

Case Report

Pancreatic Ductal Adenocarcinoma in a Wistar Hannover GALAS Rat

Yusuke Kuroda^{1*}, Seigo Hayashi¹, Soichiro Hagio¹, Masayoshi Abe¹, Satoshi Furukawa¹, and Dai Nakae^{2,3}

¹Toxicology and Environmental Science Department, Biological Research Laboratories, Nissan Chemical Industries, Ltd., 1470 Shiraoka, Shiraoka-shi, Saitama 349-0294, Japan

²Department of Pharmaceutical Sciences, Tokyo Metropolitan Institute of Public Health, 3-24-1 Hyakunin-cho, Shinjuku-ku, Tokyo 169-0073, Japan

³Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

Abstract: There are no reported spontaneous cases of pancreatic ductal adenocarcinoma (PDAC), and there are few reports about chemically-induced PDAC in rats. We encountered a PDAC in a Wistar Hannover GALAS rat that had been subjected to a medium-term multiorgan carcinogenicity bioassay. This article describes the histological and histochemical findings of the tumor. The tumor was located in the pancreatic tissue and had not invaded the liver parenchyma or the mucosal layer of the alimentary tract. The tumor cells were atypical and were mainly arranged in small tubules. In addition, abundant stroma and mucus production were observed in the tumor. In an immunohistochemical examination, the tumor cells were positive for cytokeratin, Sox9 and pancreas duodenum homeobox 1 and negative for amylase 2A and insulin. Therefore, the tumor was diagnosed as a PDAC based on its histological and histochemical findings. We considered that the tumor was caused by the carcinogens administered during the abovementioned bioassay. (DOI: 10.1293/tox.2013-0068; J Toxicol Pathol 2014; 27: 147–151)

Key words: DMBDD treatment, pancreatic ductal adenocarcinoma, Wistar Hannover GALAS rat

Pancreatic acinar cell carcinomas and pancreatic ductal adenocarcinomas (PDAC) are pancreatic tumors that originate from exocrine glands. Pancreatic acinar cell carcinoma rarely develops spontaneously in rats, but it can be induced by azaserine or 4-hydroxyaminoquinoline-1-oxide^{1–3}. On the other hand, as far as we know, no spontaneous cases of PDAC in rats have been reported, and there are few reports about chemically-induced cases^{1,4–6}. We encountered a chemically-induced PDAC in a Wistar Hannover GALAS rat. This article describes the histological and histochemical findings of this tumor.

The animal was one of 114 rats in the treated group of a study in which the toxicity of copper gluconate and the preventive effects of green tea catechins were evaluated using a medium-term multiorgan carcinogenicity bioassay⁷. In this group, 6-week-old male Wistar Hannover GALAS rats (BrlHan:WIST@Jcl, GALAS) (CLEA Japan, Inc., Tokyo, Japan) were subjected to combined treatment with 5

carcinogens targeting different organs: a single intraperitoneal injection of 100 mg/kg body weight of *N*-nitrosodiethylamine at commencement, 4 intraperitoneal injections of 20 mg/kg body weight of *N*-methylnitrosourea (MNU) during the first 2 weeks, 4 subcutaneous injections of 40 mg/kg body weight of 1, 2-dimethylhydrazine during the second 2 weeks and continuous administrations by admixing into drinking water with *N*-butyl-*N*-(4-hydroxybutyl) nitrosamine at a concentration of 0.05% for the first 2 weeks and 2,2'-dihydroxy-di-*n*-propylnitrosamine at a concentration of 0.1% for the second 2 weeks (DMBDD treatment). Subsequently, all rats were given a diet containing copper gluconate and green tea catechins for 25 weeks before being sacrificed under light ether anesthesia. Their major organs/tissues were obtained⁷, fixed in 10% neutrally buffered formalin solution, embedded in paraffin and sectioned into 4- μ m-thick specimens. All experiments were conducted according to the Guidelines for Animal Experimentation developed by the Japanese Association for Laboratory Animal Science (1987).

The specimens of the tumor were stained with hematoxylin and eosin (HE) and also subjected to periodic acid-Schiff (PAS), Alcian blue and Masson's trichrome staining. In immunohistochemical examination, the specimens were stained for cytokeratin (CK) (monoclonal; MNF116; Dako, Kyoto, Japan), SRY-related high-mobility group box 9 (Sox9) (polyclonal; Millipore, Temecula, CA, USA), amy-

Received: 29 December 2013, Accepted: 31 March 2014

Published online in J-STAGE: 28 May 2014

*Corresponding author : Y Kuroda (e-mail: kurodayu@nissanchem.co.jp)

©2014 The Japanese Society of Toxicologic Pathology

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

lase 2A (AMY2A) (polyclonal; Proteintech, Chicago, IL, USA), insulin (polyclonal, Dako), pancreas duodenum homeobox 1 (PDX1) (polyclonal; TransGenic, Kumamoto, Japan), vimentin (monoclonal, VIM 3B4, Millipore), desmin (monoclonal, D33, Dako), S-100 (polyclonal, Dako) and proliferating cell nuclear antigen (PCNA) (monoclonal, PC10, Dako) using the avidin-biotin complex method (Vectastain ABC Kit; Vector Laboratories, Burlingame, CA, USA) and for C-ERC/mesothelin (polyclonal; Immuno-Biological Laboratories, Fujioka, Japan) using the streptavidin-biotin method (Histofine Simple Stain MAX-PO; Nichirei, Tokyo, Japan).

At necropsy, a whitish solid tumor (gross size: approximately 3 cm × 2 cm × 2 cm) was found in the proximity of the greater curvature of the stomach. The tumor involved the pancreas and adhered to the liver, stomach and duodenum.

Histologically, the tumor had replaced some of the pancreatic tissue (Figs. 1 and 2A). Although the tumor cells had partly invaded the hepatic capsules and the muscular layer of the stomach, they had not invaded the liver parenchyma or the mucosal layer of the alimentary tract including the stomach (Figs. 2B and 2C). The tumor cells were mainly arranged in small tubules within the abundant stroma (Fig. 2D), and some of them showed a clumpy and solitary arrangement. The tubules were usually irregular in shape and sometimes contained debris (Fig. 2E). The tumor cells were atypical and had eosinophilic cytoplasm, polymorphic nuclei and indistinct borders. Mitotic figures were often observed in the tumor cells, and some of them contained intracytoplasmic vacuoles (Fig. 2F), which were positively stained by PAS (Fig. 2G) and Alcian blue. The stroma was characterized by proliferation of fibroblasts and abundant collagenous fibrous tissue (Fig. 2H) with infiltration of lymphocytes, neutrophils and macrophages. Myxoid regions were scattered throughout the stroma (Fig. 2F), and the spaces in these regions were positively stained by PAS (Fig. 2G) and Alcian blue.

The results of the immunohistochemical examination are summarized in Table 1. The tumor cells were positive for CK, Sox9 and PDX1 and negative for AMY2A, insulin, vimentin, desmin, S-100 and C-ERC/mesothelin (Figs. 3A-E). PCNA-positive tumor cells were frequently detected. Among the normal pancreatic tissues in the tumor specimen, the ductal, acinar and islet cells were positive for Sox9, AMY2A and insulin/PDX1, respectively. In addition, the bile duct cells in the liver were positive for Sox9 and negative for PDX1. CK MNF116 antibody reacts with CK5, 6, 8, 17 and 19 and shows an especially broad pattern of reactivity with epithelial tissue^{8, 9}. Sox9 is a transcription factor implicated in the control of diverse tissue development and carcinogenicity¹⁰. PDX1 is expressed in islet cells of the pancreas and posterior foregut in the fetus¹¹.

As for other organs, the serous surfaces of the seminal vesicles contained masses (up to 4 mm in diameter) with histological findings similar to those of the abovementioned tumor. These masses were considered to be metastatic le-

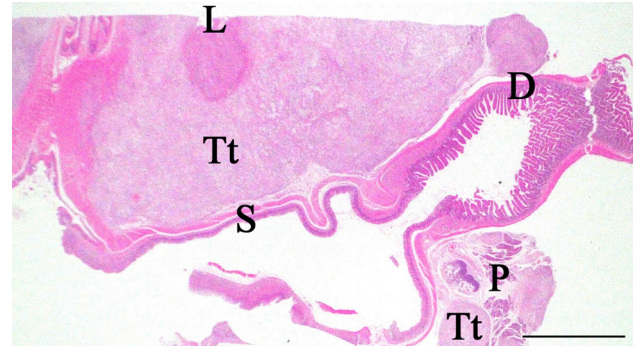


Fig. 1. The tumor found in the abdominal cavity of the Wistar Hanover GALAS rat. HE stain. The tumor (Tt) is in contact with the pancreas (P), liver (L), stomach (S) and duodenum (D). Bar = 4 mm.

sions.

The striking histological findings of the present tumor were that the tumor cells formed duct-like structures with abundant stroma in the pancreas and often produced mucus. These histological features are consistent with PDAC^{1, 3}. The tumor cells were positive for CK, Sox9 and PDX1. Sox9 is specifically expressed in ductal cells and centriacinar cells in the normal pancreatic tissue of the adult rat. In addition, Sox9 and PDX1 have been detected in the human PDAC^{12, 13} and in regenerative proliferating ductules after partial pancreatectomy in adult rats¹⁴. In contrast, the tumor cells did not contain zymogen granules, which are a feature of acinar cells, and were negative for AMY2A. Therefore, the present tumor was diagnosed as a PDAC.

Regarding the possible differential diagnoses for the present tumor, cholangiocarcinomas have similar histological features to PDAC¹, and in the present case, the bile duct cells in the normal liver were positive for Sox9, but the tumor had not invaded the liver parenchyma. Although it was reported that Sox9 and PDX1 were also expressed in pyloric glands and gastric carcinomas in humans^{15, 16}, gastric carcinomas were excluded for the same reasons as cholangiocarcinomas. Mesothelioma was also suspected because the tumor was located in the abdominal cavity. However, both the tumor and stromal cells were negative for C-ERC/mesothelin, and no continuity was detected between the tumor and the normal mesothelium (C-ERC/mesothelin-positive) around the invasive area.

It was reported that precursor lesions of PDAC are tubular complexes resulting from transdifferentiation of acinar cells in animal models of PDAC^{17, 18}. Although acinar to ductal metaplasia was observed in some of the residual pancreatic tissues in the present tumor, the association in the above reports was unclear.

Regarding cases of chemically-induced PDAC in rodents, PDACs were induced in hamsters subcutaneously injected with *N*-nitrosobis(2-oxopropyl)amine^{1, 3, 19, 20} or intraperitoneally injected with MNU²¹. In rats, it was reported that PDACs were induced by direct implantation of

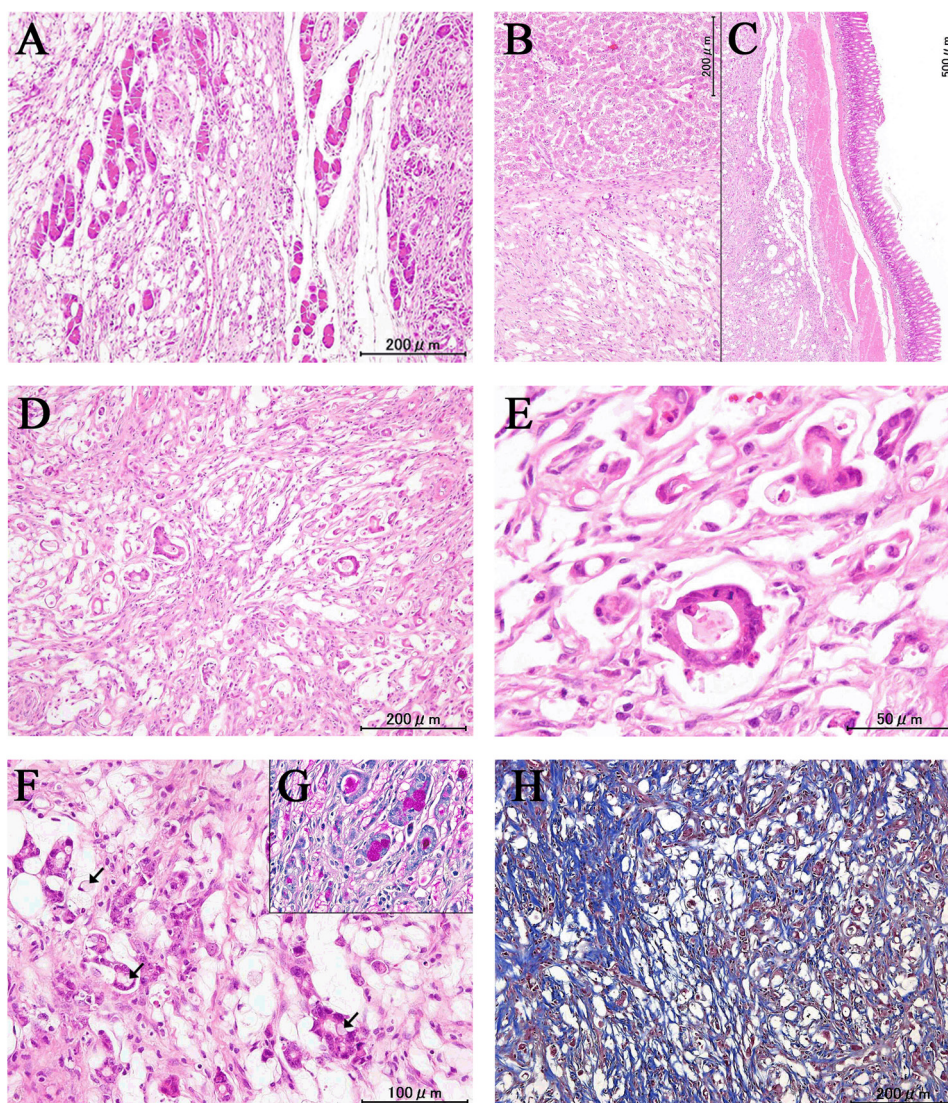


Fig. 2. The tumor found in the abdominal cavity of the Wistar Hannover GALAS rat. HE stain (A–F), PAS stain (G) and Masson's trichrome stain (H). The tumor replaced some of the pancreatic tissue (A). It did not invade the liver parenchyma (B) or the mucosal layer of the alimentary tract (C). The tumor cells are mainly arranged in small tubules within abundant stroma (D). The tubules are irregular in shape and sometimes contain debris (E). Some tumor cells with intracytoplasmic vacuoles (arrows) and myxoid regions are observed in the stroma (F). The vacuoles and spaces of the myxoid regions are positively stained with PAS (G). An abundant amount of collagenous fibrous tissue in the stroma is stained blue by Masson's trichrome (H).

Table 1. The Results of Immunohistochemical Examination of the Tumor, Pancreas and Liver

	Tumor		Pancreas			Liver
	Tumor cells	Stromal cells	Ductal cells	Acinar cells	Islet cells	Bile duct cells
CK	+	–	+	±	+	+
Sox9	+	–	+	–	±	+
PDX1	+	–	±	±	+	–
Amylase 2A	–	–	–	+	–	–
Insulin	–	–	–	–	+	–
Vimentin	–	+	NE	NE	NE	NE
Desmin	–	–	NE	NE	NE	NE
S-100	–	–	NE	NE	NE	NE
C-ERC/mesothelin	–	–	NE	NE	NE	NE
PCNA	+	–	NE	NE	NE	NE

Grade symbols –, ± and + represent negative, slightly positive and strongly positive, respectively. NE: Not examined.

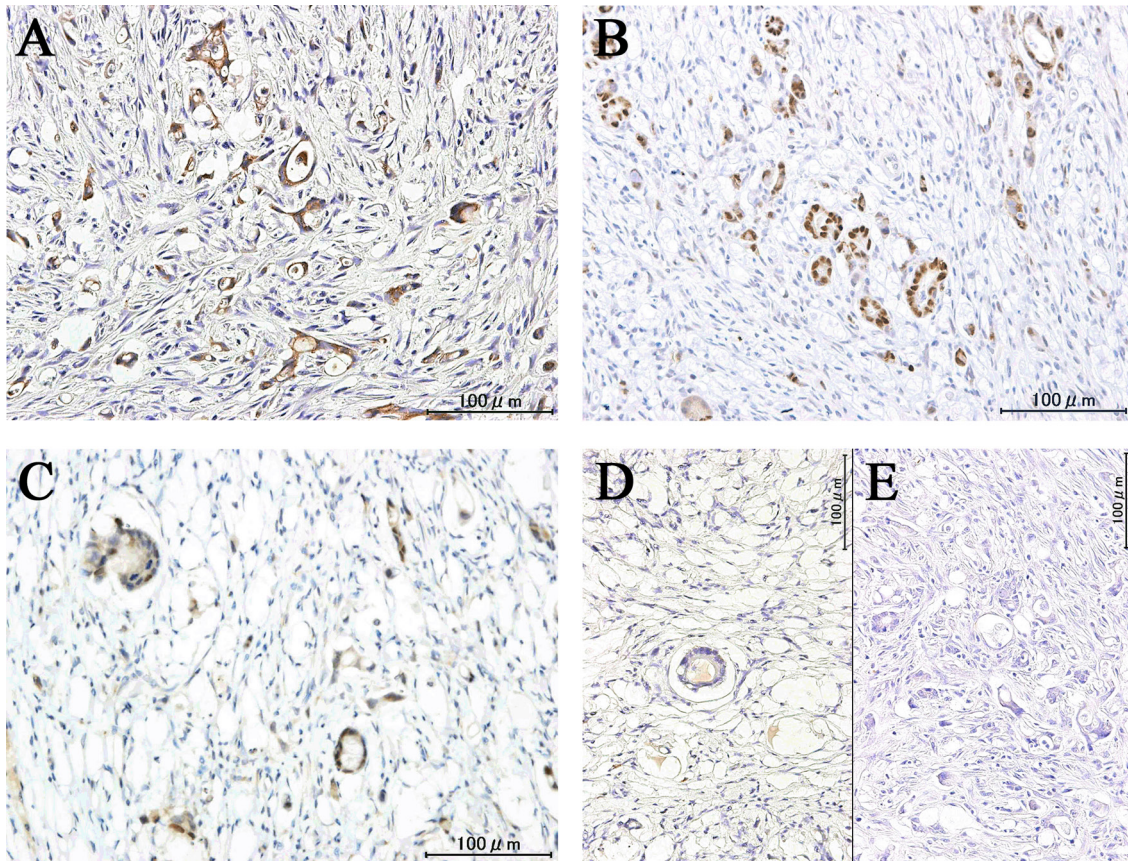


Fig. 3. Immunohistochemical staining of the tumor for CK (A), Sox9 (B), PDX1 (C), AMY2A (D) and insulin (E). The tumor cells are positive for CK, Sox9 and PDX1 but negative for AMY2A and insulin.

dimethylbenzanthracene into the pancreas⁴⁻⁶. Although to the best of our knowledge there are no reports about PDAC being induced in rats by the carcinogens used in DMBDD treatment, the histopathological features of the present case were quite similar to those of the PDACs that developed in the abovementioned studies. In conclusion, we considered that the present tumor was a PDAC that had been induced by the administration of the carcinogens used in DMBDD treatment in the Wistar Hannover GALAS rat.

Acknowledgments: The authors would like to thank Dr. Kiyokazu Ozaki (Department of Pathology, Faculty of Pharmaceutical Sciences, Setsunan University, Osaka, Japan) and Ms. Mika Nagaike (Safety Science Laboratory, Central Research Institute, Ishihara Sangyo, Ltd., Shiga, Japan) for their valuable advice and help with the immunohistochemical examinations (Sox9 and C-ERC/mesothelin) and Mr. Kiyoshi Kobayashi, Ms. Kaori Maejima, Ms. Hiromi Asako, Mr. Atsushi Funakoshi and Mr. Yoshinori Tanaka for providing excellent technical assistance. This study was supported in part by a Health and Labour Sciences Research Grant (awarded to Dai Nakae) from the Ministry of Health, Labour, and Welfare of Japan.

References

1. Tutumi M, and Konishi Y. Pancreas (Exocrine). In: Toxicological histopathology. JSTP (eds). JSTP, Aiti. 215-221. 2000.
2. Majeed SK. Studies of the incidence of spontaneous pancreatic tumours in ageing CD rats. *Arzneimittelforschung*. **47**: 879-884. 1997. [[Medline](#)]
3. Scarpelli DG. Experimental carcinogenesis, exocrine pancreas, hamster and rat. In: Digestive System (Monographs on Pathology of Laboratory Animals), 2nd ed. Jones TC, Popp JA, and Mohr U (eds). Springer-Verlag, Berlin. 274-288. 1997.
4. Rivera JA, Graeme-Cook F, Werner J, Z'graggen K, Rustgi AK, Rattner DW, Warshaw AL, and Fernández-del Castillo C. A rat model of pancreatic ductal adenocarcinoma: targeting chemical carcinogens. *Surgery*. **122**: 82-90. 1997. [[Medline](#)] [[CrossRef](#)]
5. Jimenez RE, Z'graggen K, Hartwig W, Graeme-Cook F, Warshaw AL, and Fernandez-del Castillo C. Immunohistochemical characterization of pancreatic tumors induced by dimethylbenzanthracene in rats. *Am J Pathol*. **154**: 1223-1229. 1999. [[Medline](#)] [[CrossRef](#)]
6. Tan XG, and Yang ZL. Expression of Ezrin, HGF, C-met in pancreatic cancer and non-cancerous pancreatic tissues of rats. *Hepatobiliary Pancreat Dis Int*. **9**: 639-644. 2010. [[Medline](#)]

7. Abe M, Suzuki N, Yoshida M, Usuda K, Furukawa S, Juneja LR, Okubo T, and Nakae D. Possible carcinogenic risks of copper gluconate and their prevention by co-administered green tea catechins evaluated by a rat medium-term multi-organ carcinogenicity bioassay protocol. *Food Chem Toxicol.* **46**: 1760–1770. 2008. [[Medline](#)] [[CrossRef](#)]
8. Prieto VG, and McNutt NS. Immunohistochemical detection of keratin with the monoclonal antibody MNF116 is useful in the diagnosis of epidermolysis bullosa simplex. *J Cutan Pathol.* **21**: 118–122. 1994. [[Medline](#)] [[CrossRef](#)]
9. Moll R, Franke WW, Schiller DL, Geiger B, and Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell.* **31**: 11–24. 1982. [[Medline](#)] [[CrossRef](#)]
10. Guo QQ, Hui CH, Zhao DH, Yu XL, Sheng BY, Ya QH, Liang Z, Hao F, Jie XL, Fu NJ, and Wei DZ. Combined overexpression of HI VEP3 and SOX9 predicts unfavorable biochemical recurrence-free survival in patients with prostate cancer. *OncoTargets and Ther.* **7**: 137–146. 2014.
11. Kim SK, and Hebrok M. Intercellular signals regulating pancreas development and function. *Genes Dev.* **15**: 111–127. 2001. [[Medline](#)] [[CrossRef](#)]
12. Kopp JL, von Figura G, Mayes E, Liu FF, Dubois CL, Morris JP 4th, Pan FC, Akiyama H, Wright CV, Jensen K, Hebrok M, and Sander M. Identification of Sox9-dependent acinar-to-ductal reprogramming as the principal mechanism for initiation of pancreatic ductal adenocarcinoma. *Cancer Cell.* **22**: 737–750. 2012. [[Medline](#)] [[CrossRef](#)]
13. Gandou C, Harada K, Sato Y, Igarashi S, Sasaki M, Ikeda H, and Nakanuma Y. Hilar cholangiocarcinoma and pancreatic ductal adenocarcinoma share similar histopathologies, immunophenotypes, and development-related molecules. *Hum Pathol.* **44**: 811–821. 2013. [[Medline](#)] [[CrossRef](#)]
14. Li WC, Rukstalis JM, Nishimura W, Tshipashvili V, Habener JF, Sharma A, and Bonner-Weir S. Activation of pancreatic-duct-derived progenitor cells during pancreas regeneration in adult rats. *J Cell Sci.* **123**: 2792–2802. 2010. [[Medline](#)] [[CrossRef](#)]
15. Sashikawa Kimura M, Mutoh H, and Sugano K. SOX9 is expressed in normal stomach, intestinal metaplasia, and gastric carcinoma in humans. *J Gastroenterol.* **46**: 1292–1299. 2011. [[Medline](#)] [[CrossRef](#)]
16. Sakai H, Eishi Y, Li XL, Akiyama Y, Miyake S, Takizawa T, Konishi N, Tatematsu M, Koike M, and Yuasa Y. PDX1 homeobox protein expression in pseudopyloric glands and gastric carcinomas. *Gut.* **53**: 323–330. 2004. [[Medline](#)] [[CrossRef](#)]
17. Rooman I, and Real FX. Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? *Gut.* **61**: 449–458. 2012. [[Medline](#)] [[CrossRef](#)]
18. Bockman DE, Guo J, Büchler P, Müller MW, Bergmann F, and Friess H. Origin and development of the precursor lesions in experimental pancreatic cancer in rats. *Lab Invest.* **83**: 853–859. 2003. [[Medline](#)] [[CrossRef](#)]
19. Kitahashi T, Mutoh M, Tsurusaki M, Iinuma G, Suzuki M, Moriyama N, Yoshimoto M, Wakabayashi K, Sugimura T, and Imai T. Imaging study of pancreatic ductal adenocarcinomas in Syrian hamsters using X-ray micro-computed tomography (CT). *Cancer Sci.* **101**: 1761–1766. 2010. [[Medline](#)] [[CrossRef](#)]
20. Kitahashi T, Yoshimoto M, and Imai T. Novel immunohistochemical marker, integrin $\alpha(V)\beta(3)$, for BOP-induced early lesions in hamster pancreatic ductal carcinogenesis. *Oncol Lett.* **2**: 229–234. 2011. [[Medline](#)]
21. Furukawa F, Sato H, Imaida K, Toyoda K, Imazawa T, Takahashi M, and Hayashi Y. Induction of pancreatic tumors in male Syrian golden hamsters by intraperitoneal N-methyl-N-nitrosourea injection. *Pancreas.* **7**: 153–158. 1992. [[Medline](#)] [[CrossRef](#)]