activity has been demonstrated in breast cancer. A correlation was observed between the activity of HK II and tumor size and increased invasiveness, as well as higher activity of hexokinases in the case of triple-negative breast cancer. Based on the obtained results, the authors considered HK II to be a useful prognostic marker for breast cancer [12]. In another study, increased HK II activity was detected in 52 out of 118 samples collected from patients during mastectomy. There was no significant increase in HK II activity found in benign breast tissue changes or in the normal tissue. As in the previous study, increased risk of recurrence of the disease was shown to be associated with an increase in the enzyme activity, and HK II was determined to be a potential prognostic factor. Moreover, as in gastric cancer, the relationship between HK II and HIF-1α was demonstrated, indicating the possibility of inducing HK II by HIF-1α [39].

Another group of cancers in which the level of HK II activity can be of prognostic importance are tumors of the female reproductive system. A relationship between HK II expression and chemotherapy resistance was found in the case of epithelial ovarian cancer [43]. In the study, HK II activity was immunohistochemically determined in 111 samples taken from patients [43]. The increase in the activity of the enzyme was associated with chemoresistance and reduced progression-free survival and shorter time to relapse [43]. A study of cervical squamous cell carcinoma demonstrated a relationship between HK II activity and resistance to radiotherapy [19]. High activity of HK II had a negative prognostic value for survival, indicating not only tumor malignancy but also its resistance to radiotherapy [19].

The potential role of HK II as a treatment target has been demonstrated for central nervous system tumors [15]. In the case of medulloblastoma in mice, the deletion of HK II resulted in increased survival, differentiation of tumor cells and their slower growth [15]. In-vitro studies of human glioblastoma multiforme cells showed that increased HK II activity promoted proliferation, resistance to apoptosis and production of lactate [15]. In contrast, blocking HK II in combination with decreased activity of HIF-1α and VEGF (vascular endothelial

growth factor) resulted in the restoration of glucose oxidative metabolism, leading to a disruption in the growth of tumor cells and reduced angiogenesis [15]. What is more, it sensitized the cells to the pro-apoptotic effect of radiotherapy and temozolomide [54]. Interestingly, the addition of hexokinase I to cells lacking hexokinase II resulted in an increase in the total activity of hexokinases; however, it did not promote the process of glycolysis, which could indicate the special importance of HK II in the growth of this type of cancer [54].

A similar possible application of the treatment directed to HK II inhibition has been demonstrated for head and neck cancers [11, 56]. In a study conducted on samples taken from 140 patients with nasopharyngeal cancer, HK II activity was blocked by the use of 3-BrOP (3-bromo-2-oxopropionate-1-propyl ester, bromotris (dimethylamino) phosphoniumhexafluorophosphate), which is its inhibitor [56]. In this way, glycolysis was inhibited, the ability of the cells to proliferate and invade was impaired, and the cell arrest at the G_1 phase of mitosis and their apoptosis were induced. The combined use of 3-BrOP and cisplatin intensified apoptosis of tumor cells [56]. Similar conclusions were drawn from a study conducted on larynx cancer cells, where the expression of HK II was also blocked [11].

HEXOKINASE AND OTHER DISEASES

Heksokinase II is of interest not only in the context of oncology but also in other areas of medicine. In cardiology, research has been undertaken to establish its role in protecting the myocardium from the effects of post-reperfusion syndrome [10]. In ophthalmology, hexokinase II has been studied in the context of its potential neuroprotective effects on retinal cells [35]. It has been suggested that drugs which enhance the binding of HK II to mitochondria could inhibit cellular apoptosis in retinal degenerative diseases such as diabetic retinopathy or retinitis pigmentosa [35].

In microbiology, the association between hexokinase II in the host and the inhibition of apoptosis and resistance to treatment of *Chlamydia trachomatis* infection has been studied [1]. Similar research has been carried out with regard to another intracellular parasite – *Toxoplasma gondii* [29].

In the case of infectious diseases, the effects of increased HK II activity during HCV (hepatitis C virus) infection have been investigated [36].

Hexokinase II may also play an important role in the field of gynaecology. A study conducted on equine endometrial cells demonstrated the key role of HK II in controlling glycogen accumulation in the cells during pregnancy [6].

CONCLUSION

Hexokinase II is an enzyme whose expression is characteristic of tumor cells. Its prognostic significance has already been demonstrated for such tumors as breast cancer and colorectal cancer. Removal of HK II from the mitochondrion or inhibition of its activity disrupts tumour growth and proliferation and increases cell sensitivity to chemotherapeutics. The results of these studies may indicate the potential predictive significance of hexokinase II for the application of appropriate targeted therapy aimed specifically at the process of glycolysis of tumor cells, which is the driving force for their growth, proliferation and invasion. Proving the prognostic value of HK II may in the future be an important component in the selection process of therapy for patients with various types of cancer. However, numerous large-scale studies still need to be undertaken to state this definitively. Perhaps their results will prove to be a breakthrough in the field of oncology and a guarantee of recovery for many oncological patients.

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