ORIGINAL ARTICLE

Expression of *HK2* Gene is Deregulated in Prostate Cancer

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Abstract

Aim: Prostate cancer (PC) is the second most common cancer in men. Therefore, the search for genes that could be potential targets for therapy as well as diagnostic and prognostic markers is an important task. **Materials and Methods:** In this work, the expression of genes encoding the enzymes of the first stage of glycolysis, hexokinases (HK1, HK2, and HK3), was analyzed by quantitative polymerase chain reaction (qPCR). **Results:** In 35% of PC samples, up to 7-fold increase was revealed in the expression of the *HK2* gene, whereas in 15% of cases, up to 5-fold decrease was observed. The *HK1* mRNA level was unchanged in most of the examined samples. The *HK3* expression was very low and could not be detected by qPCR. **Conclusion:** Thus, obtained results indicate that the activation of glycolysis in PC is likely caused by the *HK2* upregulation. The increased mRNA level of *HK2* gene could be a marker of this process in PC.

Key words: Gene expression, glycolysis, hexokinases, prostate cancer, qPCR

INTRODUCTION

Prostate cancer (PC) is one of the most commonly diagnosed malignancies in men. In developed countries, about 150,000 people with PC die every year. If PC is diagnosed in time, a 5-year survival rate of patients is quite high (>90%), as there are a wide range of treatment options for early-stage PC. Recent research considers that PC is primarily driven by genetic and molecular alterations. Thus, molecular basis of PC is extensively studied worldwide.[1,2]

Carcinogenesis is a complex multifactor process that is characterized by alterations in metabolic and signaling pathways. One of the universal characteristics of malignant tumors is a disturbed energy metabolism.[3-5] There is a shift from mitochondrial phosphorylation to glycolysis even in the presence of oxygen (Warburg effect).[6] As a consequence of the Warburg effect, cancer cells secrete large amounts of lactate to the extracellular microenvironment, which breaks down the collagen matrix, and causes the connections between cells to be lost. This is one of the mechanisms of cancer metastasis.[7-9]

The causes and mechanisms of glycolysis activation in tumor cells are still not fully understood.[10,11] It has been shown that some glycolytic genes were characterized by the tumor-specific activation or inactivation and are involved in carcinogenesis.[8,11-13]

Hexokinases catalyze the first step of glycolysis, in which a molecule of glucose is phosphorylated to glucose-6-phosphate. These important enzymes are encoded by four genes, *HK1, HK2, HK3*, and *HK4* (glucokinase [GCK]). GCK is mainly expressed in liver and pancreatic beta

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cells. Hexokinases HK1, HK2, and HK3 are constitutively expressed in all human tissues. Despite the fact that these hexokinases are characterized by a high affinity for glucose and have many common biochemical properties, it has been shown the involvement of HKs in carcinogenesis.[14,15] HK2 is associated with the voltage-dependent anionic channel on the outer mitochondrial membrane and is involved in the inhibition of apoptosis.[4]

In this work, we have analyzed the expression of *HK1, HK2*, and *HK3* genes in PC using quantitative polymerase chain reaction (qPCR). The deregulation of *HK2* gene expression was detected. Obtained results will help clarify molecular mechanisms of PC and provide an area for further investigations.

MATERIALS AND METHODS

Tissue samples

A total of 59 samples of PC (II-III stages) and adjacent morphologically normal tissues (conventional "normal" tissues) were collected after surgical resection. Each sample was frozen and placed in liquid nitrogen immediately after surgery. Most of the specimens were obtained from patients with locally advanced PC, who had not received neoadjuvant chemotherapy. All patients had an elevated level of prostate-specific antigen (PSA) and lymphogenous dissemination. The Gleason score was reported as 3 + 3 = 6, 3 + 4 = 7, and 4 + 3 = 7. Tumor samples were characterized according to the International System of Classification of Tumors, based on the tumor-node-metastasis and staging classification of the Union for International Cancer Control (UICC, Version 2009). Only samples with 70% or more tumor cells were studied. The study was approved by the Ethics Committee of Herzen Moscow Cancer Research Institute, the Ministry of Health of The Russian Federation. The study was done in accordance with the principles outlined in the Declaration of Helsinki (1964).[16]

Nucleic acid isolation and reverse transcription

Total RNA was isolated from tumor and conventional "normal" tissues by MagNA Pure Compact RNA Isolation Kit (Roche, Switzerland) using MagNA Pure Compact Instrument (Roche, Switzerland) according to the manufacturer's instructions. Purified RNA was quantified using Qubit 2.0 (Invitrogen, USA). RNA quality was measured with the RIN method (RNA Integrity Number) on Agilent RNA Bioanalyzer 2100 (Agilent Technologies, USA). cDNA synthesis was done using M-MLV Reverse Transcriptase (Thermo Fisher Scientific, USA) and random hexamers according to the standard manufacturer's protocol.

qPCR

qPCR was performed with Applied Biosystems commercial primer-probe sets for target genes (*HK1*: Hs00175976_m1, *HK2*: Hs00606086_m1, and *HK3*: Hs01092850_m1) and reference ones (*RPN1*: Hs01092850_m1 and *GUSB*: Hs00939627_m1) using Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific, USA).[17,18] Each reaction was repeated three times. qPCR procedure was performed as described.[19]

qPCR data were analyzed using the relative quantification (ΔΔCt) method.[20,21] Relative mRNA level of the genes was calculated using ATG program compatible with relative quantification software (Thermo Fisher Scientific, USA).[22] At least 2-fold mRNA level changes were considered as significant.

Nonparametric Wilcoxon test was used to compare mRNA expression differences of target and reference genes in PC samples. Spearman's rank correlation analysis was used to check the dependence between target gene expression levels. $P < 0.05$ was considered statistically significant.

RESULTS

We found an increase (from 2 to 7-fold) of *HK2* mRNA level in 35% (21 of 59, $P < 0.05$) of PC samples [Figure 1]. Up to 5-fold downregulation of *HK2* expression was observed in 15% (9 of 59) of the cases. The mean value of relative mRNA level was 2.5.

The *HK1* mRNA level was not changed in most (85%, 51 of 59) of the PC samples. A decrease in *HK1* expression (from 2 to 7-fold) was detected in 15% (9 of 59) of examined samples. The mean value of relative mRNA level was 1.9.

The mRNA level of *HK3* gene was very low and could not be detected by qPCR method.

We found no significant correlation between expression levels of *HK1* and *HK2*. The Spearman's correlation coefficient was $r_s = 0.04$.

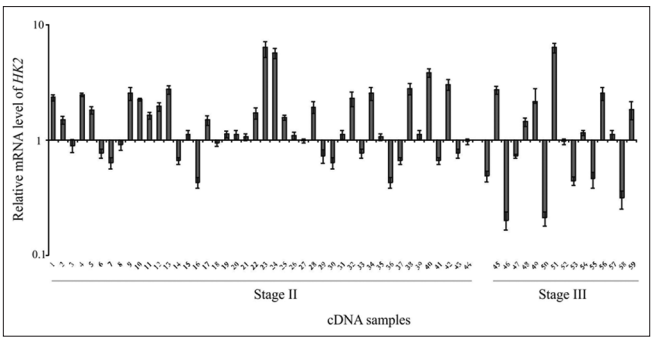

Figure 1: Relative mRNA level of *HK2* gene in prostate cancer. QPCR data

DISCUSSION

The increase of *HK2* gene expression is consistent with the results obtained by other scientists, which have shown elevated protein and mRNA levels of *HK2* in PC. However, there have been no studies on quantitative estimation of the mRNA level of the *HK2* gene in the tumor tissues compared with conventional "normal" ones.[23] Moreover, the comparison of *HK2* expression at mRNA and protein levels has also not been studied.

Interestingly, the increase in the *HK2* expression may correlate with the total PSA level.[24,25] In several clinical studies, the combination of *HK2* expression and PSA level values (4-10 ng/ml) was shown as an important diagnostic and prognostic marker of PC.[26-29]

On xenograft models of PC (mice), the mechanisms of an increase in the *HK2* expression were studied. A selective positive regulation of *HK2* through combination of Pten and p53 protein inhibition was shown in PC. Deletions of Pten gene stimulate an increase of *HK2* gene expression via activation of AKT-mTORC1-4EBP1 pathways. The loss of p53 functionality mediates the stable mRNA level of HK2 by inhibiting the biogenesis of miR143. Thus, HK2-mediated aerobic glycolysis (Warburg effect) is associated with the loss of Pten-/p53 activity.[30] Verification of the hypothesis could provide an opportunity to select a particular targeted treatment, based on inhibition of HK2 through Pten-/p53 proteins, for some patients. Currently, potential drugs that are inhibitors of HK2 are known. The most famous of these is 3-bromopyruvate, which inhibits HK2-mediated cell death by activating the mitochondrial route of apoptosis or necrosis.[31] Thus, the obtained data allow us to suggest HK2 as a potential marker for diagnosis and a target for therapy of PC.

CONCLUSIONS

We showed a significant increase in the expression of *HK2* gene in PC. The obtained results indicate that HK2 might be involved in the alterations of energy metabolism in PC. Further investigation is required to determine a possibility of application of *HK2* expression level as a marker of PC.

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