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HYPOXIA-INDUCIBLE FACTOR-1α LEVEL IN BLOOD PLASMA, THYROID TUMORS AND METASTASES*

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Metastases are the leading cause of death from malignant tumors. The process of metastasis involves the exit of cancer cells from the primary tumor, their transition into the blood or other transport system and, finally, colonization and proliferation in distant organs [1–6].

Hypoxia is a hallmark of all solid tumors and their metastases, including papillary thyroid carcinoma (PTC). This leads to activation of the hypoxia-inducible factor (HIF) family of transcription factors, which modulate gene expression within both tumor cells and immune cells within the tumor microenvironment, influencing tumor progression and treatment response [7-9].

Hypoxia-inducible factor-1 is a key regulator for balancing oxygen in the cells. It is a transcription factor that regulates the expression of target genes involved in oxygen homeostasis in response to hypoxia. Recently, research has demonstrated the multiple roles of HIF-1 in the pathophysiology of various diseases, including cancer. It is a crucial mediator of the hypoxic response and regulator of oxygen metabolism, thus contributing to tumor development and progression. HIF-1α is suggested to contribute to the Warburg effect by stimulating many genes that mediate glycolysis, including glucose transporter GLUT1, which possess hypoxia-response elements (HREs) in its promoter. Studies showed that the expression of the HIF-1α subunit is significantly upregulated in cancer cells and promotes tumor survival by multiple mechanisms. In addition, HIF-1 has potential contributing roles in cancer progression, including cell division, survival, proliferation, angiogenesis, and metastasis. Moreover, HIF-1 has a role in regulating cellular metabolic pathways, particularly the anaerobic metabolism of glucose. Given its significant and potential roles in cancer development and progression, it has been an intriguing therapeutic target for cancer research.

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Several compounds targeting HIF-1-associated processes are now being used to treat different types of cancer [9, 10].

The aim of the study was to determine level of HIF-1α in blood and postoperative samples of follicular adenoma, goiter, multinodular goiter, papillary thyroid carcinoma, metastases, and conditionally normal tissue.

MATERIALS AND METHODS

The research protocol was approved by the Ethics Committee of the Institute. Protocol 48/4-KE dated 03.25.24. All patients signed informed consent for further diagnostic and scientific research on their biomaterials.

Postoperative samples of follicular adenoma, 2 types of goiter, PTC, metastases, and conditionally normal (non-tumor or morphologically unchanged tissue) tissue, obtained from the surgical department of the Institute's clinic, were used for research. Blood plasma were also analyzed. Blood was obtained by standard venipuncture and stored in EDTA tubes. Plasma was separated by centrifugation within 10 minutes after blood sampling. The concentration of protein in cell lysates was determined according to [11]. Blood controls were taken from healthy individuals without thyroid and comorbid diseases.

Samples were stored at -80°C until use. The amount of HIF-1α was determined using enzyme immunoassay kits EH0551 (FineTest®, China). Measurements were performed at an optical wavelength of 450/630 nm on an immunoenzymatic plate analyzer Stat Fax 3200 (Awareness Technology, USA).

Patients with PTC, PTC + metastases (Mts), follicular adenoma and goiters (88 samples in total) participated in the study. Group 1 included 8 samples with follicular adenoma, group 2 included 8 samples with nodular goiter, group 3 included 8 samples with multinodular goiter, group 4 included 16 samples with PTC without Mts, group 5 included 24 samples with PTC and Mts. The concentration of HIF-1α in plasma of 9 patients with PTC without Mts and 10 patients with PTC and Mts was also determined. Plasma from 5 individuals without thyroid disease and other chronic diseases, representative for age, was used as a control.

Statistical analysis and data presentation were performed using Origin 7.0 software. The results of the study are presented as M ± SE. Student's *t*-test was used to compare data groups. Values of $P < 0.05$ were considered significant.

RESULTS AND THEIR DISCUSSION

The Fig. 1 presents the results of the HIF-1α detection in the postoperative tissue of patients with PTC, FA and goiter. No significant differences were observed between the concentration of HIF-1α in adenoma tissue and in the corresponding conditionally normal tissue. The level of HIF-1α in the tissue of nodular goiter exceeded the levels in conditionally normal tissue by 2 times. A more then 2.5 ratio was observed in PTC tissues without metastases. The concentration of HIF-1α in the tumor tissue of the PTC with metastases was higher than in the conditionally normal tissue by more then 3.5 times. The level of HIF-1α in metastases significantly differ from the corresponding conditionally normal tissue but was much lower comparing tumor tissue. There was also significant difference between PTC tissues without and with metastases (see Fig. 1).

In blood plasma of patients with PTC without metastases, the concentration of the HIF-1α significantly (~ 1.5 times) exceeded its level in control plasma (Fig. 2). The most important fact is that the concentration of HIF-1α in the blood of patients with PTC and metastases was more then 3 times higher than in of conditionally normal tissue and more then 2 times higher than in tumor tissue of PTC without metastases, which is very important for predicting the occurrence of metastases in the preoperative period.

An urgent task facing surgeons in the treatment of PTC is the search for specific markers of Mts. The number of such markers is already several dozen, but it is still difficult to determine whether Mts will form in thyroid carcinomas, as well as to predict the development of radioiodine resistance of Mts. According to our

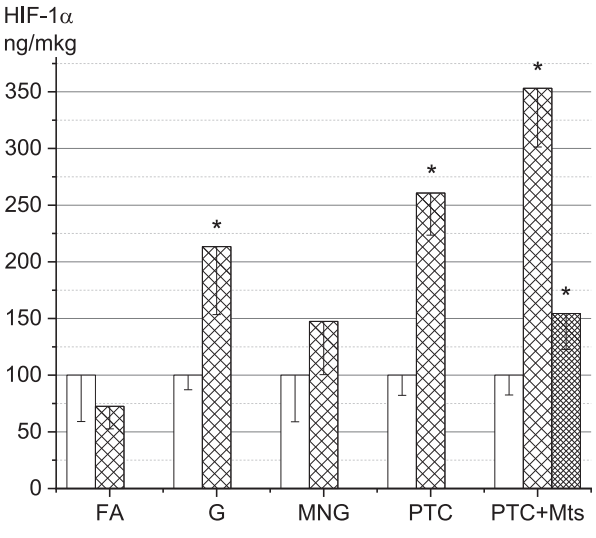

Fig. 1. HIF-1α quantity in the thyroid tissue of patients with PTC, FA and goiter.
Notes: transparent column — conditionally normal (histologically unchanged) tissue;
shaded column — tumor (changed) tissue,
heavily shaded column — metastases.
FA — follicular adenoma, G — nodular goiter,
MG — multinodular goiter, PTC — tumor tissue,
PTC+ — tumor tissue with metastasis.
* — significantly different from conditionally normal tissue,
P < 0.05; + — significantly different from all other samples, P < 0.05.

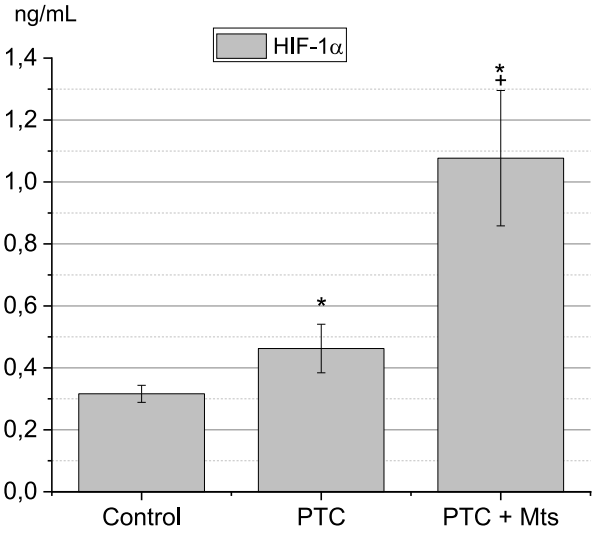

Fig 2. HIF-1α quantity in the blood plasma of patients with PTC.
Notes: * — significantly different from control samples,
P < 0.05;
+ — significantly different from PTC samples.

data, proliferating cell nuclear antigen (PCNA) [12, 13], the expression of a rare isoform of ribosomal kinase S6K – p60S6K [14], overexpression of matrix metalloproteinases (MMP) and ZEB1 transcription factor [15, 16] are markers that can indicate the aggressiveness and metastatic potential of thyroid tumors.

Our data indicate significant differences in the concentration of HIF-1α between tumor tissues of PTC without and tissues of PTC with metastasis. It is interesting to note that the level of HIF-1α in metastases was significantly lower. It should be emphasized the fact that in the blood plasma the level of HIF-1α was also significantly higher in PTC patients with Mts than in plasma of healthy people and PTC patients without metastases. The latter circumstance can become the basis for the preoperative prognosis of the development of metastases in PTC patients.

Other authors have shown that HIF-1α expression are closely related to thyroid cancer progression and invasion [8, 9, 17, 18].

Activated HIF-1α regulates the response of tumor cells to changes in oxygen concentration through transcriptional activation of genes [10]. Induction of transcription of proangiogenic factors such as VEGF (vascular endothelial growth factor) stimulates the development of blood vessels and the supply of cells with oxygen. In addition, HIF-1 stimulates metastatic activity of the tumor - migration of cells to distant and more oxygen-rich tissues. Cancer development and progression depend mainly on the presence of hypoxia and activation of HIF-1α and inflammation (activation of NF-κB). The expression of HIF-1α and HIF-2α in thyroid cancer is higher than in normal thyroid tissue or its benign lesions. Hyperexpression of HIF-1α and 2α has been associated with capsular invasion and the presence of MLV. Tumors with high levels of HIF-1α and 2α had a higher TNM (tumor, nodus и metastasis) stage. Evidence suggests that HIF may promote the migration and aggressiveness of PTC, FTC, and ATC [17]. Therefore, HIF-1α may serve as a marker and target for the treatment of thyroid cancer and radioiodine resistance [19].

CONCLUSION

One of the most important tasks facing endocrine surgery is the determination of reliable markers of metastasis in the treatment of papillary thyroid carcinoma. Of particular value are markers that can be determined at the stage of preoperative research. It is possible that HIF-1α can be one of these markers.

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Metastases are the leading cause of death from malignant tumors. Hypoxia is a hallmark of all solid tumors and their metastases, including papillary thyroid carcinoma (PTC). This leads to activation of the hypoxia-inducible factor (HIF) family of transcription factors, which modulate gene expression within both tumor cells and immune cells within the tumor microenvironment, influencing tumor progression and treatment response.

The **aim** of the study was to compare the levels of HIF-1α in blood and tissue samples of follicular adenoma, goiter, papillary thyroid carcinoma, metastases, and conditionally normal tissue.

Materials and methods. Postoperative samples of tissue and blood plasma, obtained from the surgical department of the Institute's clinic, were used for research. Patients with PTC, PTC + metastases, follicular adenoma and goiters (88 samples in total) participated in the study. Plasma from 5 individuals without thyroid disease and other chronic diseases, representative for age, was used as a control. The amount of HIF-1α was determined using enzyme immunoassay kits. Student's *t*-test was used to compare data groups. Values of $P < 0.05$ were considered significant.

Results. The level of HIF-1α in the tissue of nodular goiter exceeded the levels in conditionally normal tissue by 2 times. A more then 2.5 ratio was observed in PTC tissues without metastases. The concentration of HIF-1α in the tumor tissue of the PTC with metastases was higher than in the conditionally normal tissue by more then 3.5 times. The level of HIF-1α in metastases was much lower comparing tumor tissue. In blood plasma of patients with PTC and metastases the concentration of the HIF-1α was more then 3 times higher than in control (healthy people) and more then 2 times higher than in tumor tissue of PTC without metastases.

Conclusions. Our data indicate significant differences in the concentration of HIF-1α between tumor tissues of papillary thyroid carcinoma with and without metastasis. It should be emphasized the fact that in the blood plasma the level of HIF-1α was also significantly higher in papillary thyroid carcinoma patients with metastasis than in healthy people and papillary thyroid carcinoma patients without metastases. The latter circumstance can become the basis for the preoperative prognosis of the development of metastases in papillary thyroid carcinoma.

Keywords: papillary thyroid carcinoma, metastases, hypoxia-inducible factor 1-alpha.

РІВЕНЬ ІНДУКОВАНОГО ГІПОКСІЄЮ ФАКТОРА-1α У ПЛАЗМІ КРОВІ, ПУХЛИНАХ ЩИТОПОДІБНОЇ ЗАЛОЗИ ТА МЕТАСТАЗАХ

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Метастази є основною причиною смерті від злоякісних пухлин. Гіпоксія є ознакою всіх солідних пухлин та їх метастазів, включаючи папілярну карциному щитоподібної залози (ПКЩЗ). Це призводить до активації родини транскрипційних факторів індукованого гіпоксією фактора (HIF), які модулюють експресію генів як у пухлинних клітинах, так і в імунних клітинах у мікрооточенні пухлини, впливаючи на прогресування пухлини та реакцію на лікування.

Метою дослідження було порівняння вмісту HIF-1α у зразках крові та тканин фолікулярної аденоми, зобу, папілярної карциноми щитоподібної залози, метастазів та умовно нормальної тканини.

Матеріали та методи. Для дослідження використовували післяопераційні зразки тканин та плазми крові, отримані з хірургічного відділення клініки Інституту. У дослідженні взяли участь пацієнти з ПКЩЗ, ПКЩЗ + метастази, фолікулярною аденомою та зобом (загалом 88 зразків). Як контрольну групу використовували плазму 5 осіб без захворювань щитоподібної залози та інших хронічних захворювань, репрезентативних за віком. Кількість HIF-1α визначали за допомогою наборів для імуноферментного аналізу. Для порівняння груп даних використовували t-критерій Стьюдента. Значення $P < 0,05$ вважалися значущими.

Результати. Рівень HIF-1α у тканині вузлового зобу перевищував рівні в умовно нормальній тканині у 2 рази. У тканинах ПКЩЗ без метастазів спостерігалося співвідношення понад 2,5. Концентрація HIF-1α в пухлинній тканині ПКЩЗ з метастазами була вищою (більш ніж у 3,5 рази), порівняно з умовно нормальною тканиною. Рівень HIF-1α в метастазах був значно нижчим порівняно з пухлинною тканиною. У плазмі крові пацієнтів з ПКЩЗ та метастазами концентрація HIF-1α була у понад 3 рази вищою у порівнянні з контрольною групою (здорові особи), та у понад 2 рази вищою, ніж у пухлинній тканині ПКЩЗ без метастазів.

Висновки. Наші дані свідчать про значні відмінності в концентрації HIF-1α між пухлинними тканинами папілярної карциноми щитоподібної залози з метастазами та без них. Слід підкреслити той факт, що в плазмі крові рівень HIF-1α також був значно вищим у пацієнтів з папілярною карциномою щитоподібної залози з метастазами, ніж у здорових людей та пацієнтів з папілярною карциномою щитоподібної залози без метастазів. Остання обставина може стати основою для передопераційного прогнозу розвитку метастазів при папілярній карциномі щитоподібної залози.

Ключові слова: папілярна карцинома щитоподібної залози, метастази, гіпоксія-індукований фактор 1-альфа.