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SHORT COMMUNICATION

A rare case of giant panda cancer: Pancreatic ductal adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant cancer of the digestive system common among humans. However, it is rarely reported in wild animals. In 2018, a giant panda died in the Beijing Zoo. During subsequent histological observation of the pancreas, it was discovered that the glandular epithelial cells had lost the pancreatic acinar structure, tubular areas with obvious structure in the pancreas, and the ductal epithelium was substituted by high columnar mucus cells. Masson staining showed that there were several fibrous tissue proliferative reactions around the ductal adenocarcinoma and immunohistochemical staining revealed that CK7 and CK19 were positively expressed in the pancreatic tissue. Therefore, the pathological diagnosis indicated that the panda had PDAC. In this paper, the panda's living conditions and pathological diagnosis results are examined, with the aim of providing a reference point for the future diagnosis of wild animal tumors.

KEYWORDS
giant panda, pancreatic ductal adenocarcinoma (PDAC), the pathological diagnosis of wild animals

1 | INTRODUCTION

The giant panda is regarded as one of China's national treasures and is an endemic species in China, with its primary habitat in the mountainous areas of Sichuan, Shaanxi, and Gansu. Over the years, China has taken many steps to conserve giant pandas using different disciplines and approaches. According to results released by the China Giant Panda Breeding Technical Committee in November 2019, the number of captive giant pandas worldwide reached 600 by 2019. Data published by the National Forestry and Grassland Administration shows that the wild population of giant pandas in China has increased to 1864 as of January 2021. The International Union for Conservation of Nature (IUCN) decreased the threat level for giant pandas from 'endangered' to 'vulnerable' in 2021 (endangered level and standard of IUCN Red List Categories and Criteria).

However, although the threat level has decreased, the number of pandas in captivity and the wild is still small, and unanswered questions about the reasons for their death and endangerment

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remain. While the poor adaptability of giant pandas to their current environment is a vital internal factor that endangers them, disease (for example, parasites, viral diseases and cancer) is also a crucial element.[1–3]

In recent years, research on animal tumors and animal models of cancer has developed rapidly, but research on wild animal tumors is still relatively rare,[4,5] and there are very few reports of giant panda cancer.[6]

Pancreatic ductal adenocarcinoma (PDAC) is a common histological classification of pancreatic cancer and is a highly malignant digestive system tumor. PDAC accounts for more than 90% of human pancreatic cancers.[7]

Recently, with the increase in the number of captive wild animals and the consequent in-depth diagnosis and treatment of their disorders, the diagnostic rate of wild animal tumors shows an increasing trend. However, PDAC is uncommon in wild animals. This paper introduces a pathological diagnosis of PDAC in a dead giant panda in Beijing Zoo, which provides a reference point for the diagnosis of wild animal tumors in the future.

2 | MATERIALS AND METHODS

2.1 | Case presentation

In the case presented here, the giant panda was a female born on September 6, 1986, and raised in the Beijing Zoo. Starting in June 2018, the panda showed weakness in her hind legs when exercising and could not support her hindquarters. In addition, she could eat only a small amount of soft food. By July 2018, the panda could no longer stand up after lying down. She rapidly weakened and could not chew food, relying on the breeder to feed her a mix of milk, porridge, and other foods via a syringe. The panda died on July 7th.

2.2 | Histological processing

Immediately after her death, an autopsy was performed, and the pancreatic tissues were fixed in 10% formalin solution and were preprocessed for pathological tissue section.

2.3 | Hematoxylin and Eosin staining

Paraffin sections were dewaxed using xylene I and II for 5 min, respectively, then immersed in an absolute ethyl alcohol/xylene (1:1) mixture for 5 min, and then slowly rehydrated using absolute ethyl alcohol I, absolute ethyl alcohol II, 95% ethanol, 85% ethanol, 75% ethanol, and 50% ethanol. They were then immersed in double distilled water, dyed with Hematoxylin for 10 min, separated using 1% hydrochloric alcohol for a number of seconds, and finally washed back to blue with water for 15 minutes. They were then dehydrated using 50% ethanol, 75% ethanol, 85% ethanol, and 95% ethanol, dyed with Eosin for 1 min, washed with 95% ethanol three times, and then treated with absolute ethyl alcohol I and II for 5 min, before being made transparent using xylene I and II for 5 min and finally sealed using neutral gum. The sections were dried overnight in a 37°C incubator and then observed under the microscope.

2.4 | Masson staining

Paraffin sections were dewaxed, rehydrated, dyed using Weigert iron hematoxylin for 10 minutes, washed using water, differentiated using 1% hydrochloric acid alcohol, washed back to blue with water for 15 min, dyed with Ponceau Acid Magenta for 10 min, washed with water followed by phosphomolybdic acid for 5 min, dyed with Toluidine Blue for 5 min, treated with 1% glacial acetic acid for 1 minute, dehydrated and made transparent, and then were sealed using neutral gum for observation under a microscope.

2.5 | Immunohistochemical staining

Paraffin sections were dewaxed and rehydrated. Normal mouse serum (C-0005, BIOSS, China) was incubated at room temperature, the rabbit anti-CK7 (bs-1610R, BIOSS, China) and CK19 (bs-1028R, BIOSS, China) antibodies were incubated overnight at 4°C, biotinylated goat anti-rabbit IgG (bs-0295G, BIOSS, China) was incubated at 37°C for 30 min, HRP-labeled biotin-streptavidin complex (bs-0311P, BIOSS, China) was incubated at 37°C for 30 min, and DAB (C-0003, BIOSS, China) was developed using color. The Hematoxylin staining solution was slightly re-stained, dehydrated, made transparent, and sealed using neutral gum for observation under a microscope.

3 | RESULTS

3.1 | Pathological findings

Under the microscope, it was discovered that the pancreatic tissue tumors exhibited three varying forms. (1) Many foam-like tubular adenocarcinoma structures were found in the pancreas, and glandular epithelial cells lost the typical pancreatic acinar structure, showing a hyaline cell-like appearance (with vacuolated cytoplasm and the nucleus squeezed to one side), with an enlarged nucleus and a clear and prominent nucleolus. The tubular structure of the gland was also irregular, and a difference in nucleus sizes was evident, which was thought to indicate moderately differentiated or poorly differentiated ductal adenocarcinoma (Figure 1A). (2) Tubular areas with the obvious structures formed in the pancreas exhibited papillary growth. The surface of the papillary structure was a monolayer of epithelial cells, but no columnar epithelial structure was found, and several blood vessels were formed in the papillary structure. This was

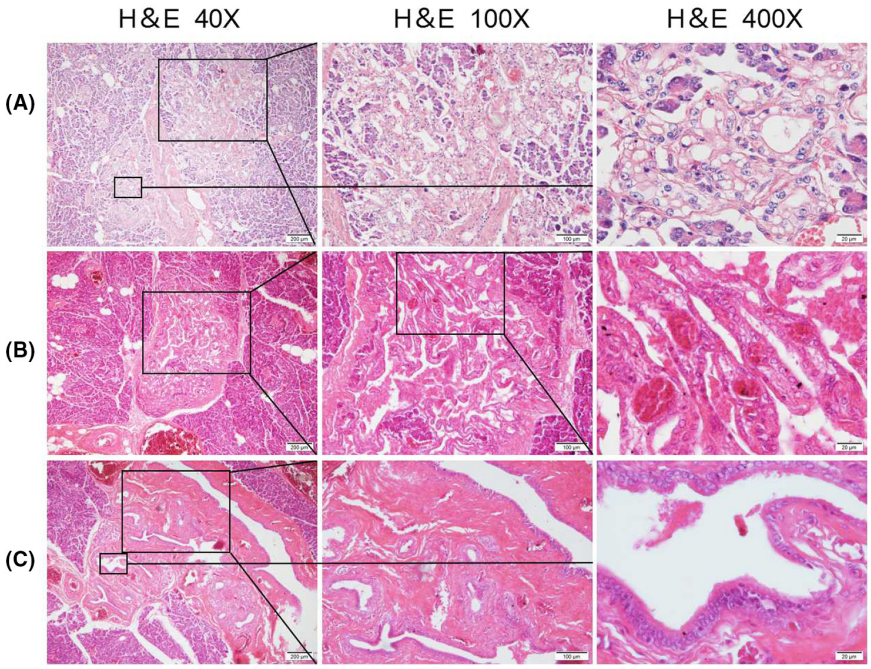

FIGURE 1 Pancreatic tissues tumors showing three different forms. (A) Several glandular tubular adenocarcinoma structures can be seen in the pancreatic tissue. The glandular epithelial cells have a clear cell-like structure at high magnification, losing the original pancreatic vesicle structure, and the nuclei are squeezed to one side. (B) A tubular area can be seen in the pancreas, showing papillary growth. The papillary structure is lined with monolayer cells, without the columnar epithelial structure, and numerous blood vessels can be seen in the papillary structure. (C) The ductal epithelium in the pancreas is substituted by high columnar mucous cells, which showed pseudostratified columnar epithelial cells. The nuclei are aggregated and enlarged, and the nucleolus is evident, accompanied by papilla formation. The connective tissue around the duct has proliferated.

thought to indicate a highly differentiated or moderately differentiated ductal adenocarcinoma (Figure 1B). (3) In addition to the above two areas, the ductal epithelium was substituted by high columnar mucus cells, which are pseudostratified columnar epithelial cells accompanied by papilla formation (Figure 1C). This was believed to indicate a precancerous lesion of the duct, i.e., pancreatic intraepithelial neoplasia (PanIN).

During the histological observation of the pancreas, Masson staining was used. After staining, it was noted that there were several fibrous tissue proliferative reactions around the ductal adenocarcinoma (fibrous tissues are dyed blue in Masson staining) (Figure 2A). In ductal adenocarcinoma, adenocarcinoma tubules were wrapped and supported by a thin but regular collagen trabecular structure (Figure 2B). In addition, the obvious difference in nuclear sizes, nuclear atypia, mitotic image, and an irregular, disordered glandular tubular structure in the tumor tissues could be seen (Figure 2C).

As the pathological changes in PDAC are similar to those of chronic pancreatitis, immunohistochemical staining of CK7 and CK19 was performed. The results indicated that CK7 and CK19 were positively expressed in the pancreatic tissue (Figure 3). Therefore, it was judged that this giant panda suffered from PDAC.

4 | DISCUSSION AND CONCLUSIONS

In humans, research indicates that PDAC occurrence may be directly related to smoking, excessive drinking, familial inheritance, and chronic pancreatitis; however, the pathogenic factors leading to this tumor are still unclear.[8–12] As the pancreas is a retroperitoneal organ with a hidden location, and the early stage of PDAC lacks specific clinical signs, there are no simple and effective early screening and diagnostic tools in the clinic, and it is difficult to find abnormalities through routine examination. Most patients with PDAC cannot undergo surgical resection after diagnosis, even though surgical resection is still the first treatment choice for curing pancreatic cancer. The peak age of this tumor is 50–70 years, with rare cases occurring earlier, and the incidence rate in males and females is equal.[13] In the case reported in this study, no abnormality consistent with the hidden characteristics of PDAC was seen in past physical examinations or in the anatomy of the giant panda, and we believe that there was a correlation between age and PDAC in this giant panda.

Recently animals suffering from PDAC have been seen more commonly in experimental and model animals (such as mice) for this tumor.[14,15] Research reports have also concentrated on studying tumor pathogenesis and related tumor markers. There have been few recent reports on animals suffering spontaneously from PDAC, with more literature appearing 10 years ago or even earlier, primarily focusing on pancreatic exocrine tumors, which are more common in dogs and cattle than in cats and pigs.[16–19] No reports of pancreatic tumors in wild animals have been found, relevant research needs to be further strengthened.

The pathological features of PDAC include infiltrating ducts and tubular structures which can be seen in many fibrous connective

tissue proliferative stromas. The diagnosis of PDAC is similar to chronic pancreatitis and distinguishing between them is the biggest problem. In chronic pancreatitis, stones or embolic substances appear in the tissues, there is complete duct structure, a regular arrangement of ducts, the nuclei are a consistent shape, and there is no expression in the nucleolus, and no obvious mitosis. Alternatively, PDAC is characterized by no stones or embolic substances, incomplete or damaged duct structure, disordered arrangement of ducts, polymorphic nuclei, clear nucleoli, and obvious mitosis. Immunohistochemistry can also be used as a technique for differential diagnosis, as CK7, 8, 18, 19, EMA, CEA, and CA19-9 are positively expressed in PDAC.[20]

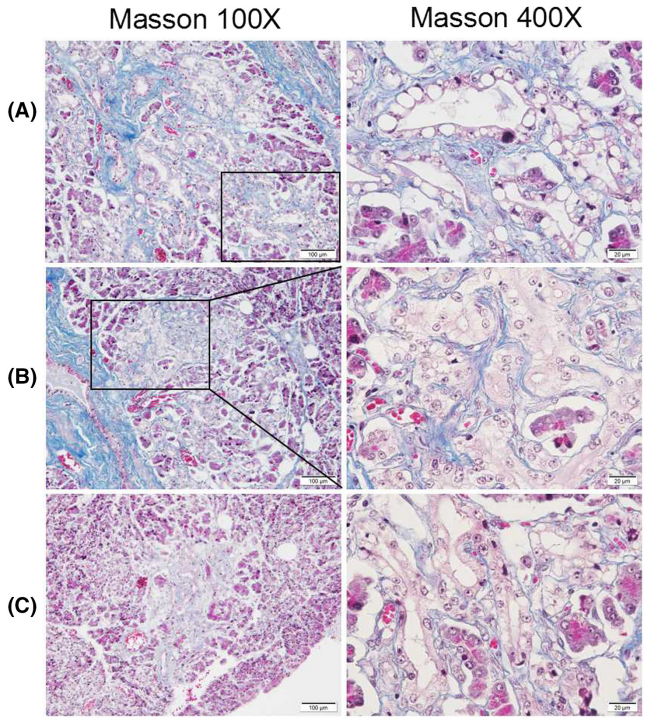

FIGURE 2 Masson staining. (A) Microscopically, it can be seen that many fibrous tissues proliferate around the pancreatic ductal adenocarcinoma (collagen fibers appear blue), and the pancreas is characterized by fibrosis. (B) At high magnification, in tubular adenocarcinoma, the ductal adenocarcinoma structure is wrapped and supported by collagen fibers. (C) In addition, a difference in nuclear sizes, nuclear atypia, mitotic images, and a irregular and messy glandular tubular structure in tumor tissues can be seen clearly.

In the diagnosis and treatment of disease in wild animals (especially wild animals in captivity), a tumor is a non-negligible problem. Increasing evidence shows that tumor occurrence in wild animals is closely related to pathogenic microorganisms, environmental pollution, and animal immunity.[21] Additionally, the intrinsic relationship between animal age and tumorigenesis should also be considered. Generally speaking, giant pandas have a life span of 18–20 years in the wild and over 30 years in captivity. The longest-lived giant panda on record was 'Panda bath' (the panda providing the prototype for the mascot of the 1990 Beijing Asian Games), who lived for 37 years. The giant panda, in this study was born in June 1986 and died in July 2018. She lived in a good breeding environment at Beijing Zoo for 32 years, a great age for a panda. In humans, the highest incidence of pancreatic cancer occurs after 50 years, which suggests that the occurrence of pancreatic cancer in giant pandas is similar to that in humans, and the incidence in giant pandas may increase with age.[13] However, because of the scarcity of giant pandas and lack of statistics, this conclusion can only be a hypothesis.

Currently, research reports on giant pandas focus on diverse living environments and intestinal microbes. Pathological reports on diseases of giant pandas are rare, and studies on tumors of giant pandas are even fewer. This highlights the challenges faced in the pathological diagnosis of wild animals (there are practical challenges, such as the lack of useful background data on sick animals, the weak applicability of specific protein targets and antibodies for differential diagnosis, and the scarcity of pathological practitioners specializing in wild animals, etc.).[22] The risk of giant pandas developing tumors is currently unknown, and the factors leading to the occurrence of tumors in giant pandas are unclear. Further studies are required to progress this field of research.

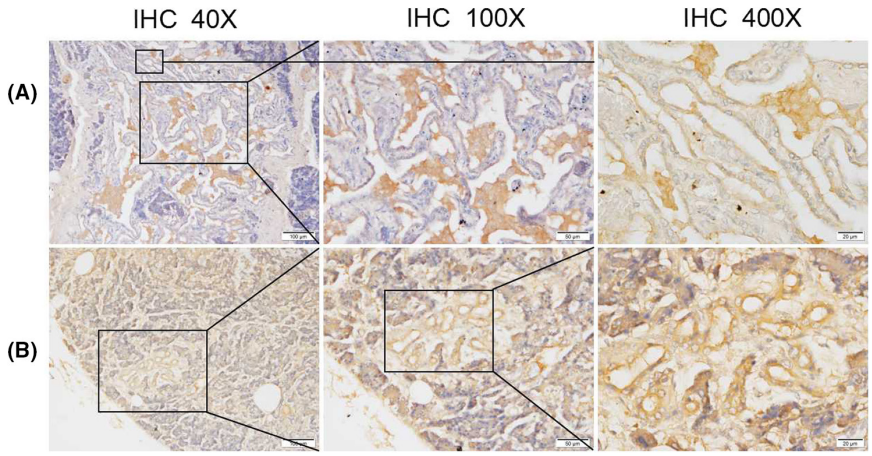

FIGURE 3 Immunohistochemical staining. CK7 (A) and CK19 (B) were positive in pancreatic tissues, and CK7 and CK19 were abundant in ductal adenocarcinoma cells.

AUTHOR CONTRIBUTIONS

Yunsheng Wang managed the case, wrote the paper and provided Figure 1. Maohua Xia performed the necropsy and wrote the corresponding section of case presentation. Xiangxiang Li and Xinxin Guo and Sufen Zhao performed Masson and immunohistochemical analysis, and provided Figures 2 and 3. Yan Lu and Tianchun Pu helped in the case management, edited the paper and provided funding for this article. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ETHICS STATEMENT

This study was approved by the Beijing Municipal Committee of Animal Management before sample collection. All experiments were performed in accordance with the approved guidelines and regulations.

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