STRESZCZENIE W JĘZYKU ANGIELSKIM

Title: microRNA-dependent regulation of the sodium iodide symporter NIS and its implication in thyroid tumorigenesis.

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Number of newly diagnosed thyroid cancer cases is constantly increasing. It is estimated that the cancer is diagnosed in approx. 3,000 people in Poland each year. Thyroid cancers can be divided into four main types: papillary, follicular, medullary and anaplastic carcinomas. Papillary Thyroid Carcinoma (PTC) is the most common malignant thyroid cancer subtype, accounting for 85% of all thyroid cancer cases.

Treatment of thyroid cancer consists in a surgical removal of the thyroid tissue with regional lymph nodes. Residual or metastatic thyroid cancer tissue is additionally ablated with radioactive iodine I131. Once radioiodine is taken up by the tumor cells, the gamma rays and beta particles are released, destroying the cells. However, this therapy is often ineffective, as a number of patients have reduced uptake of iodine and this fact results in a significantly worse clinical outcome. Transport of iodine to the thyroid cell is mediated by the Na-Iodide Transporter (NIS), localized in the basolateral plasma membrane of the thyroid cell. NIS is often lost in thyroid cancer, and its insufficient levels lead to ineffective radioiodine treatment.

The gene encoding for NIS (*SLC5A5*) is located on chromosome 19, 19p13 and consists of 15 exons and 14 introns. NIS belongs to a large family of anion-transporting proteins (SLC5, Solute carrier family 5). *SLC5A5* is expressed in almost every tissue, but its expression in the thyroid gland is significantly higher than elsewhere. Numerous studies have shown that the expression of *SLC5A5* is lowered in PTC compared to control tissue. A number of studies aimed at restoration physiological expression of *SLC5A5*, for example using recombinant human thyreotropin, or the transfer of *hNIS* gene into thyroid cell line with retroviral vector. Still, the mechanisms underlying deregulation of NIS in thyroid cancers have not been fully elucidated. In our previous studies we have shown that thyroid cancers are accompanied by significant upregulation of numerous microRNAs.

MicroRNAs (miRs) are a class of small, noncoding RNAs with the length of approx. 22 nucleotides. Each of the mature miRNAs is formed from one arm of the hairpin, named 5p or -3p. The action of mature microRNAs consist in a negative regulation of expression

of the protein - coding genes. MiRs bind to 3'UTR (*UnTranslated Region*) of target messenger RNAs. The sequence responsible for this binding, named a "seed" region, encompasses 7 nucleotides from the second to eight nucleotide of mature microRNA and must be fully complementary to the binding sequence in 3'UTR of mRNA. Binding of a miR to mRNA induces mRNA cleavage or inhibition of translation, in each case resulting in decreased synthesis of protein. Our previous studies suggested the crucial role of the miR-146 family in thyroid tumorigenesis. MiR-146b-5p is the most upregulated microRNA in thyroid cancer, while a single nucleotide polymorphism (SNP G>C, rs2910164) in the sequence of a gene encoding for miR-146a-3p is a risk factor predisposing to PTC. Because the SNP is located within the "seed" region of miR-146a-3p, each mature miR binds to a distinct set of target genes. The SNP *locus* undergoes somatic mutations in PTC, and the patients who are heterozygous for rs2910164 express all the 3 molecules, encoded by the gene: miR-146a-5p, miR-146a-3p*C and miR-146a-3p*G. In this unique situation, all the genes targeted by the miR are regulated.

The major aim of this study is to identify microRNAs that bind the *SLC5A5* transcript, regulate its expression and contribute to its aberrant levels in papillary thyroid carcinoma (PTC).

A Real-time PCR analysis performed in 47 pairs of PTC and control, non-cancerous tissue showed a 9-fold decrease of *SLC5A5* in cancer ($p=8x10^{-7}$). Further clinicopathological analysis revealed negative correlation between *SLC5A5* expression and tumor size (r=-0.32, p=0.026). Thus, the subsequent analyses aimed at identification of mechanisms underlying this phenomenon. *In silico* analysis identified microRNAs putatively binding and regulating *SLC5A5*: miR-129-2-3p, miR-146a-5p, miR-146a-3p, miR-146b-5p, miR-146b-3p, miR-21-5p, miR-221-3p, miR-29b-3p and miR-339-5p. Direct interaction between identified microRNAs and *SLC5A5* was determined in luciferase assay. Significant reduction of luciferase activity was demonstrated for miR-146b-3p (34%, p=0.03), miR-146b-5p (25%, p=0.006), miR-146a-5p-3p*C (22%, p=0.0001), miR-146a-5p-3p*G (17%, p=0.0004), miR-339-5p (21%, p=0.006) and miR-129-2-3p (29%, p=0.004). Results for microRNAs miR-21-5p, miR-221-3p and miR-29b-3p were not statistically significant, indicating that these microRNAs did not bind the 3'UTR of *SLC5A5*.

Moreover, *SLC5A5* levels were significantly (10,77-fold, p=0,01) lower in tissue samples, which were heterozygous for the rs2910164 in miR-146a-3p compared to the GG homozygotes.

A Real-time PCR analysis performed in 47 pairs of PTC and control, non-cancerous tissue showed a 15.7-fold increase of miR-146b-3p ($p=3.3x10^{-8}$), 15.6-fold increase of miR-146b-5p ($p=2.1x10^{-8}$). Furthermore, NIS levels were negatively correlated with levels of miR-146b-3p (r=-0.40, p=0.01) and miR-146-5p (r=-0.47, p=0.002). Results for miR-129-2-3p, miR-146a-5p and miR-339-5p were not statistically significant. Due to its important implications in thyroid tumorigenesis, miR-146a was not excluded from the further studies.

The cell-line based experiments were performed in a retinoic acid – hydrocortisone (tRA/H) MCF7 cells, which serve as a well - studied model for the functional analyses of NIS. The cells were transfected with microRNA inhibitors in order to analyze the impact of microRNA silencing on the expression of NIS. Significant increase of *SLC5A5* mRNA levels was demonstrated during inhibition of miR-146b-3p (1.76 fold, p=0.02), miR-146b-5p (1.77 fold, p=0.0009), both miR-146b-3p and miR-146b-5p (miR-146b, 3 fold; p=0.0029) and all the products of polymorphic miR-146a: miR-146a-5p-3p*C-3p*G (2.86 fold, p=0.0001).

Moreover, the use of inhibitors for miR-146b and miR-146a-5p-3p*C-3p*G resulted in an increased uptake of radioactive iodide. Inhibition of miR-146b resulted in 1.22 fold change of iodine uptake (p=0.03) and of miR-146a-5p-3p*C-3p*G resulted in 1.24 fold change of RAIU activity (p=0.006). The use of the miR-146b inhibitor resulted in a decreased proliferation rates of the cells and influenced their motility. The miR-146b-3p inhibitor resulted in 1.4 fold decrease of motility (p=0.002), miR-146b-5p inhibitor resulted in 1.3 fold decrease of motility (p=0.008) of the analyzed cells.

This study showed the microRNA-dependent regulation of the *SLC5A5* expression and the role microRNA inhibition in restoration of proper sodium-iodide symporter expression and function, including the radioiodine uptake. In our previous study, we have shown that the miR-146 family regulates the expression of retinoic acid receptor RARβ. Thus, inhibition of the miR-146 family results in concomitant induction of RARβ and NIS, resulting in restoration of radioiodine uptake and increased sensitivity to retinoic acid treatment. In the future, the obtained results may serve as a basis for elaboration of a novel adjuvant therapy for thyroid cancer.