Abstract. Pancreatic cancer is classified as ductal, acinar, neuroendocrine carcinoma or pancreatoblastoma. Ductal and acinar cells derive from exocrine glands and neuroendocrine cells from endocrine glands; however, mixed acinar-neuroendocrine-ductal carcinoma has different histological carcinomas coexisting within a nodule. The mixed pancreatic carcinoma forms from different developmental origins and therefore requires investigation. The current case report presents a 50-year-old male who had a tumor within the body of the pancreas. Pathological examination clarified the tumor as a mixed acinar-neuroendocrine-ductal carcinoma. The ductal and acinar/neuroendocrine tumor components were isolated using laser-capture microdissection, and next-generation sequencing analysis was performed. Consequently, TP53 frameshift (p.N210fs) and KRAS missense (p.G12R) mutations were identified in both ductal and acinar/neuroendocrine tumors. These results suggested a pancreatic mixed acinar-neuroendocrine-ductal carcinoma was derived from a founder tumor clone, and supports the notion that a founder tumor clone may differentiate and transform into a diverse histological type and form a pancreatic mixed carcinoma.

Introduction

Pancreatic cancer is one of the most lethal malignancies. Approximately 35,000 cases were reported in Japan in 2014, of whom 32,000 died, and the 5-year survival rate has been estimated as 7% (1). Malignant neoplasms of the pancreas are classified based on the cellular direction of differentiation into ductal, acinar, or neuroendocrine carcinomas (NEC), or pancreatoblastoma (2). Pancreatic ductal adenocarcinomas comprise about 90% of cases, NEC comprise 5%, while acinar cell carcinomas (ACC) are the rarest at 1-2% (3). Although most pancreatic tumors arise as a single cell type, either from the endocrine or exocrine pancreas, mixed neoplasms are listed under WHO classifications as carcinomas with mixed differentiation including mixed acinar-neuroendocrine carcinoma, mixed acinar-ductal carcinoma, and mixed acinar-neuroendocrine-ductal carcinoma (4). These mixed carcinomas are extremely rare, so their clinical and genomic features are poorly understood. Furthermore, it is not clear whether mixed carcinomas derive from the same or distinct tumor clones within a nodule.

Here, we report a case of mixed acinar-neuroendocrine-ductal carcinoma for which we performed genetic analysis. We describe the pathological, immunohistochemical, and molecular characterizations of a pancreatic mixed acinar-neuroendocrine-ductal carcinoma.

Subjects and methods

Patient and sample preparation. Written informed consent for the research study and publication was obtained from this case, which was performed in accordance with the protocols approved by the Institutional Review Board at our hospital. The study complied with Declaration of Helsinki principles and its later amendments ethical standards. Peripheral blood samples were obtained and buffy coats were isolated. Buffy coat DNA was extracted using the QIAamp DNA blood mini QIAcube kit (Qiagen, Hilden, Germany) (5).

Immunohistochemical analysis and laser-capture microdissection. The sections were deparaffinized before the antigens were retrieved by heat treatment in an EDTA solution at pH 8.0. Protein expression was evaluated using 3-µm-thick formalin-fixed and paraffin-embedded (FFPE) sections with anti-chromogranin A (1:50 dilution; clone 5H7, NCL-CHROM-430; Novocastra, Newcastle, UK), anti-trypsin...
A 50-year-old male was referred to our hospital with intermittent symptoms of epigastralgia and back pain. He had no history of smoking or drinking, and underwent an appendectomy at 10 years of age. Physical examination revealed no anaemia or icteric findings. No tumor was palpable, but slight tenderness was felt at the epigastrium without rebound tenderness. Laboratory data showed slight elevation of amylase at 20.3 U/ml, Dupan-2 at <25 U/ml, and Span-1 at <11 U/ml. Abdominal computed tomography revealed a tumor measuring 3 cm in diameter within the body of the pancreas (Fig. 1A). Tumor enhancement from the splenic artery was indicated by magnetic resonance angiography (MRA) (Fig. 1A). The postoperative course was uneventful for 1 year.

**Results**

The pancreas is composed of both exocrine and endocrine gland components: The exocrine part is formed from ductal and acinar cells, whereas the endocrine component is made up of endocrine cells. Most pancreatic tumors arise from a single cell type; therefore, ductal carcinomas and ACCs are considered to derive from the exocrine gland, and NECs from the endocrine gland. However, the existence of carcinomas with mixed differentiation complicates the situation. Additionally, few reports have defined the biological behaviour of mixed carcinomas. For example, pancreatic NEC and ACC appear to be distinct entities, but their pathological and morphological appearances can be similar. ACC has been reported to express neuroendocrine markers such as synaptophysin and chromogranin A, while the coexistence of ACC and NEC has also been reported. These observations suggest that the origin of the three types of pancreatic cancer (ductal, ACC, and NEC) is not clearly defined as either exocrine or endocrine glands.

Genetic profiles provide the direct evidence of tumor origins in the different histological components. While gene sequencing has demonstrated shared mutations in the different histologic components of mixed carcinomas (e.g., MANEC) of other digestive organs (7,8), it has not been proven in mixed acinar-ductal adenocarcinoma of the pancreas. Here, in one lymph node (data not shown). Immunohistochemical analysis revealed positive expression of chromogranin A and trypsin as markers of NEC and ACC, respectively. These protein expressions were mostly overlapped in NEC and ACC, but not stained in ductal adenocarcinoma (Fig. 1G and H). Ki67 protein expression was 50% in ductal adenocarcinoma and 80% in NEC and ACC, suggesting Ki67 index was high in both components (Fig. 2A and B). Although MUC production was seen only in ductal adenocarcinoma (data not shown), MUC1 was positive in all tumor components (Fig. 2C and D). TP53 protein expression is negative in both ductal and ACC/NEC components (Fig. 2E and F). Mixed adenoneuroendocrine carcinoma (MANEC) was neglected because of the presence of ACC.

**Discussion**

**Pathological findings.** Pathological examinations showed that the tumor was histologically well to moderately differentiated adenocarcinoma. Eosinophilic cytoplasm was also observed in solid neoplasm, mixed with aforementioned ductal adenocarcinoma. This eosinophilic cytoplasm pattern resembled acinar cell and/or islet cell. Approximately 50% of each histological component was present in tumor (Fig. 1E and F). In the solid component, vascular invasion was observed. Lymph node metastasis from the ductal adenocarcinoma was observed...
we present a case that showed the coexistence of ACC and NEC which were clearly seen together with ductal carcinoma. To investigate whether the different histological types derived from the same or a distinct tumor origin, we performed next-generation sequencing analysis and revealed genetic alterations in these distinct tumor histologies. We identified the same TP53 and KRAS mutations in both tumor samples from ductal carcinoma and mixed ACC/NEC carcinoma. Of note, there are the same genetic patterns between the two components, which were the distinct differentiation patterns. These results suggest that the same tumor clones are related to the development of pancreatic mixed acinar-neuroendocrine-ductal carcinoma. In line with this findings, previous studies also suggested the mixed neoplasm derived from same
tumor origin in endocrine-exocrine tumors of the gut and MANEC of the gastrointestinal tract (7,8).

Genetic alterations in pancreatic cancer are different among the histological types. For instance, \textit{KRAS}, \textit{TP53},
SMAD4, and CDKN2A genes are most commonly mutated in pancreatic ductal cancer (9,10). In particular, KRAS mutations are observed about 90% of pancreatic ductal adenocarcinoma. Contrary, TP53 can be mutated in a subset of ACCs and, more frequently, in NECs (11,12). However, KRAS mutations are rarely observed in ACCs (13,14). Therefore, it is particularly noteworthy that a KRAS mutation was detected in the mixed ACC/NEC tumor component of the present case. To understand this, it is necessary to consider previously-reported step-wise pancreatic tumorigenesis models. The most common model is that pancreatic intraepithelial neoplasia is a neoplastic precursor of invasive pancreatic ductal adenocarcinoma (15). Another is that pancreatic cancer develops from acinar cells (16). In the genetic pancreatic cancer mouse model, KRAS activation induces the dedifferentiation of acinar cells, which transform and lose the expression of typical marker proteins; thus, acinar to ductal metaplasia (ADM) is a precancerous form of pancreatic ductal carcinoma (17-19), and acinar cell hyperplasia has been observed in the human pancreas (20). In this study, we identified TP53 and KRAS mutations that were shared by ductal and ACC/NEC tumors. This indicates that KRAS activation in acinar cells, as well as other genetic or epigenetic changes, triggers ADM and results in subsequent cell differentiation into ductal adenocarcinoma. To our knowledge, this is the first report of common mutational profiles in ductal and acinar carcinomas in a human pancreatic tumor, which may provide genetic evidence of pancreatic tumorigenesis involving ADM.

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