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A REVIEW

HEXOKINASES IN BREAST CANCER

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ABSTRACT

Hexokinases are one of the key enzymes involved in the process of glycolysis. The level of expression of hexokinases is widely studied in breast cancer as a possible marker of unfavorable prognosis and aggressiveness of tumors. The level of expression of hexokinase may reflect the level of glycolysis activation and, thus, indicate samples with the most altered cellular metabolism.

Keywords: breast cancer, glycolysis, hexokinases, expression

INTRODUCTION

Breast cancer is one of the most frequent malignant neoplasms in women and remains the most important problem of modern oncology. World statistics show that each year more than 1.2 million women develop breast cancer. The emergence of distant metastases is the main cause of death of breast cancer patients under the age of 55 (Scully *et al*., 2012).

Tumors of the breast are characterized by considerable variability in the clinical course. There are many factors affecting the prognosis and long-term results. Among them, first of all experts distinguish age, reproductive and hormonal status, histological form and some others. The search for triggers and mechanisms of the breast cancer development, including relapse and metastasis, is conducted by many researchers, but there is still no consensus on the causes of their occurrence (Bhatelia *et al*., 2014; Redig and McAllister, 2013).

Metabolism of cancer cells

Carcinogenesis is a complex and multifactorial process in which metabolic and signaling pathways undergo significant disturbances.

However, there are general characteristics common for most malignant tumors, one of which is the disturbance of energy metabolism (Fedorova *et al*., 2015; Haraldson *et al*., 2012; Krasnov *et al*., 2013a; Kudryavtseva *et al*., 2016; Oparina *et al*., 2013; Sawayama *et al*., 2014; Snezhkina *et al*., 2016). In most cases, it switches from mitochondrial respiration to glycolysis and activation of the latter (Krasnov *et al*., 2013b; Kudryavtseva *et al*., 2014). Glycolysis is accompanied by the synthesis of ATP and is the main way of glucose catabolism used by organisms, usually with a lack of oxygen. However, malignant cells continue to use glycolysis to produce energy even when oxygen is present in the tissues in sufficient quantities (Warburg *et al*., 1927). Biochemist Otto Warburg in 1926 discovered this phenomenon, and it was named in his honor by the "effect of Warburg". As a result of the activation of glycolysis, accumulation of lactate takes place, which destroys the intercellular collagen matrix, which is one of the metastatic factors (Gatenby and Gillies, 2007; Lee *et al*., 2011; Moreno-Sánchez *et al*., 2009). Moreover, it seems that the activation of glycolysis and its utilization as the main source of ATP occurs not only as a forced measure due to the conditions of hypoxia and the disturbance of the functions of mitochondria, but also because of the necessity to use intermediate products of glycolysis for active growth and division of malignant cells (Pavlova and Thompson, 2016).

In tumors of various cancer types, switching to glycolysis as the main source of energy and the Warburg effect is observed in different degrees. In addition, the activation of glycolysis occurs through various mechanisms and the expression of a different set of enzymes participating in this process is disturbed. Furthermore, there are various regulatory mechanisms facilitating energy exchange. In cells, glycolysis occurs in the cytosol. Individual reactions and intermediates of glycolysis are well studied, and the enzymes of glycolysis are conserved for all organisms. Most glycolysis reactions are reversible, but the equilibrium is moved towards lactic acid formation (Mansouri *et al*., 2017). Glycolysis involves two stages and ten basic enzymatic reactions.

Hexokinases take part in the first stage of glycolysis. The first enzymatic reaction of glycolysis is phosphorylation - the transfer of the orthophosphate residue to the glucose molecule due to

the energy of ATP. The reaction is catalyzed by hexokinase enzymes. The formation of glucose-6-phosphate by hexokinase is an irreversible process and is accompanied by the release of a significant amount of free energy. The most important property of hexokinases is their inhibition by glucose-6-phosphate, i.e. the latter serves simultaneously as a reaction product, and an allosteric inhibitor. Hexokinases are able to catalyze the phosphorylation of not only glucose, but also other hexoses, in particular fructose, mannose, etc. In the liver, in addition to hexokinases, there is an enzyme glucokinase, which catalyzes the phosphorylation of only glucose. Glucokinase is absent in the muscle tissue.

Correlations between hexokinase expression level and pathophysiological features

Brown *et al* (2002) showed the lack of correlation between the expression of hexokinase II and the clinical stage of the disease (Brown *et al*., 2002). The association of increased expression of HK2 with the size of the tumor and the presence of metastases in this study was not revealed. Expression of hexokinases (in particular HK2) was analyzed in 24 lobular and ductal carcinomas of the breast. Hexokinase 2 was visualized by immunohistochemistry. Physiological amounts of hexokinase 2 were practically not detected, and excessively accumulated hexokinase 2 was detected in cancer cells. The analysis showed that 19 samples contained excess HK2, in the remaining 5 samples, hexokinase was expressed at the level of control. Correlations between the clinical stage of the disease and elevated levels of hexokinase were not detected. The number of tumor cells containing an increased amount of hexokinase was evaluated. It was shown that an increase in the expression of hexokinase 2 is observed in 5-100% of cells. Correlation between the relative number of cells with an increased content of hexokinase 2 and the stage of the disease was not detected also.

However, some studies indicate that increased expression of hexokinase correlates with the aggressiveness of breast cancer (Coelho *et al*., 2015). Coelho and colleagues examined 54 malignant tumors at various clinical stages, the samples grouped on the basis of expression of standard molecular markers: progesterone and estrogen receptors (PR and ES, respectively), epidermal growth factor receptor (HER-2), Ki- 67, as well as the p53 protein. The sample was analyzed for the

absence / presence of a correlation between increased expression of hexokinase 2 and the size of the primary tumor. The samples were grouped in three different ways.

The grouping by clinical stages of analysis of both mitochondrial and free hexokinase (Total HK, total hexokinase) allowed confirming a positive correlation between increased activity of this cause and stage of diseases. The highest activity was detected in tumors of stage IV. Separately from the assessment of the total activity of hexokinases, a study was also conducted for the fraction of HK associated with mitochondria. A similar nature of dependence is revealed, and more clearly.

When the samples were grouped according to the size of the primary tumor (less than 2 cm, 2 to 5 cm, more than 5 cm), both in the total and in the mitochondrial-related fractions, a positive correlation was also observed between tumor size and enzyme activity.

Assessing the dependence of hexokinase activity on the histological subtype of tumors, a traditional division into 6 subgroups was carried out based on the most commonly used markers. It is known that a subgroup with a triple negative phenotype (PR-, ER-, HER-2-) is characterized by a more aggressive course of the disease. It was shown that the level of activity of hexokinase associated with mitochondria in this type of breast cancer is the highest. Correlation of expression of Ki-67 protein with activation of hexokinases in this study was not revealed. However, this relationship was found in another paper (Sato-Tadano *et al*., 2013) - a positive correlation of the level of expression of hexokinase 2 with HIF-1a and Ki-67 was revealed. HIF-1a is a transcription factor that activates under hypoxic conditions. More than 60 genes, controlled by this factor, in particular, activation of the HK2 gene (Harris, 2002; Semenza, 2003) are described. During the rapid growth of the tumor, the cells inside it are under hypoxic conditions. According to some data, in the process of tumor growth, the increase in HK2 expression appears due to the action of factor HIF-1a (Denko, 2008). It is also shown that increased expression of HK2 in the primary tumor is associated with a decrease in the overall and disease-free survival of patients.

Currently rapidly accumulates the data about the expression of a number of genes associated with energy metabolism in breast metastases (Palmieri *et al*., 2009). The miR155 /

miR143-cascade controlling the expression of HK2 in breast cancer cells (Jiang *et al*., 2012) is described, and the participation of HK1 in the "Warburg effect" is confirmed.

Thus, the level of expression of hexokinases is widely studied in breast cancer as a possible marker of unfavorable prognosis and aggressiveness of tumors. However, large-scale retrospective studies are required on large sample, independently for each histological subtype of breast cancer. The level of expression of hexokinase may reflect the level of glycolysis activation and, thus, indicate samples with the most altered cellular metabolism.

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