

PATTERN OF GLUTAMINASE EXPRESSION IN PRIMARY COLORECTAL CANCER

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Abstract

Objective: Expression of glutaminase isoforms (LGA and KGA) in human primary colorectal cancer compared to normal colon mucosa.

Methods: The studies were performed on a group of 20 patients with primary colorectal cancer. Tumor tissue and surrounding colon mucosa taken 6-7 cm out of the tumor border (control) were used for studies. Expression of kidney-type (KGA) and liver-type (LGA) glutaminase isoforms was determined by RT-PCR (reverse transcriptase PCR).

Results: KGA-mRNA expression was observed in all cases with CRC, in both tumor and normal mucosa. No significant differences were observed in its expression between tumor and normal colon mucosa. LGA-mRNA was present only in 10 out of 20 tumors, and it was absent from normal mucosa.

Conclusion: KGA is the main glutaminase isoforms expressed in normal and cancerous human mucosa. More studies are needed to explain why LGA is expressed only in some CRC tumors.

Key-words: Glutaminase, isoforms, primary colorectal cancer, colon mucosa.

Introduction

It is well established that the glutamine content of neoplastic tissues is low, because it is rapidly utilized and poorly synthesized. Glutaminase (GLS, EC 3.5.1.2) is a mitochondrial enzyme, which represents the most important reaction for glutamine utilization, because it hydrolyzes the splitting of glutamine into glutamate and ammonia [7]. Physiological functions of glutaminase include supply of nitrogen for urea cycle in liver, renal ammoniogenesis and synthesis of neurotransmitter glutamate during glutamine-glutamate cycle in brain [9, 15]. Glutaminase is the first enzyme of glutaminolysis – the metabolic pathway involved in the energy supply for rapidly proliferating cells, such as enterocytes and lymphocytes [14]. Glutaminolysis is also an important source of energy in cancerous cells. There are two major isoforms of glutaminase encoded by different genes: liver-type (LGA) and kidney-type (KGA). Both enzymatic isoforms display different kinetic, immunological, molecular and regulatory properties [10]. The *Gls* gene is located in chromosome 2 and encodes the kidney-type glutaminase [1], that is activated by high phosphate level (phosphate-dependent), inhibited by glutamate and expressed in normal tissues (except adult liver) and many tumours [7] including hepatocellular carcinoma [11]. The *Gls2* gene is

located on chromosome 12 and encodes liver-type glutaminase[1], that is activated by low concentration of phosphate (phosphate-independent) and is not inhibited by glutamate [7]. Its expression was identified in liver, brain, pancreas and some malignancies such as breast cancer and leukemia [8]. Till now there are no available data on the expression of glutaminase isoforms in human primary colorectal cancer (CRC) and in normal colon mucosa.

In the present work we studied the expression of glutaminase isoforms on the level of mRNA in human primary colorectal cancer.

Materials and methods

The studies were performed on a group of 20 patients with primary colorectal cancer. Tumour tissue and surrounding colon mucosa taken 6-7 cm out of the tumour border (control) were used for studies of glutaminase isoforms expression by RT-PCR (reverse transcriptase PCR).

The total RNA was isolated using TRIzol reagent (Invitrogen) according to manufacturer's protocol [6]. Expression levels of LGA-mRNA and KGA-mRNA were defined by RT-PCR. Specific oligonucleotide primers for glutaminase isoforms were based on nucleotide sequences in NCBI accession No. NN014905 for KGA, and NN013267 for LGA, and were as follows: for glutaminase kidney-type: GCAGG-

AAGACCAACATGGAA (forward), CCAG-AAGGCACAGACATGGT (reverse) for glutaminase liver-type: TTGCCTCCAGTGAGCT-CTT (forward), TGCAGACATCAGATCCTCG (reverse). The specific mRNA sequence for beta 2-microglobulin (housekeeping gene) was amplified [5] and served as an internal control (forward primer: CCAGCAGAGAATGGGAA-AGTC, reverse primer: GATGCTGCTTACAT-GTCTCG). PCR products were separated on 1.5% agarose gel with ethidium bromide. The level of specific mRNA was measured and expressed in semi-quantitative way as the ratio of optical density band of KGA and LGA to optical density band of beta 2-microglobulin. The assay was repeated 2 times for each sample and performed in duplicate. System UVI-KS4000 (Syngen Biotech.) was used for densitometric analysis of the results.

Results were expressed as mean \pm S.D and median. Quantitative comparison between studied groups was performed by non-parametric Mann-Whitney U test using Statistical software (StatSoft 9.0).

Results

The expression of KGA-mRNA was observed in all studied cases, in both primary colorectal cancer and control colon mucosa, whereas LGA-mRNA was present only in 10 out of 20 studied tumours, and it was absent from the normal mucosa (Fig.1).

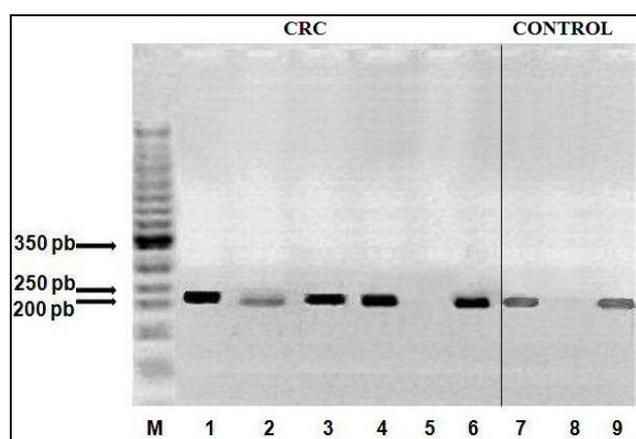


Figure 1. Expression of glutaminase isoforms in human primary colorectal cancer (CRC) and in normal mucosa (control).

RT-PCR was performed as described in Materials and Methods. Similar amount (13 μ l) of PCR product was run in each lane. Lane M -

molecular weight marker (50pb), lanes 1, 4, 7 - β_2 -microglobulin, lanes 2 and 3 - LGA and KGA from colorectal cancer of patient no 1, respectively, lanes 5 and 6 - LGA and KGA from colorectal cancer of patient no 2, respectively, lanes 8 and 9, LGA and KGA from normal colon mucosa, respectively.

The mean value of KGA-mRNA expression in colorectal tumour was 1.33 ± 0.49 and the median was 1.205. Both values were slightly lower than in control mucosa, where the mean was 1.63 ± 0.77 and the median 1.345 (Fig. 2). The differences between the normal and cancerous tissues were not statistically significant. The mean expression of LGA-mRNA in colorectal tumour was 0.51 ± 0.29 , and the median was 0.45, so it was much lower than that of KGA-mRNA (Fig.2). However, big differences in KGA-mRNA expression between individual cases were observed (from 0.6 to 3).

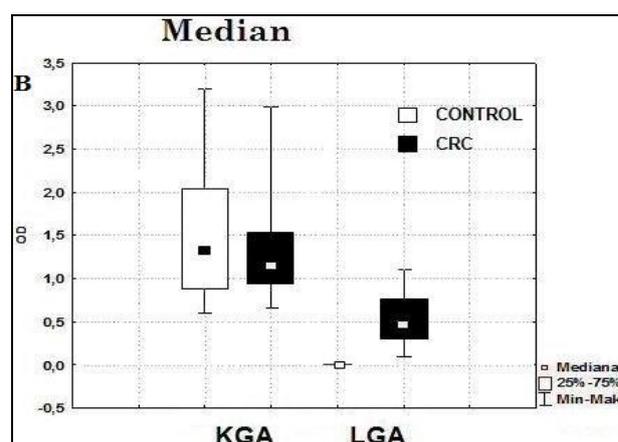
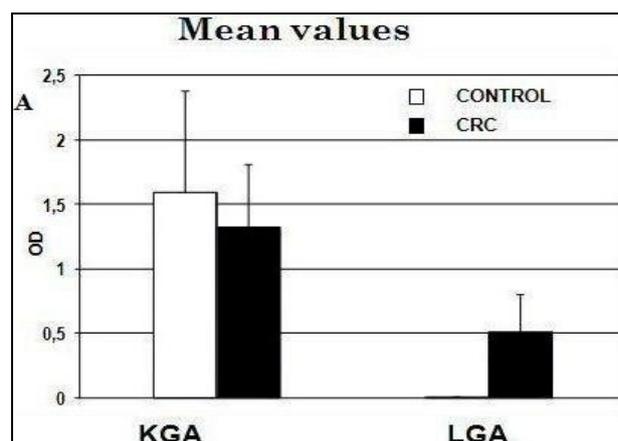


Figure 2. Mean and median values of KGA and LGA expression in CRC and control mucosa

Discussion

Colorectal cancer is a one of the most common malignancies in developed countries and the second-leading cause of cancer-related

deaths in Europe [4]. Symptoms of CRC are often non-specific and occur lately in the course of the disease. Highly sensitive and specific marker is needed to diagnose the disease, the more that patients with colorectal cancer are at high risk for metastases. Tumour cells consume a large amount of glutamine, so they compete with the host tissue for this amino acid. Thus, the presence of tumour changes the metabolism of glutamine, as well as the metabolism of nitrogen in normal host tissue. In mitochondria, glutamine is a substrate for glutaminase, the enzyme requiring high phosphate concentrations for its full activity. High concentrations of inorganic phosphate found in mitochondria of tumour cells can explain the high activity of tumour glutaminase *in vivo* [13]. A correlation between glutaminase activity and the extent of malignant proliferation was observed by many authors [12, 16, 2]. Although the importance of glutaminase in cancer has been recognized, few studies have been done on expression of this enzyme in tumours. The changes in glutaminase expression and activity, as early as 24 hrs after tumour implantation into kidney or liver were noticed [1].

In our preliminary study, we have shown that the kidney-type glutaminase is the main isoform expressed in human primary colorectal cancer. However, we have found no significant differences in its expression between tumour and normal colon mucosa. The lack of significant difference in expression of KGA between CRC and normal colon mucosa may result from the fact that colonocytes, normal cells of colon mucosa, exhibit high constitutive KGA expression, as they also utilize high amounts of glutamine as a source of energy [3]. High variability in KGA expression observed among individual patients may result from specific microenvironment (hypoxia) in tumour tissue *in vivo*, as it is known, that glutaminolysis is activated by the *Myc* gene, expression of which is oxygen-dependent [17]. The presence of liver-type glutaminase in some CRC may enhance utilization of glutamine as a source of energy for cancer cells in conditions of adequate glutamine supply.

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