Whole Exon Sequencing of Primary Lesion and Liver Metastatic Lesion in Pancreatic Neuroendocrine Tumor: A Case Report and Review of the Literature

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Case Report

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Abstract

**Background:** Pancreatic neuroendocrine neoplasms (p-NENs) are classified into neuroendocrine tumors (NET) G1, G2, G3, and neuroendocrine carcinoma (NEC) according to WHO classification. NET and NEC are different pathogenesis. The two kinds of tumors that occurred in the same part have not been reported. We found 4 foci of NEN G3 in a primary pancreatic NET G2. The cell atypia was obvious with Ki67 index of 50-70%, focal necrosis, there were 12 hepatic metastatic nodules with similar morphology to NEN G3, which is difficult to identify NEC and NET G3.

**Case presentation:** A patient with pancreatic NET was selected to perform whole exome sequencing on primary pancreatic NET G2 and liver metastatic NEN G3 paraffin tissues. NET G2 had 13 somatic mutations, while NEN G3 had 72 somatic mutations and Copy number variation in 4 genes. PS493N point mutation of TRIOBP gene was detected in NET G2 and NEN G3. 5-fold amplification of MDM4 is found in the metastatic liver lesion.

**Conclusion:** NET G2 and NEN G3 are closely related to TRIOBP gene. Oncogene amplification (MDM4) in liver metastases may be associated with morphological malignant transformation.

**Background**

Pancreatic neuroendocrine neoplasms (pNENs) are a group of rare heterogeneous pancreatic tumors originating from pancreatic neuroendocrine cells. Most of pNENs are sporadic. The etiology and specific pathogenesis are still unclear. The incidence of pNENs has significantly increased in the past three decades [1–3]. According to clinical manifestations, pNENs can be classified into functional pancreatic neuroendocrine neoplasms (F-pNENs) and non-functional pancreatic neuroendocrine neoplasms (NF-pNENs), and most of which are non-functional. F-pNENs are characterized by its ability to secrete one or more hormones mainly including insulin, gastrin, glucagon, somatostatin and vasoactive intestinal peptide. Clinical manifestations are related to excessive secretion of hormones. NF-pNENs are usually asymptomatic or mild, often presenting with nonspecific symptoms such as abdominal pain, abdominal distension, or weight loss. Forty to ninety-three percent of patients have metastases at the time of diagnosis. The most common is multi-focal liver metastases, and can also be transferred to lung, bone and other sites [4].

Pathological graded was conducted in accordance with the NEN nomenclature and classification criteria developed by WHO in the fifth edition of 2019. According to mitotic number and/or Ki67 positive index, NEN was classified as NET (G1, G2, G3 grade), NEC, and mixed neuroendocrine-non-neuroendocrine neoplasm (MiNEN). NET G3 is consistent with the number of mitotic figures (>20/2mm²) and/or Ki67 positive index (>20%) in NEC diagnostic standard. Morphological differentiation is the key to differential diagnosis. NET G3 still retains NEN organ-like structure, has poor efficacy against platinum drugs, and the overall survival time is longer than that of NEC. So it is of great significance to distinguish NET G3 from NEC. However, morphological identification is difficult in about 2/3 of NEN G3 cases (NEN G3 refers to
high-grade neuroendocrine tumors, including NET-G3 and NEC) [5]. Whether NET and NEC are different stages of differentiation in the same spectrum of lesions or two different types of tumors is also a long-standing topic.

Case Presentation

The patient

A 52-year-old female began to suffer from lower limb weakness, palpitations and hunger without obvious cause in April 2018. The symptoms started at 5~6 am every morning, which could be quickly relieved after meals. In April 2019, she was admitted to the department of endocrinology of our hospital. Abdominal MRI showed abnormal signal nodules in the pancreatic tail, which were highly suspected of neuroendocrine tumors. Multiple abnormal signals in the liver suggested metastatic tumors. Ultrasound-guided liver biopsy was performed. Pathological diagnosis supported neuroendocrine tumor (NET G3) based on the history and immunohistochemistry. In the same month, “Distal pancreatectomy + Ultrasound-guided radiofrequency ablation of hepatic mass” were performed. Intraoperative frozen diagnosis of pancreatic tail-mass revealed that neuroendocrine tumors were highly suspected and required further confirmation by routine pathology.

Pathological examination

Pathological examination observed a solid mass on the section of the pancreas, v1.8×1.5×1.2 cm, grayish-brown, soft, clearly bounded with surrounding tissues and adjacent to the pancreatic capsule (figure 1A), and the spleen is not involved. Microscopic observation showed pancreatic tumor with strong heterogeneity. The tumor cells in most regions were shape of ribbons and flower rings with rich blood supply and consistent cell size (figure 1B). There were about 3 nuclear fission/2 mm² and Ki67 index of 10% (figure 1C). In the tumor center, there were four high-grade lesions with a diameter of 0.1~0.2cm. The cells were obviously atypia and focal with necrosis (figure 1D). Nuclear fission was about 15/2 mm², and Ki67 was about 60% (figure 1E). Multiple neuroendocrine tumors were scattered around the pancreas (NET G1; Microtumor: diameter ≤0.2cm, Ki67 is about 2%, glucagon expression). Metastatic tumors were found in 1/13 of the peripancreatic lymph nodes (the metastatic tumors were NET G2). No tumor was identified in the spleen. There were 12 metastatic nodules in the liver. The tumor cells were arranged in a cluster like nest with rich blood supplied. The cells were obviously heterogeneous with multiple focal necrosis (figure 1F and G). Nuclear fission was about 30/2 mm², and Ki67 is ranged from 50% to 70% (figure 1H). Immunohistochemical demonstrated chromogranin A (CgA) and synapsin (Syn) expression in pancreatic and liver tumors (figure 1I and J), but stains for MGMT and SSTR2 were all negative. The cells in the highly differentiated region of pancreatic tumor showed P53(-) and RB(+) expression. The cells in poorly differentiated regions of the pancreas and liver metastases revealed P53(+) and RB(+) expression (figure 1K and L). The microtumor in the surrounding pancreatic tissue displayed glucagon expression, but not insulin and somatostatin.
Diagnosis

The final pathological diagnosis was: “Pancreatic neuroendocrine tumor (1.7cm in diameter). The highly differentiated region was NET G2. The low-differentiation region in the tumor center was considered to be high-grade neuroendocrine tumor (NEN G3), with a tendency toward NET G3. There were many nodules around the pancreas (NET G1; Microtumor: diameter ≤ 0.2 cm, Ki67 was about 2%, glucagon expression). Metastatic carcinoma was found in 1/13 of the peripancreatic lymph nodes (metastatic tumor was NET G2). Multiple metastatic high-grade neuroendocrine tumors were seen in the liver (NEN G3; 12). The cell atypia was obvious. It was difficult to differentiate NEC from NET G3 by morphology, and inclined to NET G3 based on the patient history. Considering the hypoglycemia (2.06 mmol / L), high insulin and C-peptide levels, the patient was diagnosed as functional pancreatic neuroendocrine tumor (insulinoma) with multiple liver metastases.

Whole exon sequencing

Whole exon sequencing was performed on the corresponding paraffin tissues of primary pancreas NET G2 (Ki67 10%) and liver metastatic NEN G3 (Ki67 70%). The correlation pathway was analyzed by bioinformatics method. The results showed that there were 13 individual cell mutations in pancreas NET G2 (figure 2), while metastatic liver NEN G3 had 72 somatic mutations and Copy number variation in 4 genes (figure 3). PS493N point mutation of TRIOBP gene was detected in both primary NET (NET G2) and metastatic foci (NEN G3) (figure 4). At the same time, the MDM4 oncogene was amplified 5 times.

Treatment and follow-up

The patient was hospitalized for surgery in April 2019. The surgery was distal pancreaticosplenectomy combined with ultrasound-guided radiofrequency ablation of hepatic mass. There were still multiple small lesions in the liver after the operation, the largest lesion was 1.3 cm. In June 2019, 5 mg everolimus was administered orally every day, and 30 mg of long-acting octreotide was injected intramuscularly every 21 days. In August 2019, the disease progressed, and the regimen was changed to Tiggio and Temozolomide combined with long-acting octreotide. As of August 2021, the disease has remained stable.

Discussion And Conclusion

In the study of pancreatic NEC, mutations of P53 and RB genes were found to be very common, while smad4/Dpc4, DAXX and ATRX1 remained unchanged. In contrast, in 45% of NET, DAXX and ATRX1 were absent, while P53 and RB remained unchanged. The overexpression of BCL2 was also mainly found in NEC and related to the proliferation activity, while NET was more related to the loss of heterozygosity of MEN-1 and 11q. These studies suggested that small cell carcinoma and large cell carcinoma have similar genetic change characteristics, and there is a great difference between them and well-differentiated NET. Therefore, in the study of NET and NEC, it is believed that the two are not continuous processes in the
disease spectrum, but two different types of diseases with different molecular biological mechanisms [6, 7].

In our study, the main component of NEN in this patient’s pancreas was NET G2, in which 4 foci (0.1~0.2cm) of advanced neuroendocrine tumors (NEN G3) appeared, and it was difficult to differentiate NET G3 from NEC morphologically. Immunohistochemistry showed that P53 gene was mutated in the NEN G3 region and RB1 gene was not absent. In addition, the morphologic features of liver metastases were all high-grade neuroendocrine tumors, and the immunohistochemical expression was consistent with that of NEN G3 in the pancreas. Therefore, it should be diagnosed that the presence of NEC in the pancreas NET G2 and with NEC liver metastasis. However, according to the current theory, NET and NEC have different molecular biological mechanisms. So, this diagnosis was still in doubt. Whether NET and NEC shared a common pathogenesis remained to be discussed. To further analyze the relationship between the two types, we performed whole exon sequencing on the corresponding paraffin tissues of primary pancreas NET G2 (Ki67 10%) and liver metastasis NEN G3 (Ki67 70%). Thirteen individual cell mutations were found in pancreas NET G2 and 72 in liver NEN G3. Bioinformatics analysis revealed that P.S493N point mutation of TRIOBP gene was detected in both primary NET and metastatic foci (NEN G3).

The TRIOBP gene encodes multiple proteins, which together play crucial roles in modulating the assembly of the actin cytoskeleton. TRIOBP-1 (also known as TARA or TAP68) is a mainly structured protein that is ubiquitously expressed and binds to F-actin, preventing its depolymerization. It has been shown to be important for many processes including in the cell cycle, adhesion junctions, and neuronal differentiation. Few studies have been conducted on the correlation between TRIOBP gene and tumors. At present, no studies have been conducted on the correlation between TRIOBP gene and the NENs. This study suggests that TRIOBP gene may be involved in the occurrence and development of the NENs.

At the same time, the MDM4 oncogene in liver metastases was amplified 5 times. The human MDM4 gene is in chromosome 1q32 and consists of 10 introns and 11 exons. The relative molecular weight of the protein product is 54864 [8]. The human full length MDM4 consists of 490 amino acids, and contains 3 conserved domains: P53-binding domain and Zn finger domain at N terminal, RING finger domain at C terminal. It also contains a rich cid residue structure domain (acidic domain). When MDM4 is phosphorylated, its P53-binding domain can bind to the transcriptional activation domain of wild-type and mutated proteins to form the MDM4/P53 complex and inhibit the transcriptional activity of P53 [9, 10]. As a crucial regulatory factor in the upstream of p53, MDM4 plays an important role in normal tissue growth and tumor development. MDM4, as an oncogene, was less activated in the reproductive peak age, but increased rapidly with age. The functions of MDM4 splices, such as their significance in the process of tumor genesis and development and its role in the development of chemotherapy resistance, will be the focus of future research. In this case, MDM4 was significantly amplified in high-grade NEN compared with NET in the pancreas. Is it suggested that MDM4 is similar to P53 gene? If the obvious amplification represents a higher degree of malignancy, this will be one of the focuses of our next research. In the future, whether MDM4 inhibitor provide a new option for NEN treatment, or whether they will be more effective for NEC treatment of P53 mutation.
Abbreviations

p-NENs: Pancreatic neuroendocrine neoplasms; NET: Neuroendocrine tumors; NEC: neuroendocrine carcinoma; F-pNENs: Functional pancreatic neuroendocrine neoplasmas; NF-pNENs: Non-functional pancreatic neuroendocrine neoplasmas; WHO: World health organization; MiNEN: Mixed neuroendocrine-non-neuroendocrine neoplasm; CgA: Chromogranin A; Syn: Synapsin.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from the patient. Ethical approval was obtained from the Ethics Committee of China-Japan Friendship Hospital in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Conceptualization: Yanfen Shi and Luojie. Supervision: Dingrong Zhong and Zhaoqing Li. Writing — original draft: Yanfen Shi. Writing — review & editing: Jie Luo, Huangying Tan and Yuanliang Li. The author(s) read and approved the final manuscript.

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**Figures**

**Figure 1**

A. A solid mass seen at the section of the pancreas appears grayish brown, soft, clearly bounded. B. The highly differentiated tumor of the pancreas, NET G2, ribbon structure, uniform cell size (HE, ×40 times). C. Ki67 expression in pancreatic NET G2 tumor cells (EnVision, ×100 times). D. In NEN G3 region of the pancreas, cell heterogeneity was evident and focal with necrosis (HE, ×100 times). E. Ki67 expression in pancreatic NEN G3 tumor cells (EnVision, ×200 times). F. Liver metastasis are clumpy nests with rich blood supply (HE, ×40 times). G. The tumor cells in liver metastases are obvious heterotypic and mitotic (HE, ×100 times). H. Ki67 expression in liver metastatic tumor cells (EnVision, ×100 times). I. CgA expression in pancreatic tumor (EnVision, ×100 times). J. Syn expression in pancreatic tumor (EnVision, ×100 times). K. P53 expression (EnVision, ×100 times). L. RB1 expression (EnVision, ×100 times).
Figure 2

Representation of individual cell mutations in pancreas NET G2
Figure 3

Representation of individual cell mutations in metastatic liver NEN G3
Figure 4

PS493N point mutation of TRIOBP gene was detected in both primary NET and metastatic foci