

IMMUNOCYTOCHEMICAL CHARACTERISTICS OF THYROCYTES IN RADIOIODINE REFRACTORY METASTASES OF PAPILLARY THYROID CANCER

A. Zelinskaya*

State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism, NAMS of Ukraine»,
Kyiv 04114, Ukraine

The aim of the work was to carry out an immunocytochemical (ICC) study of thyroid peroxidase (TPO) and thyroglobulin (Tg) expression in the punctates of the radioiodine (RI) refractory and RI uptake metastases of thyroid papillary carcinoma (PTC), on the basis of which it is possible to develop new methods of preoperative prediction of RI resistance and RI therapy efficiency for thyroid papillary carcinoma metastases. **Materials and Methods:** The ICC research was carried out on a fine needle aspiration biopsy material of 104 metastases that were found after thyroidectomy and RI therapy, i.e. in postoperative period (79 — radioiodine-refractory metastases, 25 — radioiodine-uptake metastases). The ICC analysis of TPO and Tg expression in PTC was performed with the use of monoclonal antibodies against TPO and Tg. **Results:** RI-refractory (RIRM) and RI-uptake metastases of PTC significantly differ by the percentage of cells expressing TPO and Tg ($p < 0.05$, and $p < 0.05$ respectively). The effectiveness of RI therapy was different for patients with different percentages of TPO-positive cells. **Conclusion:** A statistically significant difference was demonstrated in the expression of TPO in a punctates of RI-resistant and RI-sensitive metastatic PTC, based on which a new method for preoperative prediction of RI resistance and RI therapy efficiency was developed. It was shown that ICC determination of Tg expression in metastases is effective in preoperative monitoring of RI resistance of PTC if the part of Tg-positive cells in punctates is lower than 56%. The comprehensive study of the ICC profile of thyrocytes in RI-uptake metastases punctates allows us to develop a personified approach to prediction, monitoring and therapy of patients with PTC.

Key Words: thyroid papillary carcinoma, radioiodine-refractory metastases, preoperative prognosis, thyroglobulin, thyroid peroxidase.

DOI: 10.32471/exp-oncology.2312-8852.vol-41-no-4.13705

After the accident at the Chernobyl Nuclear Power Plant a sharp increase of the thyroid cancer incidence among adolescents under the age of 18 that lived in Ukraine, Belarus and Russia in 1986 has been observed [1, 2]. For 20 years of observation, most cases of differentiated thyroid cancer (DTC) show an excellent prognosis for more than 95% of patients thanks to thyroidectomy and radioiodine (RI) therapy, based on the unique ability of the thyroid cells to accumulate RI [3, 4]. Despite the favorable prognosis for the majority of patients with DTC, in 4–20% of cases there is a development of the metastatic RI refractory (or resistant) disease, cells in such metastases are not capable of RI accumulation, and RI-therapy becomes ineffective. RI refractoriness of thyroid carcinoma correlates with a median's decrease of survival up to 2.5–3.5 years in patients with distant metastases [5–7]. The development of radioiodine-refractory metastases (RIRM) makes it impossible to diagnose DTC and treat it using RI. Therefore, it is desirable to have the possibility to predict as soon as possible the development of RIRM, because their detection in proper time increases the chances of patient's survival. Predicting the RI ability of metastatic DTC can

be based on the cytological determination of thyrocyte features in fine needle aspiration biopsy (FNAB) material (punctates) of the RIRM. There are some ideas about their morphological, immunohistochemical and molecular characteristics [8–10]. However, there were no studies on cytological characteristics of FNAB material of RIRM. Nevertheless, such research could be useful for developing methods allowing the earliest preoperative prediction of RIRM of DTC.

The study of antigen expression — thyroid peroxidase (TPO) and thyroglobulin (Tg) which are involved in the process of RI accumulation and its retention in thyrocytes, is of particular interest. Immunohistochemical studies of the thyroid metastases that do not accumulate RI showed a significant reduction of Tg, TPO, corresponding mRNA and association of TPO expression with BRAF mutation as a demonstration of thyroid carcinoma aggression [10, 11]. According to other data, a significant correlation of TPO expression with the ability to accumulate RI was found, however the authors mentioned that it is impossible to predict RI accumulation ability by the level of TPO expression [7]. There are also conflicting data about Tg expression in the RIRM, the correlation of its presence in papillary thyroid carcinoma (PTC) cells and its metastases and the RI resistance phenomenon [12–14].

Consequently, the aim of the work was to find out the role of TPO and Tg expression abnormality in PTC cells in the process of RI resistance development, on the basis of which it is possible to develop

Submitted: March 12, 2019.

*Correspondence: E-mail: ann.zelinskaya@ukr.net

Abbreviations used: DTC — differentiated thyroid cancer; FNAB — fine needle aspiration biopsy; ICC — immunocytochemical; PTC — papillary thyroid carcinoma; RI — radioiodine; RIRM — radioiodine-refractory metastases; RIUM — radioiodine-uptake metastases; Tg — thyroglobulin; TPO — thyroid peroxidase.

methods for preoperative prediction of RI resistance to RI therapy for PTC. The main task was to conduct an immunocytochemical (ICC) investigation of TPO and Tg expression in the puncture material of RIRM and radioiodine-uptake metastases (RIUM).

MATERIALS AND METHODS

The research was realized on the material of FNAB of PTC and their metastases under the control of sonography at the clinic of State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine». The age of the patients (male and female) ranged from 8 to 40 years. The informativity of native samples was assessed by microscopy during FNAB. Informational samples included at least 6–9 groups of thyrocytes per one object carrier. The research was carried out on a punctates of 104 metastases that were found during sonographic and scintigraphic examination after thyroidectomy and RI-therapy, i.e. in postoperative period (RIRM — 79, RIUM — 25).

All patients were informed and provided their consent on the use of their biological material in the research.

For ICC, punctates were fixed in methanol, stained with Romanovsky stain with following antigen recovery reaction, developed in our lab [15]. Then the detection of antigens was conducted with indirect immunoperoxidase method using mouse monoclonal antibodies (MCAB) against the following antigens: Tg — clone RBU/01 (Sigma, USA) and clone DAK-Tg 6 (DakoCytomation, Denmark), antibody dilution 1:100; TPO (TPO-47, DakoCytomation, Denmark) dilution 1:50. Monoclonal antibodies against mouse g-globulins (DakoCytomation, Denmark), dilution 1:100 were used as the second antibodies. After ICC reaction, cell nuclei were stained with a hematoxylin solution. Afterwards, at least 1000 thyrocytes were counted, and the percentage of antigen-positive thyrocytes was determined.

Statistical analysis was performed with a non-parametric Mann — Whitney U-test and correlation analysis. The calculations were done using Statistica. 11 program. The immunopositive cell percentage was compared in RIRM and RIUM groups using Mann — Whitney U-test. The differences between the groups were considered significant at p value < 0.05 .

RESULTS AND DISCUSSION

The paucity of RIUM group in comparison with the RIRM group is due to the fact that such metastases are visualized on scintigrams with RI, after which patients receive the RI treatment. The majority of patients with RIUM are not referred to the FNAB procedure for diagnosis, because such metastases are in most cases successfully treated with RI and disappear eventually. At the same time, all patients with RIRM were referred to the FNAB in case of sonographic suspicion of lymph node metastases that were not visualized during the scintigraphic study with RI. It should be noted that the studies did not require additional invasive interventions as they were performed on the material for diagnostic

cytological studies. It was possible due to the method developed earlier in the laboratory of the combination of cytological and ICC studies on the same specimens [15].

After counting the immunopositive thyrocytes in metastasis punctates, a statistically significant difference in percentages of cells expressing TPO was detected between RIRM and RIUM of PTC. According to Mann — Whitney U-test, these two groups were statistically different by this index ($p < 0.05$). A high level of TPO expression was detected in 77.5% RIUM punctuates (the percentage of immunopositive thyrocytes was 65–100%), and in 12.5% RIUM punctuates this index was 30–46%. On the contrary, in RIRM samples, TPO expression was absent in 83% of cases, and in the rest of 17% cases only 6–22% of cells were TPO-positive (Fig. 1, 2).

An analysis of the RI therapy effectiveness demonstrated that, despite the fact that all metastases of the RIUM accumulated RI, the effectiveness of RI therapy was different in patients with different percentages of TPO-positive cells. Thus, for 87.5% of patients from this group, RI therapy was successful and new metastases did not appear (the percentage of TPO-positive cells in punctates of such metastases was higher than 50%). At the same time, 12.5% of metastases lost the ability to accumulate RI (progress of secondary RIRM), and eventually were surgically removed; the percentage of TPO-positive cells was 30–46%. So, even if the intermediate percentage of TPO-expressing cells (30–46%) cause a moderate RI accumulation, their counts were insufficient for successful RI therapy. Explanation of the different RI therapy efficacy can be based on the phenotypic heterogeneity of the thyrocyte populations in metastases. Probably, a small percentage of TPO-positive cells is destroyed by RI, and TPO-negative cells give rise to secondary RIRM.

According to the data of ICC study of Tg expression in the RIRM group compared to RIUM, a high expression of this antigen was detected in 78–100% of RIUM thyrocytes. At the same time, in the RIRM group, the percentage of Tg expression varied from 0 to 100%. In 17% (10/56) of the patients with RIRM, Tg expression was observed only in isolated cells or not

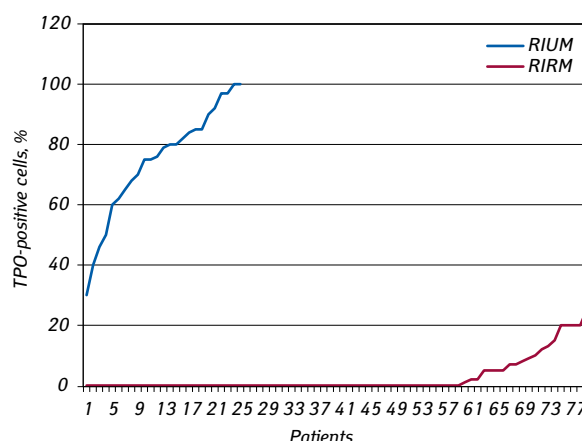


Fig. 1. Distribution of RIUM and RIRM by percentage of cells containing TPO. $p < 0.05$

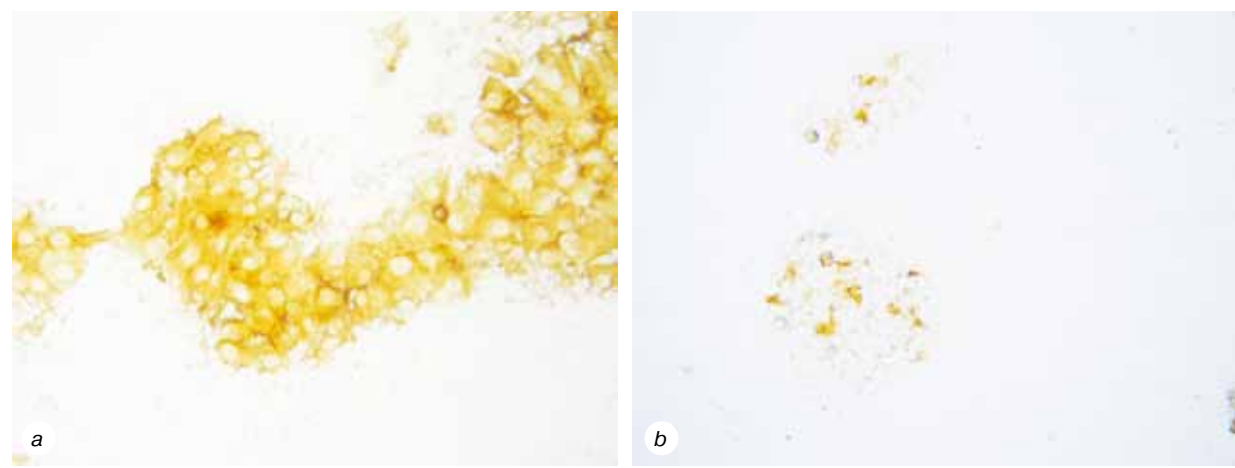


Fig. 2. Immunocytochemical detection of expression of TPO in punctuates of PTC metastases. *a* — intense reaction of epithelium of RIUM, × 400; *b* — expression of TPO in significant part of the epithelium of RIRM, × 200

observed at all. In 28% of cases, the percentage of Tg-positive thyrocytes was 78–100% (16/56), as in RIUM group. In 55% (30/56) of cases, Tg-positive cells were observed in 4–75% of the thyrocyte population. A statistically significant difference in the TG-positive cells percentage between RIRM and RIUM groups was shown ($p < 0.05$). However, in 28% of RIRM of PTC, this index did not differ from RIUM and constituted 78–100%. At the same time, there were no cases of Tg absence in the RIUM punctates in comparison with RIRM. This indicates the possibility of preoperative prediction of RIRM, detected in the postoperative period, only with the presence of a relatively low TG-positive percentage of cells (the median level for RIRM indicator corresponds to 56%) (Fig. 3, 4).

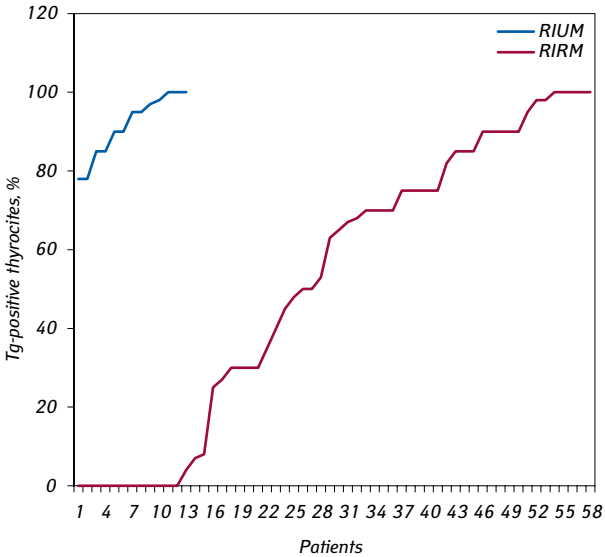


Fig. 3. Distribution of RIUM and RIRM by percentage of cells containing Tg. $p < 0.05$

Table. The ICC data on TPO and Tg expression in thyrocytes from punctates of RIAM and RIRM of PTC

ICC data	RIUM (25)	RIRM (79)
TPO-positive cells (Average ± SD, %)	74.08 ± 3.56*	2.67 ± 5.83
TPO-positive cells (median, %)	77.5	0
Tg-positive cells (Average ± SD, %)	91.31 ± 1.85*	47.53 ± 4.99
Tg-positive cells (median, %)	92.5	56

Note: The difference is significant, * $p < 0.05$.

The ICC data on TPO and Tg expression in thyrocytes from punctates of RIAM and RIRM of PTC are presented in the Table.

The results do not contradict the literature data according to which all cases with weak Tg expression of the relapse cells were iodine-negative [16]. It should be noted that there is a clear heterogeneity of the RIRM group by the percentage of TG-positive cells in comparison with the RIUM group, and there is different intensity of ICC reaction with Tg antibodies in RIRM cells — from intense to weak. In several cases, instead of uniform reaction in the thyrocytes' cytoplasm there was observed a granular atypical Tg accumulation. At the same time, in the RIUM group, the reaction of the thyrocyte cytoplasm with Tg antibodies was bright and homogeneous. Thus, not only the number of Tg-positive cells should be assessed, but also the intensity of the reaction.

The correlation analysis showed a direct correlation between the percentage of thyrocytes with positive Tg and TPO expression in the punctates of RIRM of PTC ($r = 0.42$). In that way, the assessment of both antigens expression in the punctates of postoperative metastases of PTC increases the prediction accuracy of their RI resistance in determining the doubtful percentage of cells with TPO (35–46%). If the percentage of Tg-positive cells in this case is not higher than 56%, then the probability of ineffective RI therapy increases. The high probability of RI refractoriness is related to the low percentage of thyrocytes expressing both antigens. Also, in 10% of RIRM Tg expression is totally absent or present only in single cells. In several of these cases, despite the presence of regional DTC metastases, there was no Tg increase in blood serum, which is considered as a reliable marker for the PTC recurrence in the postoperative period. It is known that the serum Tg levels depend on the number of tumor cells that synthesize Tg and on their differentiation grade [16, 17]. It motivates to monitor more carefully patients with a high probability of the development of RI resistance, determined by prognostic cytological factors, even after receiving negative scans with RI and zero-level of serum Tg.

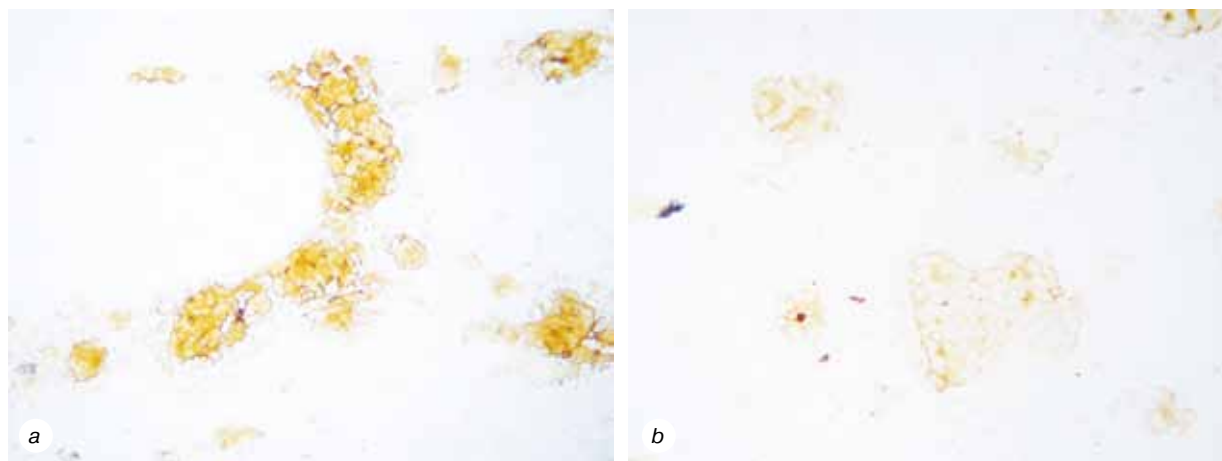


Fig. 4. Immunocytochemical detection of Tg expression in the PTC metastases punctates. *a* — intense reaction of epithelium of RIUM, $\times 400$; *b* — weak expression of Tg in the thyrocytes of RIRM, $\times 200$

In conclusion, the method of preoperative prediction of RIRM, detected in the postoperative period, was developed and based on the ICC determination of TPO expression in FNAB. If the TPO expression is absent in the punctates of postoperative metastases, it predicts the RI resistance, the RI therapy ineffectiveness and necessity of their surgical elimination. If the percentage in thyrocytes that contains TPO is more than 77.5% (median value), RI therapy will be successful. The compatible definition of Tg and TPO in the punctates of postoperative metastases of PTC increases the prediction accuracy of their RI resistance.

REFERENCES

1. Bogdanova T, Zurnadzy L, Nikiforov Y, *et al.* Histopathological features of papillary thyroid carcinomas detected during four screening examinations of a Ukrainian-American cohort. *Br J Cancer* 2015; **113**: 1556–64.
2. Drozd V, Branovan I, Shiglik N, *et al.* Thyroid cancer induction: nitrates as independent risk factors or risk modulators after radiation exposure, with a focus on the Chernobyl accident. *Eur Thyroid J* 2018; **7**: 67–74.
3. Markovina S, Grigsby P, Schwarz J, *et al.* Treatment approach, surveillance, and outcome of well-differentiated thyroid cancer in childhood and adolescence. *Thyroid* 2014; **24**: 1121–6.
4. Haugen B, Alexander E, Bible K, *et al.* 2015 American Thyroid Association management guidelines for adult patients with thyroid nodule and differentiated thyroid cancer: The American Thyroid Association guidelines task force on thyroid nodules and differentiated thyroid cancer. *Thyroid* 2016; **26**: 1–133.
5. Pacini F, Yasuhiro I, Markus L. Radioactive iodine-refractory differentiated thyroid cancer: unmet needs and future directions. *Expert Rev Endocrinol Metab* 2012; **7**: 541–4.
6. Pfister D, Fagin J. Refractory thyroid cancer: a paradigm shift in treatment is not far off. *J Clin Oncol* 2008; **26**: 4701–4.
7. Deandreis D, Ghuzlan A, Leboulleux S, *et al.* Do histological, immunohistochemical, and metabolic (radioiodine and fluorodeoxyglucose uptakes) patterns of metastatic thyroid cancer correlate with patient outcome? *Endocr Relat Cancer* 2011; **1**: 159–69.
8. Castro M, Bergert E, Goellner J, *et al.* Immunohistochemical analysis of sodium iodide symporter expression in metastatic differentiated thyroid cancer: correlation with radioiodine uptake. *J Clin Endocrinol Metab* 2001; **86**: 5627–32.
9. Min J, Chung J, Lee Y, *et al.* Relationship between expression of the sodium/iodide symporter and (131)I uptake in recurrent lesions of differentiated thyroid carcinoma. *Eur J Nucl Med* 2001; **28**: 639–45.
10. Mian C, Barollo S, Pennel G, *et al.* Molecular characteristics in papillary thyroid cancers (PTCs) with no 131I uptake. *Clin Endocrinol* 2008; **68**: 108–16.
11. Huang M, Batra R, Kogai T, *et al.* Ectopic expression of the thyroperoxidase gene augments radioiodide uptake and retention mediated by the sodium iodide symporter in non-small cell lung cancer. *Cancer Gene Ther* 2001; **8**: 612–8.
12. Bätge B, Henning Dralle H, Barbara Padberg B, *et al.* A histology and immunocytochemistry of differentiated thyroid carcinomas do not predict radioiodine uptake: A clinicomorphological study of 62 recurrent or metastatic tumours. *Virchows Archiv* 1992; **421**: 521–6.
13. Xin Zhang, Hui Li, Chen Wang. Quantitative thyroglobulin response to radioactive iodine treatment in predicting radioactive iodine-refractory thyroid cancer with pulmonary metastasis. *PLoS One* 2017; **12**: e0179664.
14. Dralle H, Schwarzrock R, Lang W, *et al.* Comparison of histology and immunohistochemistry with thyroglobulin serum levels and radioiodine uptake in recurrences and metastases of differentiated thyroid carcinomas. *Acta Endocrinol (Copenh)* 1985; **108**: 504–10.
15. Bozhok Y, Tavokina L, Epstein E. The new things in diagnostic of thyroid cancer. Optimal combination of morphological and immunocytochemistry research methods of punctious materials. *Med Announcer* 1996; **138**: 40–3.
16. Wiseman S, Griffith O, Deen S, *et al.* Identification of molecular markers altered during transformation of differentiated into anaplastic thyroid carcinoma. *Arch Surg* 2007; **142**: 717–27.
17. Latrofa F, Ricci D, Montanelli L, *et al.* Lymphocytic thyroiditis on histology correlates with serum thyroglobulin autoantibodies in patients with papillary thyroid carcinoma: impact on detection of serum thyroglobulin. *Clin Endocrinol Metab* 2012; **97**: 2380–7.