

Case Report

Spontaneous well-differentiated pancreatic islet cell carcinoma with vascular invasion in a male F344 rat

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Abstract: This case report describes a case of spontaneous pancreatic islet cell carcinoma with vascular invasion in a 110-week-old male F344 rat. Histologically, a pancreatic nodule consisting of tumor cells and many blood-rich vessels, and covered with a fibrous capsule showed local invasion in the capsule and adjacent acinar tissues, encircling a large duct-like structure (DS). The tumor was composed of well-differentiated tumor cells resembling normal pancreatic islet cells, which had small round nuclei and eosinophilic cytoplasm. Mitotic figures were rare. Immunohistochemistry revealed that the tumor cells were positive for insulin. Although endothelial cells were not detected, the DS wall showed cells positive for α -smooth muscle actin and elastic fibers, suggesting that the DS is the pancreatic artery. This is a rare case of islet cell carcinoma consisting of well-differentiated tumor cells with invasion of the pancreatic artery in a rat. (DOI: 10.1293/tox.2021-0037; J Toxicol Pathol 2022; 35: 99–102)

Key words: islet cell carcinoma, well-differentiated, vascular invasion, F344 rat, spontaneous tumor

Spontaneous islet cell carcinoma (ICC) in rats is composed of well-differentiated islet cells with pleomorphic and anaplastic large cells, showing a sheet-like, cord-like, or ribbon-like growth pattern, and is prominent in mitotic figures and capsule formation^{1–6}. The majority of tumor cells of this tumor are found to be insulin-positive in immunohistochemistry (IHC)^{6–8}. In rats, although ICC is deemed to show invasive growth, the invasive sites are generally localized around the capsule of the tumor and in the extracapsular areas adjacent to the acinar tissues^{2, 4–7}. Metastasis is sometimes found in the liver or lung^{3, 5, 6, 9, 10}. The incidence of ICC in F344 male rats was 2.1% and was 0.3% in female rats¹. There is no clear strain difference in the incidence among F344, Sprague-Dawley, and Wistar rats^{5, 7, 9, 11}. In addition, no marked differences in histological features are observed among these strains. Although there are a few reports of invasion of blood vessels by tumor cells of the ICC in rats^{8, 10}, the morphology of these tumor cells and blood vessel type invaded by the tumor cells are not described in detail. This report describes the detailed histopathological characteristics of a case of ICC with tumor cell invasion into the vascular wall and lumen.

The animal was a 110-week-old male F344/DuCrj rat

(Charles River Laboratories Japan Inc., Kanagawa, Japan) that was subjected to a scheduled sacrifice in a long-term study to obtain background data. All rats in this study were housed individually in stainless steel wire cages in barrier system rooms maintained at a room temperature of $23 \pm 3^\circ\text{C}$ with a humidity of $50 \pm 20\%$ under a 12-hour light/dark cycle. Animals were allowed free access to radiation-sterilized pellets (CRF-1, Oriental Yeast Co., Ltd. Tokyo, Japan), and tap water was provided *ad libitum*. The study was approved by the Committee for Animal Experiments of the BoZo Research Center Inc. No general symptoms were observed in this case. At necropsy, this case was macroscopically seen as a single nodular lesion ($3 \times 2 \times 2$ mm) in the pancreas. All organs and tissues were collected, fixed in 10% buffered formalin, and stained with hematoxylin and eosin (HE). The nodule was stained with HE, Masson's trichrome stain (MT), and Elastica van Gieson stain (EVG). For IHC, mouse anti-insulin monoclonal antibody (K36AC10, Sigma-Aldrich, St. Luis, USA; 1:5,000 dilution), α -smooth muscle actin (α -SMA) monoclonal antibody (1A4, DAKO, Denmark A/S; 1:100 dilution, antigen activation with microwave)¹², rabbit anti-Ki67 (Ki67) polyclonal antibody (27309-1-AP, Proteintech, USA, 1:10,000 dilution, antigen activation with microwave), and von Willebrand factor (vWF) polyclonal antibody (DAKO, Japan, Tokyo; 1:500 dilution, antigen activation with Proteinase K) were used.

Histologically, the tumor consisted of tumor cells and many blood-rich vessels, and was covered with a fibrous capsule (Fig. 1A–C). The tumor cells had small round nuclei and rich eosinophilic cytoplasm, resembling normal pancreatic islet cells. The tumor cells were compartmentalized by fine collagen fibers and capillaries, showing a nest-like growth pattern (Fig. 1D, 1E). Mitotic figures were rare

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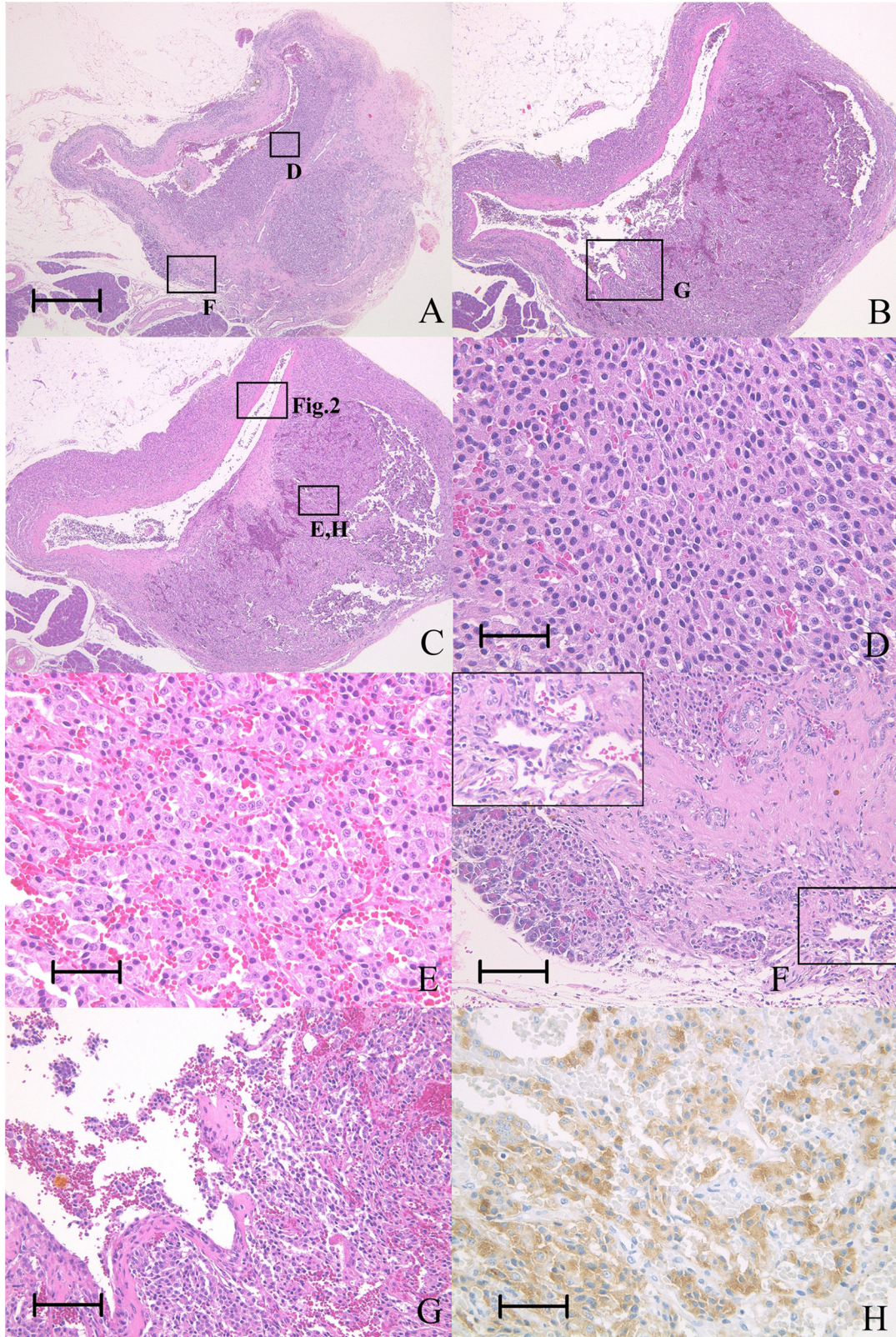


Fig. 1. Histological and immunohistochemical features of the islet cell carcinoma (A to C: consecutive sections). (A-C) A large duct-like structure (DS) is surrounded by tumor cells and forms a well-defined fibrous capsule. The tumor cells invade the DS wall. HE staining, Bar=500 μ m. (D, E) Higher magnification of Fig. 1A and C: Tumor cells, which have small round nuclei and abundant eosinophilic cytoplasm, arranged in cord and nest patterns within the fibrovascular stroma. HE staining, Bar=50 μ m. (F) Atrophic acinar cells and small ducts are scattered within the thick fibrous capsule surrounding the tumor, and the tumor expand and compress the surrounding tissue, and the tumor cells invade the capillaries or lymph vessels. HE staining, Bar=100 μ m. (G) Tumor cells show destructive invasion in all the layers of the DS wall, and erythrocytes and the tumor cells are found in the DS lumen. HE staining, Bar=100 μ m. (H) Tumor cells are positive for insulin. Immunohistochemistry, Bar=50 μ m.

in this case. The tumor had expanded and compressed the surrounding acinar cells with small ducts around it. In some areas of the tumor, the tumor cells showed invasion into the capillaries or lymph vessels (Fig. 1F). The tumor cells encircled and invaded a large duct-like structure (DS), and erythrocytes and tumor cells were found in the DS lumen (Fig. 1G). IHC revealed insulin positive tumor cells around the DS wall and lumen (Fig. 1H). The number of Ki67-positive cells in the tumor was very low (positive index: 2.6%). Therefore, the tumor was diagnosed as a well-differentiated ICC. No metastatic foci of ICC were observed in the other organs and tissues.

The DS wall was composed of smooth muscle-like cells and eosinophilic homogeneous fibrous stroma revealed using HE staining (Fig. 2A). However, the cells lining the lumen of the DS wall were unclear, and it was difficult to distinguish the pancreatic ducts from the blood vessels in sections stained with HE. MT and EVG staining and IHC for α -SMA and vWF were performed to identify the origin of DS. As a result, the majority of the cells forming the DS wall were positive for α -SMA in IHC (Fig. 2B), and many fibers stained blue in MT were observed among the α -SMA-positive cells (Fig. 2C). In the EVG stain, no structure suggesting the inner elastic lamina could be found, but layered elastic fibers were observed in a part of the DS wall (Fig. 2D).

No endothelial cells were positive for vWF on the DS wall. The DS was regarded as an artery in the pancreas because of the large number of red blood cells in the DS lumen and the presence of elastic fibers in the DS wall on EVG.

In general, histological characteristics in the acute phase of damaged blood vessels include infiltration of inflammatory cells, swelling of endothelial cells, rupture of internal and external elastic lamina, rupture of elastic fibers of the media, necrosis/disappearance of smooth muscles, thrombosis¹³. In the repair or chronic phase, proliferation of smooth muscles and myofibroblasts and production of connective tissues result in intimal thickening and medial or adventitial fibrosis¹⁴. As this case had increased collagen fibers and decreased elastic fibers, with the disappearance of the inner elastic lamina and endothelial cells, the morphological changes observed in the DS wall might have been caused by the destruction of the blood vessel due to invasive growth of the tumor and its reparative changes.

There are a few reports showing that ICC invades the blood vessels around the tumor in rats, but no report has been described so far on the occurrence of ICC accompanied by invading the relatively large blood vessel, as seen in this case. One to several arterioles enter the islets of Langerhans (IL), and occasionally, IL exists near the small arteries in rats⁶. Therefore, this tumor may originate from the IL

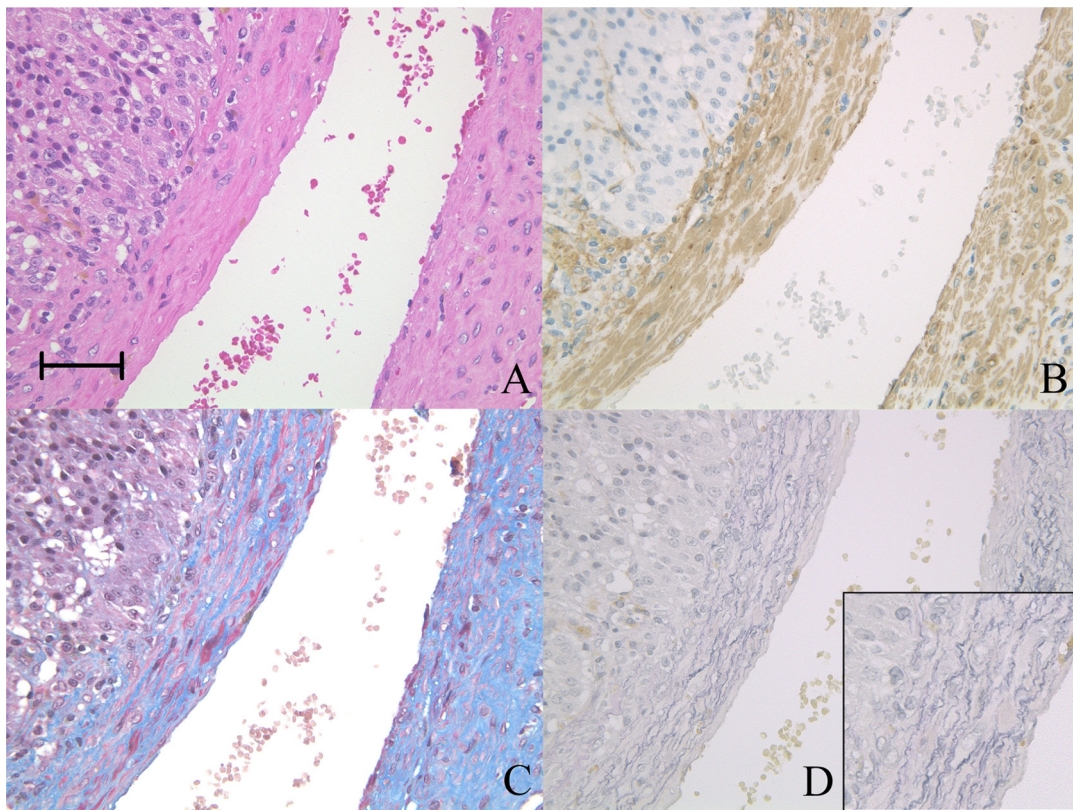


Fig. 2. Morphological features of the DS wall (consecutive sections). (A) The DS wall is composed of smooth muscle-like cells and a large amount of eosinophilic homogeneous fibrous stroma. HE staining, Bar=50 μ m. (B) The majority of the cells constructing the DS wall are positive for α -SMA. Immunohistochemistry. (C) The fibrous matrix constructing the DS wall is stained blue with MT staining. (D) Layered Elastic fibers are found in a part of the DS wall. EVG staining.

located in the vicinity of the pancreatic artery and invades the arterial wall. In addition, in the classification of islet cell tumors in rats, cellular anaplasia and two or more mitotic figures per high-power field are used as criteria for malignancy, but there is uncertainty about these criteria so as to reflect the true biological feature of ICCs⁴. Therefore, the diagnosis of malignant tumors should not be based solely on light microscopic findings. The results of the present study suggest that well-differentiated tumor cells that are morphologically similar to normal pancreatic islet cells may also exhibit malignant behavior. In dogs, even a small ICC composed of well-differentiated tumor cells may show invasive growth into the lymph and blood vessels and metastasis to the liver and lymph node¹⁵. Considering the morphology and proliferative activity of tumors in the guidelines adopted by the European NET societies (ENETS), North American NET societies (NANETS), and World Health Organization (WHO), human neuroendocrine tumors of the pancreas (PanNETs) are classified into the following three types of tumors: well-differentiated PanNETs (Grade 1 and 2), and poorly differentiated PanNETs (Grade 3)¹⁶. It has been reported that well-differentiated PanNETs in humans can invade/metastasize to other organs/tissues, and in such cases Ki67 shows a higher positive index¹⁶. However, pathological features in such human case are clearly different from those found in the present case showing vascular invasion with rare mitotic figures and low Ki67 proliferative activity.

In conclusion, this case is a spontaneous well-differentiated ICC with invasion of not only the surrounding tissues of the tumor but also the pancreatic artery, which is a rare type of rat ICC.

Disclosure of Potential Conflicts of Interest: The authors declare that they have no conflicts of interest.

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