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Expression of tight junction zonula occludens-1 protein in thyroid carcinomas

P.P. Zynych,
V.M. Pushkarev,
N.I. Levchuk,
E.A. Shelkovoy,
M.Yu. Bolgov

State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine»

Abstract. Epithelial-mesenchymal transition (EMT) has a decisive influence on the process of metastasis (Mts) formation. A key event of EMT is the reorganization of tight junctions (TJs). The TJ membrane is based on scaffold, adapter and signaling proteins, which include ZO-1-3 (zonula occludens) proteins. ZO proteins play an important role in inflammation, tumorigenesis, cancer progression, etc., affecting cell proliferation and motility and are considered tumor suppressors. It has been repeatedly proven that ZO proteins are diffused or lost in almost all types of inflammation, during tumor formation and Mts. **The aim** was to compare the amount of ZO-1 in the tissues and blood of patients with papillary thyroid carcinoma (PTC) without Mts and with Mts to the lymph nodes. **Material and methods.** Postoperative samples of tumor tissue, Mts and conditionally normal tissue were used for the studies. Blood was obtained by standard venipuncture and stored in EDTA tubes at -80 °C. The amount of ZO-1 was determined using E-EL-H1516 enzyme immunoassay kits (Elabscience, USA). The study involved patients with PTC and goiter. Group 1 included patients with PTC without Mts, group 2 – with PTC and Mts, group 3 – with goiter. **Results.** The obtained data indicate that the level of ZO-1 in the conditionally normal thyroid tissue is 2 times higher than in tumor tissue of PTC without Mts. In PTC with Mts, the amount of ZO-1 is significantly lower than in the conditionally normal thyroid tissue, both with and without Mts. In goiter tissue, the amount of ZO-1 does not differ from that in conditionally normal carcinoma tissue, but is significantly higher than in tumor tissue and Mts. The level of ZO-1 in the blood of PTC patients is significantly lower than in the blood of healthy people. The amount of ZO-1 in the blood of PTC patients with Mts is lower and significantly different from that of patients with PTC without Mts. **Conclusions.** Thus, a decrease in ZO-1 concentration in tumors and blood may be one of the markers of Mts in PTC.

Keywords: ZO-1 proteins, tight junctions, metastasis, thyroid carcinomas.

Epithelial cell plasticity, exemplified by EMT, has a crucial impact on tumorigenesis and Mts [1]. A key event in EMT processes is the reorganization of intercellular junctions, especially TJs [2].

TJs are specialized contact sites between epithelial and endothelial tissue cells that create selective semipermeable paracellular barriers, which maintain compartments of the body with different fluid

compositions. Occludin, tricellulin, and marvelD3 belong to the MARVEL family of TJ-associated proteins. Occludin and tricellulin jointly contribute to the formation of the TJ filament branch point and the maintenance of the epithelial barrier [3]. Claudins regulate the interaction between occludin, tricellulin, and marvelD3, which in turn modulate claudin oligomerization [2, 4].

Оригінальні дослідження

TJ transmembrane proteins are connected intracellularly to complexes of scaffold and adaptor proteins, which in turn are directly or indirectly linked to actomyosin and the microtubule cytoskeleton. Both scaffold, adaptor proteins, and the cytoskeleton perform architectural and regulatory functions [5, 6]. These include ZO proteins (ZO-1, ZO-2, and ZO-3), ZO-1-associated nucleic acid-binding protein (ZONAB), proteins associated with Lin Seven 1 (Pals1), multi-PDZ domain protein 1 (MUPP1), cingulin (CGN), symplekin, atypical protein kinase C, protein phosphatase 2A, Rab3b, Rab13, PTEN, 7H6, guanine nucleotide exchange factors (GEFs), and GTPase-activating proteins (GAPs), which not only provide the protein scaffold of the TJ membrane, but also influence TJ belt formation and dynamics, regulation, and repair by connecting the TJ to the cytoskeleton and its regulatory proteins. The scaffold proteins ZO-1 and ZO-2 are essential for barrier assembly, as depletion of either abolishes claudin chain assembly [2, 7].

ZO proteins are regulated mainly by the nuclear factor kappa B (NF- κ B) signaling pathway and cytokines, including tumor necrosis factor alpha (TNF- α), interleukin 1 beta, interleukin-6, interleukin-8, interleukin-9, interleukin-22, and interleukin-33 [8, 9].

The aim of the work was to compare the amount of ZO-1 in tissues and blood of patients with PTC without Mts and with Mts to the lymph nodes.

Material and methods

The study protocol was approved by the Institute's Ethics Committee. All patients signed informed consent for further diagnostic and scientific studies of their biomaterial.

Postoperative samples of tumor tissue, Mts, and conditionally normal (non-tumor, histologically unchanged) tissue obtained from the surgical department of the Institute's clinic were used for the studies. Blood plasma samples were also studied. Blood was obtained by standard venipuncture and stored in EDTA-coated tubes. Plasma was separated by centrifugation within 10 minutes after blood collection. Control blood samples were taken from healthy individuals without thyroid diseases or other chronic diseases.

Samples were stored at -80 °C until use. The amount of ZO-1 was determined using E-EL-H1516 enzyme immunoassay kits (Elabscience, USA).

Measurements were performed at an optical wavelength of 450 nm on a Stat Fax 3200 enzyme immunoassay plate analyzer (Awareness Technology, USA). Calibration curve for calculating the concentration of ZO-1 presented in the **Fig.1**.

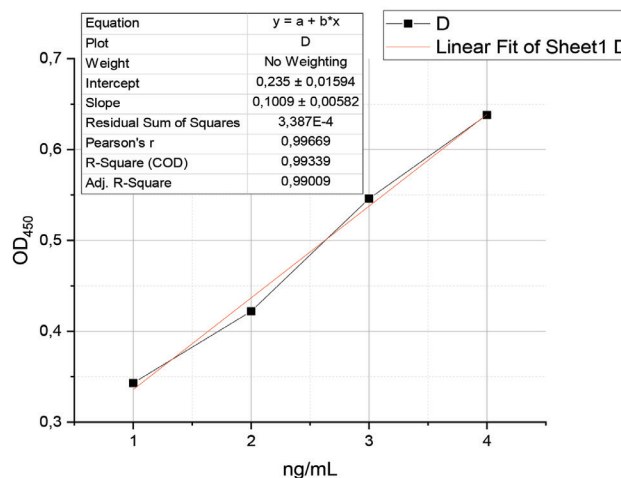


Fig. 1. Calibration curve for calculating the concentration of ZO-1 using E-EL-H1516 enzyme immunoassay kits.

Statistical analysis and data presentation were performed using Origin 2019b software. The results of the study are presented as $M \pm SE$. Student's pair t-test was used to compare data groups. Values of $p \leq 0.05$ were considered as significant.

Results and discussion

The study included patients with PTC without Mts, PTC with Mts and patients with goiter. Group 1 included 10 patients with PTC without Mts, group 2 – 14 patients with PTC and Mts, group 3 – 4 patients with goiter. The average age of the patients was 51.50 ± 0.93 years. The blood of 4 healthy individuals without chronic diseases, representative in terms of age, served as controls.

Our data indicate that the level of ZO-1 in the conditionally normal thyroid tissue is 2 times higher than in the tumor tissue of PTC without Mts (**Table 1**, groups 1 and 2), which is consistent with the data of other authors for different types of cancer [9-12]. In PTC with Mts, the amount of ZO-1 is significantly lower than in the conditionally normal thyroid tissue, both with and without Mts (**Table 1**, groups 4 and 5). In goiter tissue, the amount of ZO-1 does not differ from the conditionally normal tissue of carcinomas, but is significantly higher than in tumor tissue and Mts (**Table 1**, group 7).

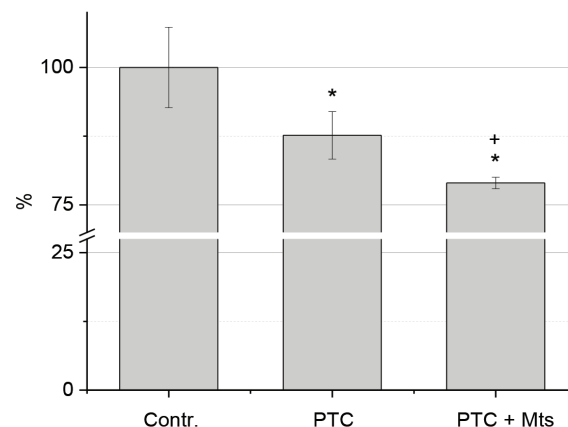
Table 1. ZO-1 concentration in normal, tumor and Mts tissues (ng/ μ g of protein), as well as in blood plasma (ng/mL) of patients with PTC

Groups	Tissue	n	M \pm SE
PTC without Mts			
1	Conditionally normal	10	0.304 \pm 0.064
2	Tumorous	10	0.152 \pm 0.027 ¹
PTC with Mts			
3	Conditionally normal	14	0.229 \pm 0.016
4	Tumorous	14	0.136 \pm 0.026 ³
5	Mts	14	0.126 \pm 0.025 ³
Goiter			
6	Conditionally normal		0.266 \pm 0.040
7	Goiter tissue	4	0.210 \pm 0.036 ^{4,5}
Blood plasma			
8	Control	4	2.01 \pm 0.15
9	PTC without Mts	5	1.76 \pm 0.09 ⁸
10	PTC with Mts	9	1.59 \pm 0.02 ^{8,9}

Note. Indices indicate significant differences from the corresponding group, $p < 0.05$.

The ZO-1 concentration in blood plasma was also determined. In the blood of patients with PTC without Mts, a decrease of ZO-1 was observed compared to blood of healthy people ($p = 0.038$) (Table 1, group 9). In the blood of patients with PTC with Mts the level of ZO-1 showed a further decline and was significantly different from the indicator of patients with PTC without Mts (Table 1, group 10). The **Fig. 2** shows the percentage of ZO-1 decrease in the blood plasma of patients with PTC with and without Mts.

ZO proteins play a crucial role in embryonic development, tissue homeostasis, injury repair, inflammation, tumorigenesis, cancer progression and Mts formation. Their structural function ensures the barrier function of TJs, and the signal regulation function affects cell proliferation and motility. ZO proteins affect cell proliferation by translocating between the nucleus and the membrane, regulating cell cycle-related proteins, such as cyclin D1 and PCNA. ZO-1 mainly inhibits cell proliferation by binding to the SH3 domain of the transcription factor ZONAB. ZONAB promotes the transcription of the cell cycle-related proteins cyclin D1 and PCNA by directly binding to their promoters. In addition, ZONAB accelerates cell cycle progression by associating with cyclin-dependent kinase 4 and cyclin D1, facilitating their entry into the nucleus [9]. ZO proteins are therefore considered tumor suppressors. Although ectopic expression or knock-

**Fig. 2.** Percentage of ZO-1 decrease in the blood plasma of patients with PTC.

Note. PTC – tumor tissue, PTC + Mts – tumor tissue with Mts. * – significantly different from control samples, $p = 0.04$; + – significantly different from PTC samples $p \leq 0.05$.

down of ZO-1 and ZO-2 did not affect the growth of lung cancer cells, they significantly regulated cell migration and invasion [13]. It has been repeatedly demonstrated that ZO proteins are diffused or lost in almost all types of inflammation. The reduction of ZO proteins in gastrointestinal cancer and hepatocellular carcinoma correlates with tumor progression and poor prognosis. Therapeutic interventions targeting ZO in gastrointestinal and liver cancer are being considered [14].

An important task facing endocrine surgeons is the search for specific markers of Mts [15-21]. According to our data, markers that may indicate aggressiveness and metastatic potential of the thyroid tumors are proliferating cell nuclear antigen (PCNA) [15, 16], expression of a rare isoform of ribosomal kinase p70S6K – p60S6K [17], expression of ZEB1 [18], MMPs [19] and TGF- β 1 in tumor tissues, plasma, and blood cells [20]. Another such marker of Mts may be ZO-1, a decrease in the concentration of which in tumor tissues and blood of patients with PTC may indicate the possibility of the Mts formation.

Conclusion

Thus, the reduction of zonula occludens-1 concentration in both tumors and blood may be one of the markers of metastasis in thyroid carcinomas. Of particular value is the clear difference of this indicator from the norm in blood plasma, which can be used for prognostic purposes in the preoperative period.

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Abbreviations

- Mts** – metastasis
PTC – papillary thyroid carcinoma
TJ – tight junctions
ZO – zonula occludens

Експресія білка щільних з'єднань ZO-1 у карциномах щитоподібної залози

П.П. Зінич, В.М. Пушкарєв, Н.І. Левчук,
 Є.А. Шелковой, М.Ю. Болгов

ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України»

Резюме. Епітеліально-мезенхімальний перехід (EMT) має вирішальний вплив на процес утворення метастазів. Ключовою подією EMT є реорганізація щільних з'єднань (TJ). В основі мембрани TJ містяться каркасні, адаптерні та сигнальні білки, до яких належать білки ZO-1-3 (zonula occludens). Білки ZO відіграють важливу роль у запаленні, пухлиногенезі, прогресуванні раку та ін., впливаючи на проліферацію та рухливість клітин і вважаються супресорами пухлин. Було неодноразово доведено, що білки ZO дифундують або втрачаються майже при всіх типах запалень, під час пухлиноутворення та метастазування. **Мета.** Метою роботи було порівняльне дослідження кількості ZO-1 у тканинах і крові хворих із папілярною карциномою щитоподібної залози (ПКЩЗ) без метастазів та з метастазами в лімфовузлах. **Матеріал і методи.** Для досліджень використовували післяопераційні зразки пухлинної тканини, метастазів та умовно-нормальної тканини. Кров отримували стандартною венепункцією і зберігали в пробірках з EDTA при -80 °C. Кількість ZO-1 визначали за допомогою наборів для імуноферментного аналізу E-EL-H1516 (Elabscience, США). У дослідженні брали участь хворі з ПКЩЗ та з зобом. До 1 групи увійшли хворі з ПКЩЗ і без метастазів, у 2 групу – із ПКЩЗ і метастазами, у 3 групу – із зобом. **Результати.** Отримані дані свідчать, що рівень ZO-1 в умовно-нормальній тканині ЩЗ був

у 2 рази вищим, ніж у пухлинній тканині ПКЩЗ без метастазів. У ПКЩЗ із метастазами кількість ZO-1 суттєво нижча, ніж в умовно-нормальній тканині ЩЗ, як із метастазами, так і без них. У зобній тканині кількість ZO-1 не відрізняється від умовно-нормальної тканини карцином, але помітно вища, ніж у пухлинній тканині та метастазах. Рівень ZO-1 у крові хворих на ПКЩЗ був суттєво нижчим, ніж у крові здорових людей. Кількість ZO-1 у крові хворих на ПКЩЗ з метастазами була нижчою та вірогідно відрізняється від показника хворих на ПКЩЗ без метастазів. **Висновок.** Таким чином, зниження концентрації ZO-1 в пухлинах і крові може бути одним із маркерів метастазування в ПКЩЗ.

Ключові слова: карциноми щитоподібної залози, білки ZO-1, щільні з'єднання, метастази.

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Correspondence address: Pushkarev Volodymyr Mykhaylovych, pushkarev.vm@gmail.com. State Institution «V.P. Komisarenko Institute of Endocrinology and Metabolism of the NAMS of Ukraine», 69, Vyshgorodska st., Kyiv 04114, Ukraine.

Information about the authors: Zynych Petro Petrovych, Cand. Sci. (Medicine), Researcher of the Department of Endocrine Surgery, ORCID: 0000-0001-8890-4343; Pushkarev Volodymyr Mykhaylovych, Dr. Sci. (Biology), Senior Scientist, Chief Researcher of the Department of Fundamental and Applied Problems of Endocrinology, ORCID: 0000-0003-0347-7771; Levchuk Nataliia Ivanivna, Cand. Sci. (Biology), Senior Scientist, Leading Research Fellow of the Department of Fundamental and Applied Problems of Endocrinology, ORCID: 0000-0003-0482-5176; Shelkovoy Yevhen Anatoliyovych, Junior Researcher of the Department of Reproductive Endocrinology, Ultrasound Diagnostic Doctor, ORCID: 0009-0005-2255-5773; Bolgov Mychailo Yuriyovych, Dr. Sci. (Medicine), Prof., Head of the Department of Endocrine Surgery, ORCID: 0000-0002-9011-9982.

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Адреса для листування: Пушкар'єв Володимир Михайлович, pushkarev.vm@gmail.com; ДУ «Інститут ендокринології та обміну речовин ім. В.П. Комісаренка НАМН України», вул. Вишгородська, 69, Київ 04114, Україна.

Відомості про авторів: Зінич Петро Петрович, канд. мед. наук, науковий співробітник відділу хірургії ендокринних залоз, ORCID: 0000-0001-8890-4343; Пушкар'єв Володимир Михайлович, д-р біол. наук, старш. наук. співроб., головний науковий співробітник відділу фундаментальних та прикладних проблем ендокринології, ORCID: 0000-0003-0347-7771; Левчук Наталія Іванівна, канд. біол. наук, старш. наук. співроб., провідна наукова співробітниця відділу фундаментальних та прикладних проблем ендокринології, ORCID: 0000-0003-0482-5176; Шелковой Євген Анатолійович, молодш. наук. співроб. відділу репродуктивної ендокринології, лікар ультразвукової діагностики, ORCID: 0009-0005-2255-5773; Болгов Михайло Юрійович, д-р мед. наук, проф., керівник відділу хірургії ендокринних залоз, ORCID: 0000-0002-9011-9982.

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