Apical iodide transporter (AIT) and its microRNA – induced silencing in thyroid malignancies

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Introduction

Apical iodide transporter (AIT) is a universally expressed protein. The gene was characterized as a tumor suppressor in numerous human cancers, including malignancies of the thyroid, colon, kidney and lungs. In the thyroid it is one of the proteins transporting iodide into the thyroid follicle, which means it plays a crucial role in thyroid hormone biogenesis. Its decreased levels observed in thyroid cancer underlie its progression and inefficiency of radioactive iodine treatment, used for ablation of post-operative and metastatic thyroid cancer cells.

Recent studies show that AIT most possibly exerts its tumor-suppresive role by decreasing the expression of an antiapoptotic protein, survivin, probably through interaction with STAT3. However, this phenomenon was investigated only in cell line models and have not yet been confirmed in any human cancer.

It is known that the presence of BRAF T1799A mutation results in hypermethylation of AIT promoter and its subsequent downregulation, but no other data explain the lowered expression of AIT in thyroid tumors. Recent data on overexpression of microRNAs in thyroid cancer suggests their possible role in deregulation of AIT.

microRNA (or miRs) are short, about 22-nucleotide, non-coding RNAs that regulate the expression of target genes through binding to their 3ʼ UTRs. MicroRNA deregulation was shown in numerous diseases, including neoplasms. They are considered as an interesting targets of therapies, and first treatments aimed at modulation of microRNA expression and activity have reached phase II clinical trials.

Aim of the study

The aim of this study was to establish the impact of microRNAs on regulation of AIT expression and to assess the role of deregulation of this process in thyroid carcinogenesis.

Materials and methods

MicroRNAs deregulated in thyroid cancer that potentially target AIT were identified *in silico* (www.microrna.org). Reporter plasmid containing 3ʼ UTR of AIT cloned downstream of the firefly luciferase was ordered from Genecopeia. HeLa cells were seeded on 12-well plates, at density 100,000 per well and after 24-hours transfected with 400-500ng of plasmid using PEI as a transfectant. Subsequently, after 6 or 18 hrs cells were transfected with 12,5pmol synthetic pre- or mimic-miRNAs, respectively (LifeTechnologies). After 18 (pre-microRNA) or 4 (microRNA mimics) hours, cells were lysed with PLB and luminescence was measured in a GloMax Multi detection system.

Tissue specimens, each consisting of the papillary thyroid carcinoma (PTC, n=48) or thyroid follicular adenoma (FA, n=19) and normal adjacent thyroid tissue (N) were collected with informed consent. Total RNA was isolated using TRIzol. Reverse transcription was conducted (Promega M-MLV reverse transcriptase) and expression of AIT compared to reference gene, HPRT, was measured in SQ-PCR (Roche SYBRgreen, LightCycler480). Expression of miRNAs was quantified using TaqMan kits (LifeTechnologies). Statistical analyses were performed using Statistica 10 software (StatSoft) and Prism (GraphPad).

Results

Transfections and luciferase assays confirmed binding of the selected microRNAs: miR-181a-5p, miR-182-5p and miR-494-3p with the 3'UTR of AIT (Table 1).

Table 1:	Decrease of luminescence in the luciferase assay	
miR	Luminescence decrease [%]	P-value
-181a-5p	20	0.02
-182-5p	18	0.05
-494-3p	15	0.01

SQ-PCR analysis in 48 tissue pairs (PTC and normal thyroid adjacent tissue) showed a 67-fold decrease of AIT (p=2x10-7) (Fig. 1) and a concomitant upregulation of miR-181a-5p (1.67-fold,p=0.001) and miR-182-5p (2.03-fold, p=0.002) (Fig. 3). Mean expression of miR-494 was increased 2.2-fold, however, this change has not reached statistical significance. 44 PTC tissues were screened for BRAF T1799A mutation, revealing the presence of the mutation in 18 tissues, each representing the classic variant of PTC, whereas 26 tissues, including 7/7 PTCfv, presented the wild type BRAF sequence. When compared between the classic (47/54) and follicular variant (7/54), the expression of AIT is 12-fold lower in the classic variant of PTC (p=0.06) (Fig. 2). This phenomenon may contribute to differences in biology of those subtypes of cancer. The presence of BRAF mutation resulted in additional 4.79-fold decrease in AIT expression (p=0.001).

Interestingly, AIT expression negatively correlates with tumor diameter (r=-0.29, p=0.03, N=54) (Fig. 4) , what may suggest that its levels decreas during the natural course of the disease and thus may be one of the reasons of weaker response to radioiodine treatment in patients with more advanced disease.

Table 2: Changes in gene expression in tissue samples			
Gene		Fold change	P-value
AIT	All (N=48)	-67	$2x10^{-7}$
	BRAF T1799A T (26/44)	-23	0.001
	BRAF T1799A T/A (18/44)	-108	
miR-181		1.67	0.001
miR-182		2.03	0.002
miR-494		2.20	>0.05

Additional analyses were performed in 19 follicular adenoma (FA) tumor/control pairs. In FA samples AIT was decreased 2.24-fold compared to adjacent normal tissue (Fig. 1), whereas the expression of miRs was strongly upregulated: miR-181a-5p (1.8-fold,p=0.02), miR-182-5p (5.4-fold,p=0.05) and miR-494-3p (6.1-fold,p=0.01) (Fig. 3). Downregulation of AIT was accompanied by upregulation of its downstream efector, antiapoptotic survivin (r=-0.45,p=0.055). We did not observed such an effect in PTC.

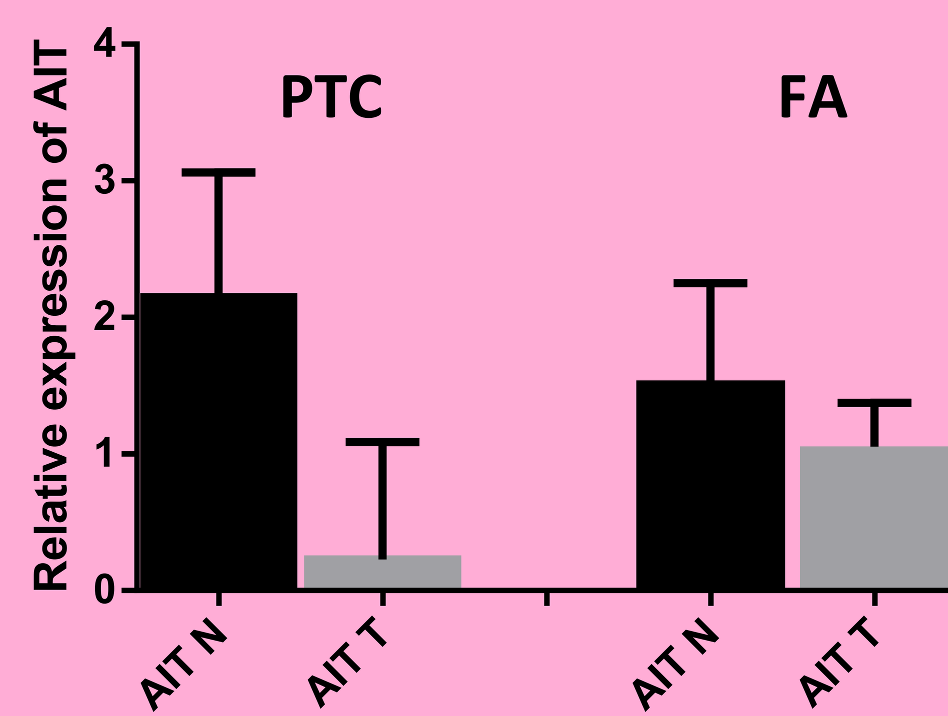

Fig. 1 Mean expression of AIT in PTC and FA samples. T-tumor, N-non-cancerous adjacent tissue

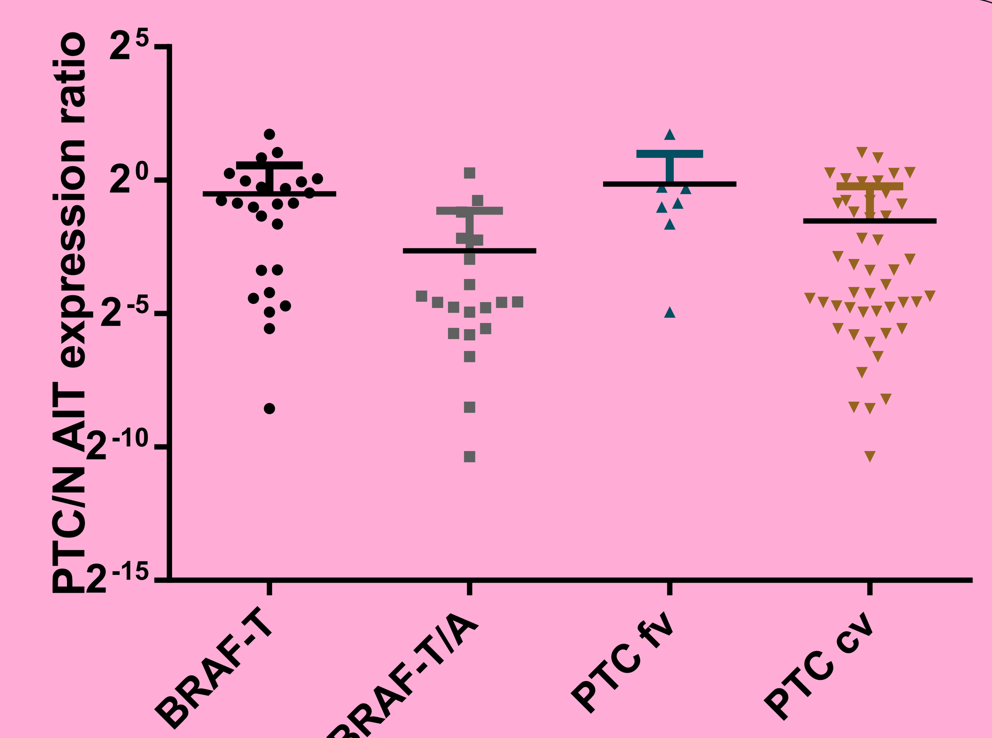

Fig. 2 AIT expression levels depending on the BRAF status and histological subtype of PTC.

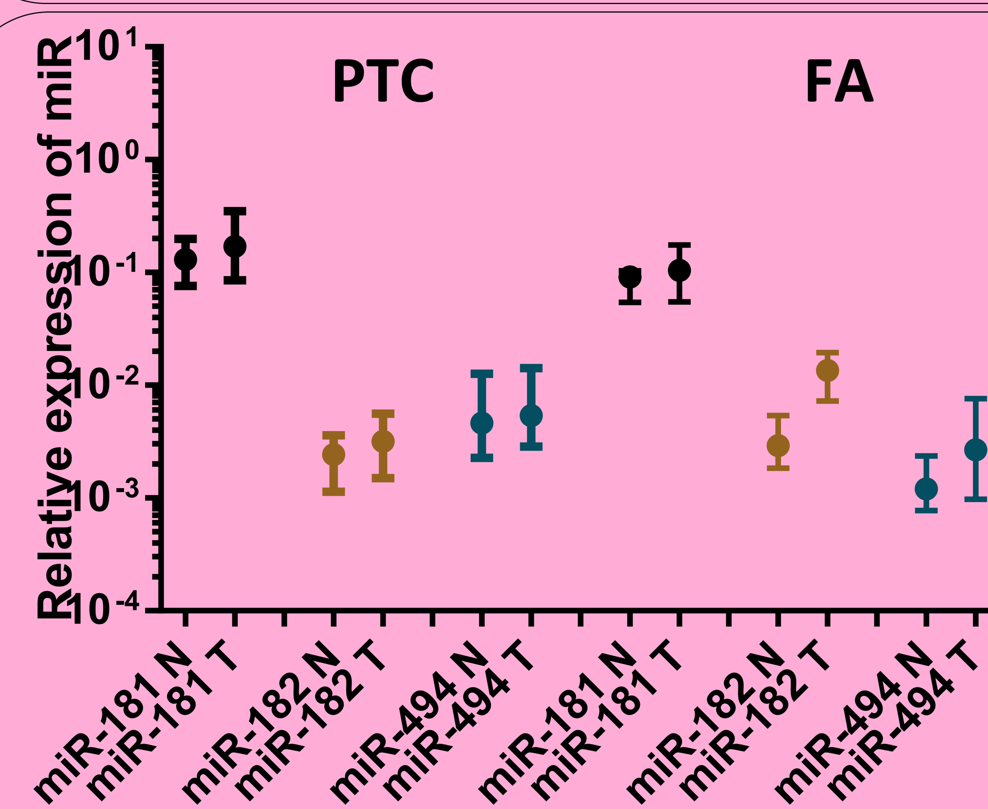

Fig. 3 Mean expression of miRs in PTC and FA samples. T-tumor, N-non-cancerous adjacent tissue

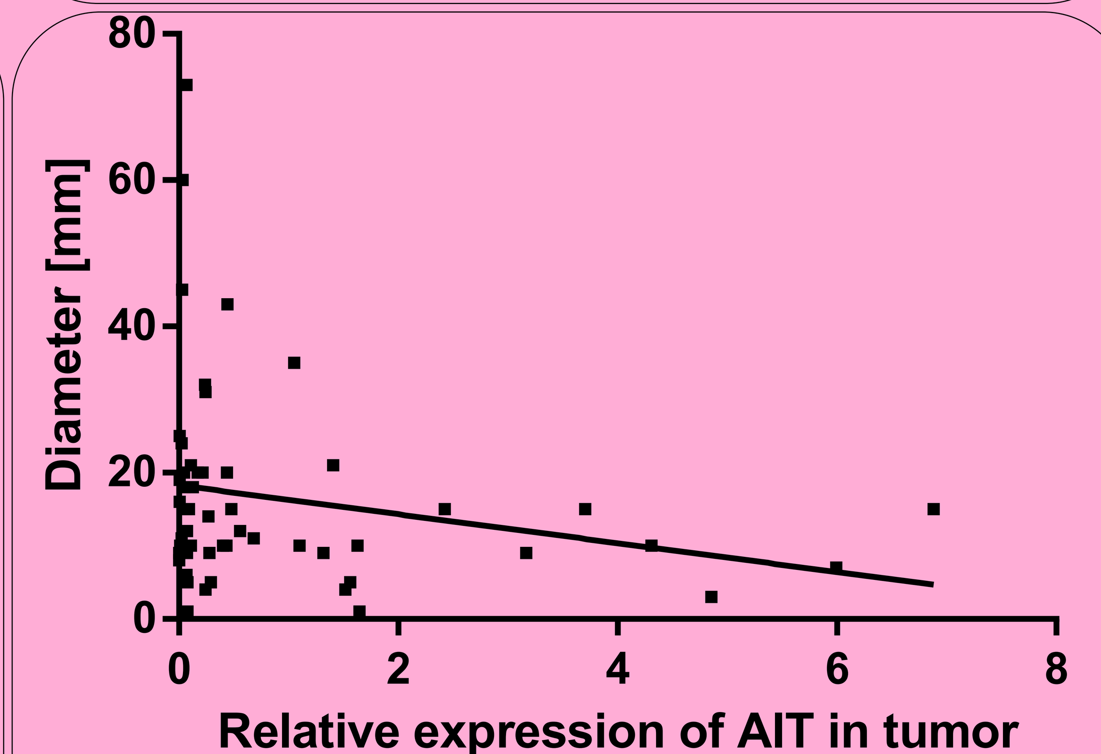

Fig. 4 Negative correlation between AIT expression and tumor size

Summary

AIT is one of the crucial genes involved in the proper functioning of the normal thyroid tissue and in the course of thyroid neoplasms. We have shown that the expression of is regulated by microRNAs, and to our best knowledge, this is the first work that confirmed this phenomenon. We have also shown that AIT levels change depending on the BRAF T1799A mutation status, histological subtype and diameter of tumor. AIT has been already characterized as iodide transporter, tumor supresor gene, here we additionaly showed that its downregulation may have antiapoptotic effect trough its downstream effector, survivin, in FA. Modulating the levels of miR-181a-5p, miR-182-5p and miR-494-3p could be used for restoration of AIT and reversal of carcinogenic process, as well as to reestablishment of radioiodine uptake by the thyroid cells.

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