

A rare case of a metastatic neuroendocrine tumor of the pancreas

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ABSTRACT

Aim. To study a rare sporadic case of metastatic gastrinoma associated with mutations in the *MEN1* and *TSC2* genes in a 25-year-old male.

Materials and methods. A retrospective analysis of the history of a 25-year-old patient with sporadic gastrinoma with a highly aggressive clinical course and high metastatic potential was performed. Sequencing of the DNA extracted from the surgical tumor biopsy was performed on the Illumina NextSeq 550 sequencer (Illumina Inc., USA) with the mean coverage of at least 100× using the AmpliSeq target panel for Illumina Comprehensive Cancer Panel for studying exons of 409 genes, mutations in which are associated with oncopathology.

Results. The article presents the results of complex diagnosis and treatment of metastatic gastrinoma using modern locoregional therapy and drugs from the group of somatostatin analogues. Using next generation sequencing and Sanger sequencing, sporadic mutations in the *MEN1* and *TSC2* genes with pronounced clinical significance were identified in the extracted DNA.

Conclusion. The identified mutations, being the drivers of the tumor process, apparently determined the atypical development of the presented clinical case – the sporadic Zollinger – Ellison syndrome. Complete morphological and immunohistochemical validation of the neuroendocrine tumor before treatment determined a successful treatment strategy, including the use of somatostatin analogues in adjuvant and neoadjuvant therapies in combination with chemoembolization of hepatic metastases.

Key words: neuroendocrine tumor, Zollinger – Ellison syndrome, next generation sequencing, metastases.

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Редкий случай метастатической нейроэндокринной опухоли поджелудочной железы

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РЕЗЮМЕ

Цель. Изучение редкого спорадического случая метастатической гастриномы, ассоциированной с мутациями в генах *MEN1* и *TSC2*, у мужчины 25 лет.

Материалы и методы. Был проведен ретроспективный анализ истории болезни пациента 25 лет с наличием спорадической гастриномы с высоко агрессивным клиническим течением и высоким метастатическим потенциалом. Секвенирование ДНК, экстрагированной из операционного биоптата опухоли, проводили на секвенаторе Illumina NextSeq 550 (Illumina Inc., США) со средним покрытием не менее 100× с применением таргетной панели AmpliSeq Comprehensive Cancer Panel for Illumina для исследования экзонных регионов 409 генов, мутации в которых ассоциированы с онкопатологией.

Результаты. Представлен результат комплексной диагностики и успешного лечения метастатической гастриномы с применением современных локорегиональных методов лечения и препаратов из группы аналогов соматостатина. С помощью секвенирования нового поколения и секвенирования по Сэнгеру в экстрагированной ДНК были выделены спорадические мутации в генах *MEN1* и *TSC2* с выраженным клиническим значением.

Заключение. Идентифицированные мутации, являясь драйверами опухолевого процесса, очевидно, определили нетипичное развитие представленного клинического случая – спорадического синдрома Золлингера – Эллисона. Полная морфологическая и иммуногистохимическая верификация нейроэндокринной опухоли до начала лечения определила его успешную тактику, включавшую применение аналогов соматостатина в адьювантном и неоадьювантном режимах в сочетании с химиоэмболизацией печеночных метастазов.

Ключевые слова: нейроэндокринная опухоль, синдром Золлингера – Эллисона, секвенирование нового поколения, метастазы.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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INTRODUCTION

At the annual meeting of the American Surgical Association in Philadelphia on April 29, 1955, Robert Zollinger and Edwin Ellison were the first to present the relationship between non-insulin-secreting tumors of the pancreas and hypersecretion of hydrochloric acid in the stomach. Due to the ability to produce a large amount of gastrin, the tumor became known as

gastrinoma. Clinical manifestations of the disease are included in the Zollinger – Ellison syndrome (ZES), although these terms are currently used as synonyms. It has been only 65 years since the first description of the syndrome, but during this time, more than 3,300 articles have been published, and unique clinical data of more than 1,000 patients have been presented. It has been established that the annual incidence of the disease is 1 case per 1 million population [1].

The initial manifestations of the disease usually appear at the age of 30–60 years. The onset of manifestations is associated with the tumor status – sporadic or hereditary cancer [2]. ZES includes ectopic secretion of gastrin by a neuroendocrine tumor, gastrinoma, primarily localized in the duodenum (60–80%) or pancreas (10–40%). The result is hypersecretion of hydrochloric acid and subsequent development of gastroesophageal reflux disease and ulcer, which are normally resistant to classical drug therapy [3].

In 30% of patients with multiple neuroendocrine neoplasia type 1 (*MEN1*), a pancreatic neuroendocrine tumor (pancreatic NET) is diagnosed, and gastrinoma in this case develops exclusively before the age of 20 and is more often localized in the duodenum [4, 5]. The treatment strategy and the outcome for sporadic gastrinomas and gastrinomas associated with *MEN1* are different. Distinctive features of the latter include their small size, multifocal growth, and high metastatic potential. The main method of treatment is surgical, but its results are still characterized by a low rate of favorable outcomes. Signs of biochemical recurrence are present in more than 95% of patients within 3 years after surgery [6].

It should be noted that a combination of ZES with other hereditary syndromes is an extremely rare phenomenon. The literature describes only one case of gastrinoma in a 34-year-old man who was diagnosed with tuberous sclerosis in childhood without a family history of the condition. At the time of diagnosis, multiple metastases were found in the liver, lungs, and spine. Being inoperable, the patient died 6 months later [7]. Early clinical manifestations and an aggressive course of the disease are not typical of sporadic gastrinomas. In this regard, the case of pancreatic gastrinoma without a family history of the disease with early development of ZES and the presence of sporadic mutations in the *MEN1* and *TSC2* genes is of particular interest.

CLINICAL CASE

In September 2018, patient A., 25 years old, went to a general practitioner in the place of residence with complaints of general weakness, dizziness, abdominal pain in the antecardium arising after eating, and nagging pain in the right hypochondrium. The performed esophagogastroduodenofibroscopy helped to detect an ulcerative lesion in the cardiac region of the stomach and erosive duodenitis. Magnetic resonance imaging (MRI) of the abdominal organs showed a neoplasm in the tail of the pancreas ($45 \times 55 \times 42$ mm) and similar

lesions in S1 (14×20 mm) and S7 ($16 \times 16 \times 10$ mm) segments of the liver (Fig. 1).

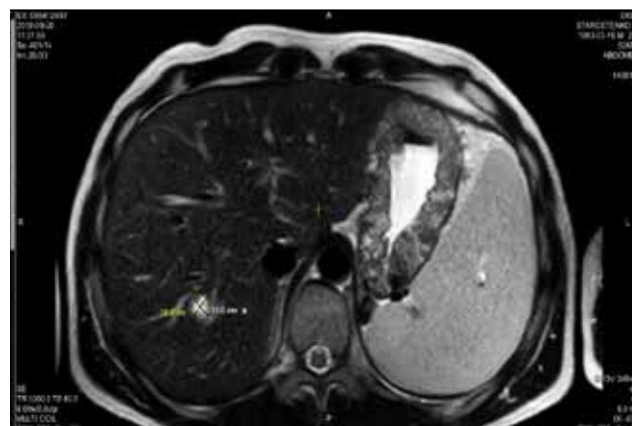


Fig. 1. MRI of the abdominal organs. Visualization of a metastatic focus in the liver parenchyma

For further examination and treatment, patient A. was referred to the National Medical Research Center for Oncology (NMRCO), Rostov-on-Don (former Rostov Research Institute of Oncology – RRIO). The morphological study of the biopsy material from the pancreas and liver made it possible to verify the neuroendocrine tumor in the tail of the pancreas. According to the immunohistochemical study, strong positive reactions were determined with the classical markers of the neuroendocrine phenotype: chromogranin A (CgA), synaptophysin, Ki-67 = 10% (which corresponds to a highly differentiated G2 pancreatic NET, according to the classification of the World Health Organization (2017)). In addition to the mandatory minimum set of markers, an expanded panel of markers to determine expression of a range of hormones was used. It identified high levels of gastrin expression. Besides, somatostatin receptors type 2A were present in large numbers on the surface of primary cancer cells.

Therefore, according to the results of histological and immunohistochemical studies, a functionally active pancreatic NET – gastrinoma – with clinical presentation of ZES was verified. The diagnosis of pT-3N1M1 (hep), stage IV neuroendocrine tumor of the tail of the pancreas with regional lymph node and liver metastases was established.

TREATMENT

The NMRCO board of the NMRCO board of doctors recommended to carry out surgical treatment including corporocaudal resection of the pancreas with

splenectomy and atypical resection of the S1 and S7 segments of the liver.

As preoperative preparation, the patient was prescribed lanreotide, which decreased CgA in the blood serum from 500 to 34 nmol / l. After 1 month of taking lanreotide, the surgery was performed. Laparotomy visualized a tumor in the tail of the pancreas, growing to the splenic hilum up to 1.5 cm in diameter (Fig. 2). The tumor conglomerate included the splenic artery and vein and was tightly adjacent to the left lateral semicircle of the superior mesenteric and portal veins. The splenic artery was tumor-free. Enlarged paracaval lymph nodes were identified. Mobilization of the ligament of Treitz and transsection of the peritoneum above the mesentery of the small intestine visualized the superior mesenteric vein. It was dorsomedial to the tumor. The surgery included corporocaudal resection of the pancreas, splenectomy, resection of the mesentery of the large intestine, perirenal fat, and left adrenal gland. Atypical resection of the S1 and S7 segments of the liver was performed.



Fig. 2. Gross specimen of the tumor in the tail of the pancreas

In the postoperative period, the patient continued to receive lanreotide, against the background of which the progression of the disease was recorded 3 months after the surgery: elective MRI showed a neoplasm in the liver S7 segment, 12 × 11 × 10 mm in size (Fig. 3).

The NMRCO board of doctors recommended to carry out transarterial chemoembolization (TACE) of the liver using lipiodol-cisplatin emulsion. Considering the progression of the metastatic process in the liver, it was decided to add chemotherapy to the ongoing treatment according to the GEMOX scheme.

The patient was included in the study of the genetic profile of pancreatic NET by the method of massively parallel sequencing (next generation sequencing,

NGS). Sequencing of DNA extracted from the surgical biopsy of the tumor was performed on the Illumina NextSeq 550 sequencer (Illumina Inc., USA) with an average coverage of at least 100 × using the AmpliSeq™ target panel for Illumina Comprehensive Cancer Panel® to study exon regions of 409 genes, mutations in which are associated with oncopathology. Analytical sensitivity for mutation detection was 5%. The pathogenicity of the identified nucleotide substitutions was assessed according to the recommendations of the American College of Medical Genetics and Genomics (ACMG) and Association of Molecular Pathology (AMP) [8].

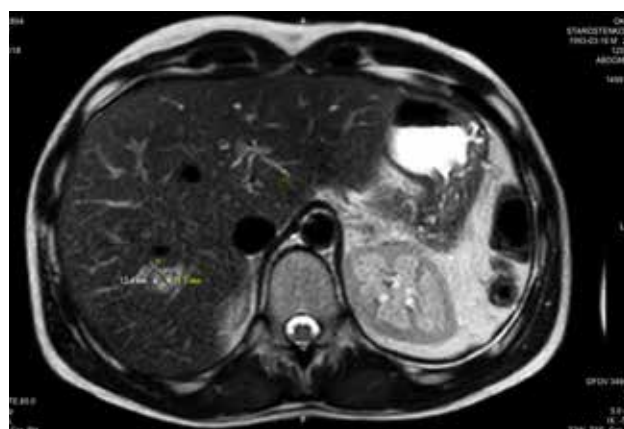


Fig. 3. MRI of the abdominal organs 3 months after the corporoduodenal resection of the pancreas and atypical liver resection with signs of the disease progression in the liver S7 segment

In the studied DNA sample, 1,041 variants of nucleotide sequences were found, two of which, in the *MEN1* and *TSC2* genes, were identified as pathogenic mutations with strong clinical significance according to the ACMG and AMP classification (Fig. 5). A frameshift mutation in the *MEN1* gene at c.248delT (p.Leu83ArgfsTer36) was described in the studies on pancreatic tumors [9, 10]. The detected variant was represented by a mosaic form (variant allele frequency (VAF) was 12.6%). The mutation c.337-1G>A (rs45517105), presented in the mosaic form (VAF = 7.6%) and associated with tuberous sclerosis, was found at the 3' acceptor splice site of intron 4 of the *TSC2* gene [11].

The identified pathogenic mutations were subsequently verified by direct Sanger sequencing. Their presence in the tumor and their absence in the blood leukocytes were confirmed. Thus, the diagnosis of sporadic gastrinoma with ZES was confirmed. Cur-

rently, the patient is under medical supervision with no signs of disease progression.

CONCLUSION

The presented case of sporadic gastrinoma is of high scientific value. The rarity of this type of neuroendocrine pancreatic tumor is combined here with its high malignant and metastatic potential and development of a complete clinical picture of ZES against the background of a fairly young age of the patient. Early development of gastrinoma is usually associated with the presence of hereditary syndromes, the manifestations of which were absent in the patient. However, using NGS and direct Sanger sequencing, sporadic mutations in the *MEN1* and *TSC2* genes with a strong clinical significance were detected, typically associated with development of multiple endocrine neoplasia type 1 (MEN1) and tuberous sclerosis, respectively. Obviously, the combination of two pathogenic mutations predetermined high aggressiveness of the disease.

Emerging new methods of locoregional therapy for hepatic metastases in NET, such as TACE, made it possible to select a personalized treatment strategy and exclude its aggressive impact on the entire liver parenchyma. High efficiency of this method is proven by the absence of disease progression in the described patient within 16 months of follow-up after TACE.

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Authors contribution

Kit O.I. – analysis of the scientific work, critical revision of the manuscript for important intellectual content. Trifanov V.S. – treating doctor, conception of the study, drafting of the article. Timoshkina N.N. – drafting of the article, carrying out of the molecular and genetic research. Kolesnikov E.N. – conception of the study, planning of treatment. Gvaldin D.Yu. – carrying out of the molecular and genetic research, bioinformatic analysis of data. Karnaukhov N.S. – analysis of the morphological material. Kutilin D.S. – bioinformatic analysis of the NGS data. Meshcheryakova M.Yu. – drafting of the article, illustrations.

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