ОБМЕН ОПЫТОМ

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HETEROGENEITY OF METASTATIC GASTRIC NEUROENDOCRINE TUMOR

The purpose of the study was to demonstrate the effect of heterogeneity of a tumor and its metastases on the choice of treatment tactics in a clinical case. A patient with gastric neuroendocrine cancer and liver metastases was examined. According to immunohistochemical analysis, the primary neuroendocrine tumor (NET) of the stomach was highly differentiated (NET, G1). In liver metastases S1, S2, S5, S8, NETs were moderately differentiated (NET, G2), and in S7 NETs were poorly differentiated (NEC, G3), demonstrating the tumor heterogeneity which progressed as the malignant process developed. Only in the low-differentiated NETs (NEC, G3), the reaction with Chromogranin A was negative, which is typical of low-differentiated NET. The reaction with SSTR2 was moderately and sharply expressed in liver metastases S7, S5, S8 and, on the contrary, was nearly undeterminable in the primary NET of the stomach and in liver S1, S2. The clinical case demonstrates the phenomenon of tumor heterogeneity and the associated ambiguity of the results of immunohistochemical research. Therefore, testing of tumors prior to the treatment is of paramount clinical importance for the correct treatment choice and disease prognosis. The development of specific molecular markers for the diagnosis of neuroendocrine tumors remains an actual problem of oncology.

Keywords: intratumoral heterogeneity, genetic instability, gastric neuroendocrine tumor, somatostatin receptors.

Background. The problem of metastatic neuroendocrine tumors (NETs) is extremely relevant in modern clinical oncology, because most NETs, being functionally inactive, often clinically manifest only at the metastasis stage. One of the main problems also lies in the peculiarities of molecular biology of metastatic foci whose characteristics do not always coincide with that of the primary tumor. The main mistake of the "default" treatment is that it considers the response to therapy only in the primary tumor based on its differentiation, hormonal status and other indicators. Hence the obvious conclusion why therapeutic treatment cannot give positive results in every case [3].

Currently, there is no doubt that the tumor is a complex dynamic system. Starting from a genetically normal cell, the tumor process leads to the formation of a population consisting of trillions of tumor cells that have formed many different phenotypes providing more aggressive behavior [1]. Thus, over time, the tumor modifies its characteristics, which leads to a number of differences in the cells of the primary tumor and metastases, which, in essence, constitutes tumor heterogeneity [2]. This phenomenon was first described by Rudolf Virchow in the mid-19th century. "The increased instability of the genome, being a necessary reason for the formation of highly aggressive cell populations within the tumor, together with the ongoing selection, underlies the intratumoral heterogeneity" [2]. This thesis by Peter Novel started the modern era of the study of this phenomenon, extremely complex and diverse in its forms.

Numerous studies of recent years report phenotypic and genotypic differences between the cells of primary tumors and metastases, as well as heterogeneity of metastatic foci of the same tumor. These differences may apply to tumor cells at both the morphological and functional levels. Meanwhile, primary and metastatic tumors are considered to be able to develop as genetically different when metastatic spread occurs at an early stage of tumor progression [1, 3].

The intratumoral heterogeneity is based on genetic instability, including both gene and chromosomal mutations and microsatellite instability [4]. Along with genetic mechanisms, there are also epigenetic factors that enhance or weaken various damage to the cell genome and are quite reversible [4, 5, 6]. Heterogeneity also inevitably arises from "noise processes" - stochasticity in the gene expression which results in the production of genetically identical cells of different levels of specific proteins at every moment [2]. The phenotypic heterogeneity of tumor cells is a consequence of both the above mechanisms and the influence of the tumor microenvironment, which is especially significant in metastatic foci [2, 6]. This means that tumor cells adapting to a new microenvironment acquire new properties that cause an increase in heterogeneity. The oncologist has to prescribe combined targeted therapy taking into account heterogeneity of the primary tumor and metastases by a number of different signs [1, 2, 4, 5].

According to the American Association for Cancer Research (AACR), the treatment for a patient in the near future may look like this: a biopsy of metastases; DNA analysis of tumor cells in the plasma by digital PCR with determination of the molecular and genetic profile of the tumor; prescribing a universal drug active for the revealed changes; subsequent assessment of the molecular and genetic profile in patients with disease progression; the appointment of a new drug that

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suppresses the activity of newly identified changes [3, 7].

Thus, all of the above determines the high relevance of studying the problem of tumor heterogeneity in all forms of its manifestation, and the development of new approaches to the treatment of malignant neoplasms is the most important task of modern oncology.

The purpose of the study was to demonstrate the effect of heterogeneity of a tumor and its metastases on the choice of treatment tactics in a clinical case.

Material and methods. A standard immunohistochemical study was performed on sections with a thickness of 3-5 μm prepared from paraffin blocks using Autostainer (Autostainer, ThermoScientific, type 480s). Deparaffinization of sections and restoration of antigenicity was performed in a buffer with pH 9 in PT Module (ThermoScientific, UK). An immunohistochemical study included antibodies to chromogranin A, synaptophysin, and nonspecific enolase (NSE). Antibodies to Ki-67 were used to evaluate the proliferative activity of tumor cells; monoclonal antibodies, Anti-somatostatin Receptor 2, were used to detect the expression of somatostatin receptors.

Results and discussion. Patient G. was admitted to Department of Abdominal Oncology No.1, Rostov Research Institute of Oncology, with the diagnosis of gastric neuroendocrine cancer, T3NxM1, stage IV, clinical group 2, secondary (metastatic) liver cancer. A council of physicians (surgeon, chemotherapist and radiologist) recommended surgical treatment according to the clinical practice guidelines for the treatment of gastrointestinal (GI) NETs by the Association of Oncologists of Russia. The surgery involved gastrectomy with extended lymphadenectomy using laparotomy access with atypical resection of S1, S2, S5, S7, S8 of the liver. The surgery and the postoperative period proceeded without complications. Tissue samples were referred for pathohistological and immunohistochemical testing.

Pathohistological testing showed: neuroendocrine tumor, sporadic ECL-cell (type III), alveolar and pseudoglandular growth with ulceration and germination into all layers of the stomach wall, invasion of subserous fatty tissue, lymphatic and blood vessels, pT3N2M1 (hep).

Immunohistochemical analysis showed that primary gastric neuroendocrine tumor was highly differentiated (NET, G1), according to the WHO classification of GI NETs from 2010, with a Ki-67 proliferation index = 1.8%. Reactions with chromogranin A, synaptophysin, NSE were highly pronounced. These markers are included in the minimum NET diagnostic panel, and positive reactions with them reliably confirm the NET diagnosis. Reactions with specific markers of the extended diagnostic panel (gastrin, insulin, glucagon, serotonin, etc.) were negative, indicating the non-functioning nature of the tumor. NET metastases of a similar nature with Ki-67 proliferative activity index up to 9.5% (NET, G2) were detected in 3 of 12 examined lymph nodes.

5 remote metastatic foci in the liver (S1, S2, S5, S7, S8) were examined immunohistochemically. The Ki-67 proliferation index calculated at the “hot spots” manually was 12.2% (S2), 14.5% (S1), 17.1% (S8), 18.3% (S5), 27.4 % (S7). Thus, in liver metastases S1, S2, S5, S8 NETs were moderately differentiated (NET, G2), and in S7 NETs were poorly differentiated (NEC, G3), demonstrating the tumor heterogeneity which progressed as the malignant process developed. The proliferative activity assessed by the Ki-67 level is an indicator of the tumor phenotype, which determines the tumor growth rate, its course and outcome. Therefore, in our clinical case, the disease outcome was determined both by the fact of liver metastases and by an extreme biological aggressiveness of the metastatic foci themselves. Only in the low-differentiated NETs (NEC, G3), the reaction with Chromogranin A was negative, which is typical of low-differentiated NETs [2, 3]. This suggests probable differences in the results of immunohistochemical reactions between the primary NET (localized in the stomach in this case) and metastatic foci in the liver, which only confirms the phenomenon of tumor heterogeneity.

For the primary tumor and metastatic nodes, a reaction was performed to establish the expression of type 2 somatostatin receptors (SSTR2), and it gave mixed results. The reaction with SSTR2 was moderately and sharply expressed in liver metastases S7, S5, S8 and, on the contrary, was undeterminable in the primary NET of the stomach and in liver S1, S2. In our case, the expression of SSTR2 in clinically significant amounts increased as the malignant potential increased. It is impossible to reliably speak of a clear relationship between the NET differentiation and the level of SSTR2expression, since these observations are not enough and studies on a large and representative sample are necessary. In any case, we recommend determining the status of SSTR2 (as the most common type of a somatostatin receptor) before starting NET treatment in a routine morphological study. In our opinion, this will expand the possibilities of therapy. Russian specialists have already gained sufficient experience with the use of somatostatin analogues for the treatment of NETs, mainly GI and pancreatic ones [1].

Conclusions. The presented clinical case clearly demonstrates the phenomenon of tumor heterogeneity and the related ambiguity of the results of immunohistochemical studies. Therefore, the immunohistochemical testing of neuroendocrine tumors is of crucial clinical importance even before the start of treatment to select the correct treatment regimen and for the disease prognosis. The development of specific molecular markers for the diagnosis of cancer remains an actual problem of oncology.

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