The objective of the study

Thyroid cancer is the most common group of cancers of endocrine glands and their number is constantly growing in Poland. Similar tendencies are observed in all developed countries of the world. The largest group of thyroid cancer develops from the organ-specific follicular cells. We divide them into differentiated cancers, including papillary and follicular, and poorly differentiated and anaplastic cancer. The mainstay of treatment of differentiated thyroid carcinomas, including papillary carcinoma (PTC), which is 85% of all cases of thyroid cancer is surgical removal of the thyroid and lymph nodes and complementary therapy involving the administration of radioiodine I131. Iodine is accumulated by thyroid tissue, which uses it for hormone synthesis and by differentiated tumors originating from it as well. While prognosis for most of the patients with thyroid cancer is good and the tumor is considered to be extremely mild, in case of some of them we observe primary or secondary resistance to treatment with radioactive iodine. In view of the limited effectiveness of other forms of treatment, such as external beam radiotherapy, chemotherapy or attempts to tyrosine kinase inhibitors, these patients die due to local advancement or metastatic disease. Therefore, it is necessary to search for new therapies.
The protein product of SLC5A8 in the thyroid plays doubly important role. It was initially identified as an iodide transporter present in the apical membrane of thyroid cells, which is adjacent to the follicle lumen. For this reason, it was called AIT (ang. Apical Iodide Transporter). As already mentioned, thyroid cells have the ability to accumulate iodine, which is then incorporated in the hormones produced by the gland – thyroxine and triiodothyronine. Transport of iodine through the apical membrane is a complex and not fully explored process that probably involves many proteins, and its maintenance is essential for proper thyroid hormone synthesis. In addition, AIT is a tumor suppressor, which means its product ensures the proper cell divisions, and its expression is reduced not only in thyroid tumors, but also many others such as colorectal, stomach, pancreas, lung, prostate cancers and gliomas. Its silencing is so early and distinctive event in carcinogenesis that the determination of its methylation - commonly occurring method of silencing particular genes - has even been proposed as a component of a diagnostic panel for PTC. But beyond that, its role in the thyroid remains relatively unclear, indicating the need for further research.
Most researches on the suppressor function of SLC5A8 refer to colorectal cancer, and focus on the ability of the AIT protein to transport short-chain fatty acids, including inhibitors of histone deacetylase type 1 and 3 (HDAC1 and 3). These enzymes, by disconnecting the acetyl residues of histones, which are proteins forming "scaffolding" for the DNA, make it difficult to read information from a certain gene. Thus, such changes in histone acetylation effect in a direct reduction in the expression of numerous proteins.
Although SLC5A8 seems to play a vital role in maintaining normal cell physiology, especially in thyroid – both through its role as tumor suppressor and transport of short-chain fatty acids, and iodine - only a few studies marginally mention its role in the regulation of the expression of other genes involved in tumorigenesis, such as TP53. They are involved in the regulation of cell proliferation and elimination, and an imbalance of these processes is considered to be the cause of cancer. The important role of SLC5A8 is also reflected in the fact that expressing it in cell lines derived from colorectal cancer inhibits their proliferation. It is surprising, therefore, that **changes induced by overexpression of SLC5A8 at the level of the entire transcriptome have not been investigated yet**. Such an analysis would enable the identification of all cellular pathway that are regulated by AIT.

A description of the methodology

Research is based on preliminary results

Previous studies have shown that the reduced expression of SLC5A8 thyroid cancer is with no doubt result of influence of many factors. It directed our attention to the role of microRNAs in regulating expression of this gene. MicroRNAs (miRNAs, miR) are noncoding, about 22-nucleotide-long RNA molecules that regulate gene expression by binding with a specific sequence in their transcripts. Our studies on the tissues obtained from patients operated due to PTC confirmed that the decrease in the gene expression is accompanied by increased expression of certain microRNAs. The studies performed by us **confirmed binding of miR-181a-5p, miR-182-5p and miR-494-3p to the 3'UTR of SLC5A8. miR-181a-5p and -182-5p are also significantly overexpressed in thyroid cancer.** miR-494-3p level is increased too. All 3 microRNAs reduced SLC5A8 mRNA level and miR-182 decreases iodide efflux. Changes in microRNA expression can be modulated by specific inhibitors, therefore, **we confirmed that the introduction to the cells synthetic inhibitor of miR-181a-5p increases SLC5A8 mRNA levels**.
In contrast to genetic changes, changes related to the microRNA are reversible. Because a single microRNA can regulate multiple target genes, we expect that the results of silencing them will differ from those caused by overexpression of SLC5A8. Review of the literature reveals that these microRNAs are overexpressed in other cancers, silencing pathways specific for them. We can therefore, by analogy with oncogenes, call them oncomiRs. Thus, **it is of interest and justified to investigate the effect of inhibiting microRNA on changes of the entire transcriptome**.

Description of the planned research

The next step will be to compare changes in gene expression caused by overexpression of SLC5A8 and inhibition of microRNA. During the project we will create a plasmid expressing the gene SLC5A8 (that is, we will clone it) and create a specific construct to silence all three studied microRNAs. This construct is called "sponge-miR" because its function is to "sucking" from the cells particles which it is directed to. Thus, their level is significantly reduced.
Samples obtained in this way will be subjected to analysis using next-generation sequencing (NGS). It is a very modern method, that enables comprehensive analysis of the expression of all genes in a cell. In this way, we will obtain a list of genes with expression altered by overexpression of SLC5A8 and (separately) microRNA silencing. Then we compare these lists in order to determine changes induced by overexpression of the gene with ones caused by silencing selected microRNAs, and consequently – will determine the potential utility of inhibitors of microRNAs in restoring the expression of SLC5A8 in papillary thyroid cancer.
It will not only provide a list of genes regulated by SLC5A8 and microRNAs, but also bring us closer to answering the question

whether the tissue-specific inhibition of microRNA could be used in the future in the treatment of papillary thyroid carcinoma.

To sum up, the objectives of this project are:

- determination of the genes which expression in the thyroid gland cells is regulated by SLC5A8,
- simultaneous silencing of microRNAs miR-181a-5p, -182-5p and -494-3p in order to increase expression of SLC5A8,
- a thorough examination of the effect of silencing microRNAs on the entire transcriptome, or the expression of all genes in the cell.

Justification for research

This will be the first project to determine the impact of SLC5A8 on the expression of other genes at the level of the entire transcriptome.

This gene is particularly interesting for us because, firstly, as iodine transporter it plays a role in hormone production by the thyroid gland and in radioiodide therapy, and secondly – a decrease in its expression is typical for numerous cancers. In the case the PTC it is even considered to be an early event and, therefore, the definition of SLC5A8 methylation patterns in blood-circulating DNA has even been proposed as a component of a diagnostic panel for thyroid cancer. Therefore, by its overexpression we are expecting to demonstrate its broad effects, including ones on many of the genes involved in the processes of cell reproduction (proliferation) and their planned death (apoptosis).

We will define also the possibility of restoring the expression of SLC5A8 using microRNA inhibitors. Notably, through use of NGS we will identify useful from a therapeutic point of view of other target genes for these microRNAs. Each of the identified microRNAs, besides SLC5A8, regulates also many other genes, and their expression is increased in other tumors. Only data from NGS is able to provide us with information about the impact of their silencing on the entire transcriptome. We do not know any attempts to study the impact of silencing several microRNAs on entire transcriptome, what further emphasizes the novel character of this project.

Finally, we will compare the changes caused by overexpression of SLC5A8 with ones caused by silencing microRNAs selected by us in order to specify the potential usefulness of silencing selected microRNAs in the treatment of patients with papillary thyroid carcinoma.