Regulation of the expression of the *SLC2A5* gene and the GLUT5 protein by selected biologically active compounds of natural origin

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Abstract

Fructose in the diet of inhabitants of developed countries often exceeds the recommended 10% share in the daily energy balance. This is an alarming phenomenon because fructose is seen as the cause of many metabolic diseases. Recent studies have also proven its relationship with a higher incidence of cancer. The GLUT5 protein, encoded by the *SLC2A5* gene, is responsible for transport of fructose into cells residing in the intestine. GLUT5 is present in the enterocyte plasma membrane on the apical side. Signaling pathways and transcription factors regulating the expression of this gene, despite the increasing number of publications on this subject, have not been well understood. The literature data also mentions the influence of natural compounds such as (-)-epicatechin gallate from green tea, rubusoside from blackberry (*Rubus suavissimus*) and astragaline glucoside from the fruit of pokeweed (*Phytolacca americana*) with the activity of GLUT5 transporter inhibitors. However, these reports indicated low activity and specificity of these compounds. Due to the role of fructose in the development of some cancers, the GLUT5 protein is indicated as an important therapeutic target in anticancer therapy.

In this doctoral thesis, the cellular model was based on the Caco-2 colon cancer epithelial cell line, in which the high level of expression of *SLC2A5* mRNA as well as GLUT5 protein was confirmed by real-time RT-PCR and Western blotting, respectively.

One of the tasks of this work was to identify transcription factors responsible for regulation of *SLC2A5* gene expression. For this reason, the 5'-flanking sequence of the human *SLC2A5* gene was cloned and analyzed *in silico*, and the activity of resultant constructs was determined in the reporter gene assay. The results suggest that the basal promoter is located in the fragment carrying the -214/+58 sequence and the enhancer is located in the region -1050/-650 counting from ATG. After a series of experiments using expression vectors for transcription factors selected during bioinformatics analysis, it was found that USF-1 and USF-2 induce the activity of a reporter gene containing the promoter of the human *SLAC2A5*. Deletion analysis revealed that the response site for USF family factors within the -214/+58 fragment of the promoter, where the E-BOX sequence was identified using Matinspector software. However, usage of siRNAs capable of silencing the expression of these two transcription factors, showed no changes in the expression of the *SLC2A5* gene. Due to the fact that the E-BOX sequence is also recognized by other factors, e.g. from the SNAI family, further studies were performed using

SNAI1 and SNAI2. Overexpression of SNAI1 and SNAI2 resulted in the inhibition of the promoter activity of the *SLAC2A5* gene as well as its mRNA and GLUT5 protein. Mutagenesis of the E-BOX sequence abrogated this effect. An electrophoretic mobility assay of the protein-DNA complex (EMSA) was also performed, confirming the direct binding of the SNAI1 and SNAI2 factors to the regulatory sequence of the *SLC2A5* gene promoter.

Identification of the SNAI1 and SNAI2 factors as regulators of *SLC2A5* gene expression, encouraged us to search for pharmacologically active compounds that would increase the expression of these factors in order to inhibit *SLC2A5*. The analysis of the scientific literature indicated that trichostatin A, which is an inhibitor of histone deacetylase, may be such a compound. Experiments performed with trichostatin A confirmed its inhibitory effect on the *SLC2A5* gene promoter in the reporter gene assay. Analysis of gene expression by real-time RT-PCR showed that trichostatin A induced mRNA expression for *SNAI1* and *SNAI2* genes and inhibited *SLC2A5* gene expression. Similar results were obtained at the protein level using the Western blot technique. The EMSA test also showed an increase in the binding of nuclear lysate proteins to the E-BOX containing fragment of the analyzed promoter. Chromatin immunoprecipitation was also performed and showed an increased binding capacity of the SNAI1 factor to the *SLC2A5* gene promoter upon stimulation with trichostatin A.

Recent publications have highlighted the fact that increased expression of *SLC2A5* is associated with the resistance of some cancer cells to chemotherapeutic agents. Thus, it was checked whether trichostatin A administration sensitizes cancer cells with high expression of the *SLC2A5* gene to chemotherapeutics used in anti-colon cancer therapy. It turned out that the pre-incubation of cells with trichostatin A led to an increased sensitivity of Caco-2 cells to platinum compounds (cisplatin and oxaliplatin), and the specificity of this effect was proven for tumors with high *SLC2A5* expression.

One of the assumptions of the doctoral thesis was also to find biologically active phytochemicals showing an inhibitory effect on the *SLC2A5* gene expression. Four compounds were tested: apigenin, quercetin, ursolic acid, and chrysin. Real-time RT-PCR analysis showed that only apigenin is able to inhibit mRNA levels for the *SLC2A5*. To check whether mechanism of action of apigenin is similar to those proven for trichostatin A we tested its effects on the expression of identified transcription factors of the SNAI family. It was shown that apigenin

inhibited the expression of *SNAI1* at the mRNA and protein levels and this indicates a different mechanism of action of apigenin and trichostatin A. The literature mentions the activity of apigenin as histone deacetylase inhibitor. However, the results of Western blot analysis showed no effect of apigenin on the acetylation status of histone H3.

Due to the high content of apigenin in chamomile, an extract from the flowers of chamomile *Matricaria chamomilla L*. was made. Interestingly, the results using these extracts showed that they inhibit the expression of *SLC2A5*. Some of the extracts were subjected to a lactic fermentation process using lactic bacteria (*Levilactobacillus brevis* ATCC 14869). Fermentation increased the inhibitory properties of the extracts toward mRNA for the *SLC2A5* gene and revealed the inducing properties toward *SNAI1*. UPLC analysis showed the absence of apigenin in the extract after fermentation, suggesting that compounds other than apigenin are responsible for the induction of the SNAI1 factor and the inhibition of *SLC2A5* gene expression.

The results obtained during the implementation of this doctoral thesis indicate that the use of histone deacetylase inhibitors has therapeutic potential against colon cancer cells with the high expression of the *SLC2A5* gene and may increase the effectiveness of cisplatin and oxaliplatin in some patients. Perhaps some phytochemicals, including those present in chamomile, may have a similar effect.