The oncogenic roles of John Cunningham virus T antigen in digestive epithelial cells with tissue specificity

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Research Article

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Abstract

Background

John Cunningham virus (JCV), a ubiquitous polyoma virus that commonly infects the human, is identified as the etiologic factor for progressive multifocal leukoencephalopathy and cancers.

Methods

Here, the transgenic mice of CAG-loxp-Laz-loxp T antigen were established and T antigen expression was especially activated in gastroenterological target cells with LacZ deletion using cre-loxp system.

Results

Gastric poorly-differentiated carcinoma was observed in T antigen-activated mice using K19-cre (stem-like cells) and PGC-cre (chief cells), but not Atp-4b-cre (parietal cells) or Capn8-cre (pit cells) mice. There appeared spontaneous hepatocellular and colorectal cancers in Alb-cre (hepatocytes)/T antigen and villin-cre (intestinal cells)/T antigen transgenic mice. Gastric, colorectal and breast cancer was observed in PGC-cre/T antigen mice. Pancreatic insulinoma and ductal adenocarcinoma, gastric adenoma, and duodenal cancer were detected in Pdx1-cre/T antigen mice. There was alternative splicing of *T antigen* mRNA in all target organs of these transgenic mice.

Conclusions

It was suggested that JCV T antigen might induce gastroenterological carcinogenesis at a manner of cell specificity. These spontaneous tumor models provide good tools to investigate the oncogenic role of T antigen in digestive cancers.

Background

John Cunningham virus (JCV) belongs to human non-enveloped polyomavirus family in combination of SV40 and BK virus, and is considered as an established etiologic factor of progressive multifocal leukoencephalopathy (PML). Among its 6 genomic genes, T antigen encodes the main oncogenic and multifunctional phosphoprotein essential for viral DNA replication because it binds to and breaks DNA to unwind double helix and recruit ATPase, helicase, and polymerase[1]. In nucleus, T antigen can inactivate p53 and pRb proteins to speed up cell cycle and subsequently proliferation, and in cytoplasm suppress the Wnt pathway by targeting βTrCP1/2 for abnormal β-catenin degradation, and disrupt IGF-IR signaling pathway[2]. Additionally, T antigen down-regulated Bag-3 expression for apoptotic suppression by blocking AP2 binding to Bag3 promoter[3].

JCV is a neurotropic virus because its replication is dependent of transcriptional factors in glial cells and neuron, including Jun, NF-1, GF-1, Sp1, Sμbp-2, Purα, and YB-1. Intravenous or intracranial inoculation of JCV into mice could result in glioblastomas, astrocytomas, medulloblastomas and neuroblastomas, and transgenic mice expressing *T-antigen* suffered from malignant peripheral nerve sheath tumors and pituitary adenoma[1 2]. However, the virus enters tonsillar tissue through intake of raw sewage and inhalation of air droplet, and persists quiescent in the lymphoid and renal tissue during latency.4 Therefore, higher copies and positive rate of T antigen were detected in oral, esophageal, gastric, colorectal, anal, head neck squamous carcinoma, lung cancer, prostatic cancer, cervical cancer and urothelial carcinoma[1–2, 4–10].

Recently, we firstly established transgenic mice and found that T antigen induced lens tumor and lung cancer, which provide direct evidence for its oncogenic role [11, 12]. Subsequently, we for the first time found that the insertion of T antigen into genome might cause lung cancer and lens tumor, but the alternative splicing of its intron was not of cell specificity11. To clarify the oncogenic role of JCV, we firstly generated CAG-loxp-LacZ-loxp-T antigen transgenic mouse, and activated T antigen expression in gastric, intestinal, and pancreatic ductal epithelium, islet β-cell or hepatocyte using various cre tool mice and observe the carcinogenesis with the alternative splicing of T antigen detected.

Materials And Methods

Animal model

pBS-JCVMad1 (kindly provided by Prof. Hirofumi Sawa, Hokkaido University), K19-COX-2 (kindly provided by Prof. Masanobu Oshima, Kanazawa University), and PBS-cre (Prof. Zhi-hong Zheng, China Medical University) was used for CAG-loxp-LacZ T antigen, K19-cre and PGC-cre mice in Shanghai Biomodel Organism Science & Technology Development Co. Ltd. To activate T antigen expression in gastric, intestinal, pancreatic ductal epithelia, islet β-cells or hepatocytes, we crossed CAG-loxp-LacZ T antigen mice with Atp-4B-cre (gastric parietal cell, kindly presented by Prof. Xiao Yang, Genetic Laboratory of Development and Diseases, Institute of Biotechnology, Beijing, China), Capn8-cre (gastric pit cell, also kindly presented by Prof. Xiao Yang), PGC-cre (gastric chief cell, unpublished), K19-cre (gastric stem-like cell), villin-cre (intestinal epithelium, Jax Lab), Pdx1-cre (pancreas, stomach and duodenum, Jax Lab) and Alb-cre (hepatocytes, Jax Lab) mice. These mice were fed in specific pathogen-free condition. All procedures and housing were approved by Animal Experiments Committee of The Affiliated Hospital of Chengde Medical University.

PCR

We extracted DNA from the mouse tail and tissues by proteinase K/phenol/chloroform method. DNA was amplified by PCR targeting T antigen (Forward: 5’-TGGCCTGTAAAGTTCTAGGCA-3’ and Reverse: 5’-GCAGAGTCAAGGGATTTACCTTC-3’), and cre (Forward: 5’-GCCTG CATTACCGGTCGATGC-3’ and Reverse: 5’-CAGGGTGTTATAAGCAATCCC-3’). Total RNA was extracted from normal tissues of transgenic mice using Trizol reagent and used for cDNA synthesis. To confirm the alternative splicing of T antigen

mRNA, we designed the primers (Forward: 5’-TCATCATCACTGGCAAAC-3’ and Reverse: 5’-GCAAAGAACTCCA CCCT-3’) as previously documented [11].

Western blot

We extracted the protein in radio-immunoprecipitation assay lysis buffer, separated the protein in 10% SDS polyacrylamide gel, and transferred the protein to PVDF membrane. After blocking membranes in 5 % milk in TBST, we incubated the membrane with mouse anti-SV40 T antigen, rabbit anti-PGC or mouse anti-GAPDH antibody in TBST, and then with anti-mouse or anti-rabbit IgG conjugated to HRP (DAKO). Bands were visualized with Azure Biosystem C300 by ECL detection kit.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was employed to quantify the serum PGC I level (Biohit, Finland). Briefly, we incubated either standard or serum sample in anti-PGC I-antibody-coated polystyrene plates for 1h. After removing the liquid, we added biotin-antibody working solution to each well and incubated the plate for 2h. After aspiration from each well and 3 rinses with 350μl wash buffer, we added 100μl HRP-avidin working solution to each well and incubated for 1h. After washed for 3 times, we added 90μl TMB into the plates, and incubated for 30 min. Finally, we dispensed stopping solution to each well and measure absorbance.

Computed tomography (CT)

Bruker CT (SkyScan1276) was used to image the spontaneous tumors. In brief,
the mice were put onto the poly-styrene bed of CT scanner. The images were taken with the scan set of the x-ray source: 20.19μm image pixel size, 388ms exposure, 0.5mm filter Al, rotation step (deg)=0.400, use 360 Rotation, 70kV tube voltage, 200μA tube current and 1344×2016 matrix. The NRecon reconstruction conditions were minimum and maximum image conversion for CT scanning =0.000000 and 0.050000.

Patients

Gastric cancer (n=358) and normal mucosa (n=130), and breast cancer (n=219) and normal tissues (n=43) were sampled from The Affiliated Hospital of Chengde Medical University. These patients never had received adjuvant treatment, radiotherapy or chemotherapy before operation. They signed the informed consent and the ethics committees of our hospital approved the study.

Histopathology

We fixed the tissues in 4% formaldehyde, embedded them in paraffin and cut them into 4μm-thick sections. The lesions were histologically diagnosed according to hematoxylin- and-eosin (HE) staining. All the human tissues were subjected to tissue microarray preparation. Consecutive sections were deparaffinized with xylene, rehydrated with alcohol, and subjected to immunohistochemistry as previously reported [6]. Anti-SV40 T antigen antibody was purchased from CST,

anti-PGC from Invitrogen, anti-Pdx1, anti-CK19, anti-ki67, anti-insulin, anti-CDX2, anti-villin, anti-CEA and anti-MUC1 from Abcam.

Bioinformatics analysis

The expression data (RNA-seqV2) of gastric and breast cancers and their normal tissues were downloaded from the Cancer Genome Atlas (TCGA) database by TCGA-assembler in R software. We integrated the raw data, analyzed CK19 and PGC mRNA expression.

Statistical analysis

Fisher test was used to compare positive rates, and student t test to do the means. We employed SPSS 10.0 software for data analysis. $P<0.05$ was considered as statistically significant

Results

T antigen was alternatively spliced and overexpressed in transgenic mice

Transgenic mice with CAG-loxp-LacZ-loxp T antigen were established (Fig. 1A). To activate the expression of T antigen in gastric parietal cells, chief, stem-like cells, pancreatic ductal cells, islet β-cells, and intestinal cells, as well as hepatocytes, the mice were crossed with Atp-4b-cre, PGC-cre (unpublished), Capn8-cre, K19-cre, Alb-cre, Pdx1-cre, and villin-cre mice, respectively. We employed tail DNA PCR to screen positive mice for the presence of cre and T antigen (Fig. 1B). We used target organ DNA PCR to verify the successful activation of T antigen targeting LacZ (Fig. 1C). Western blots revealed that T antigen was strongly expressed in the stomach, prestomach, duodenum, intestine, pancreas, liver, breast, lung, kidney, and metastatic cancer tissues of such transgenic mice, especially those expressing T antigen in target organs (Fig. 1D). To analyze the alternative splicing of T antigen, we designed primers targeting the T antigen intron as previously described [11]. Intron deletion was found in cDNA samples from stomach, lung, intestine, kidney, breast of PGC-cre/T antigen mouse, pancreas, stomach and duodenum of Pdx1-cre/T antigen mouse, stomach, lung and intestine of K19-cre/ T antigen mice, stomach of Atp-4b/T antigen and Capn8-cre/T antigen mice, liver of Alb-cre/ T antigen, and intestine of pvillin-cre/ T antigen mouse (Fig. 1E).

Gastric And Colorectal Neoplasia Was Induced In T-antigen Transgenic Mice

Grossly and histologically, we did not find gastric lesions in Atp4b-cre/T antigen and Capn8-cre/T antigen mice (data not shown), but we found a poorly-differentiated carcinoma in K19-cre/T antigen (37.5%, 3/8) mice at 16–19 months of age (Table 1). Immunohistochemically, positive expression of Pdx-1, Ki-67, and villin was noted in tumors (Fig. 2A). To check K19 promoter activity, we examined K19 protein and mRNA

expression in cancerous and matched normal tissues. Higher CK19 immunoreactivity was noted in gastric cancer compared to matched mucosal tissues, but a difference was not observed in K19 expression between breast cancer and matched normal tissues (Fig. 2B and 2C). In contrast, the converse was true for K19 mRNA (Fig. 2D). In villin-cre/T antigen mice, colorectal tumors and peritoneal metastatic foci were grossly observed (Fig. 3A). Primary and metastatic cancer cells showed T-antigen overexpression (Fig. 3B). Tumor incidence was 44.4% (8/18) in villin-cre/T antigen mice at 9–10 months of age (Table 2).

Table 1
The age and gender distribution of spontaneous gastric cancer in CK19-cre$^{+/-}$; JCV T antigen$^{+/-}$ mice

Number	Gender	Gastric cancer (months)
1		-
2		18
3		-
4		18
5		16
6		-
7		-
8		-

Table 2
The age and gender distribution of spontaneous colorectal cancer in villin-cre$^{+/-}$; JCV T antigen$^{+/-}$ mice

Number	Gender	Colorectal cancer (months)
1		-
2		-
3		-
4		-
5		-
6		-
7		-
8		10
9		9
10		9
11		9
12		9
13		9
14		-
15		-
16		-
17		10
18		9

Hepatocellular Carcinoma Was Induced In T-antigen Transgenic Mice

According to computed tomography (CT) scanning (Fig. 4A), we found diffuse swelling in the liver and ascites in Alb-cre/T antigen mice, but not in wild-type mice. Grossly, necrosis was found in large liver tumors (Fig. 4B). Grossly (Fig. 4C) and histologically (Fig. 4D), we also found primary hepatocellular carcinoma, and metastatic cancers in the spleen, lung and peritoneum, which showed strong T antigen expression. In hepatocellular carcinoma, AFP (α-fetoprotein), Hep-1, β-catenin, and Ki-67 proteins were

positively expressed, but negative expression of carcinoembryonic antigen (CEA) and cytokeratin (CK)19 was noted (Fig. 4E). Tumor incidence was 100.0% (13/13) in Alb-cre/T antigen mice at 3–10 months of age (Table 3).

Table 3
The age and gender distribution of spontaneous hepatocellular carcinoma in Alb-Cre$^{+/-}$; JCV T antigen$^{+/-}$ mice

Number	Gender	Hepatocellular carcinoma (months)
1		3
2		5
3		5
4		3
5		8
6		9
7		7
8		7
9		7
10		7
11		10
12		8
13		8

Pancreatic And Gastrointestinal Tumors Were Induced In T-antigen Transgenic Mice

According to CT images (Fig. 5A), pancreatic tumors with necrosis and grossly irregular tumors were observed in Pdx1-cre/T antigen mice (Fig. 5B). In each pancreatic tumor, we observed a pancreatic ductal cancer that was CEA positive (data not shown), and an (data not shown) insulinoma that was insulin positive and CEA negative (Fig. 5C). Additionally, we found adenoma in the gastric body or antrum and adenocarcinoma in the duodenum (Fig. 5C). The incidence of gastric adenoma, and pancreatic and duodenal adenocarcinoma was 100% (15/15) in Pdx1-cre/T antigen mice, and the incidence of insulinoma was 16.7% (3/18) at the age of 2–5 months (Table 4).

Table 4

The age and gender distribution of spontaneous tumors in Pdx1-Cre$^{+/-}$; JCV T antigen$^{+/-}$ mice

Number	Gender	Pancreatic cancer(months)	Insulinoma	Gastric adenoma	Duodenal adenocarcinoma
1		2	-	+	+
2		3	-	+	+
3		3	-	+	+
4		3	-	+	+
5		3	-	+	+
6		3.5	-	+	+
7		3.5	+	+	+
8		3	-	+	+
9		2.5	-	+	+
10		2	+	+	+
11		2.5	-	+	+
12		3	+	+	+
13		3	-	+	+
46		3	-	+	+
14		3	-	+	+
15		5	-	+	+

Gastric, colorectal, and breast cancers were found in PGC-positive cells of transgenic mice expressing T antigen

Histologically, we found gastric (Fig. 6A and 6B, 12.5%, 2/16) and colorectal (Fig. 6C, 6.25%, 1//16) adenocarcinomas in 17-month-old PGC-cre/T antigen mice (Table 5). Grossly, breast cancer was found in the abdominal area (Fig. 6D). Histologically, lobular carcinoma was observed in 37.5% (6/16) of PGC-cre/T antigen mice at 12–15 months of age (Fig. 6E). Immunohistochemically, the hyperexpression of T antigen, GATA-3, CA153, and β-catenin was noted, but we found no expression of estrogen receptor (ER), progesterone receptor (PR), c-erB2, ki-67, and p53 in breast cancer (Fig. 6F). PGC expression was weaker in gastric cancer than in normal mucosa at both mRNA and protein levels according to bioinformatics analysis and immunostaining, respectively. However, a difference in PGC expression between breast cancer and normal tissue at both mRNA and protein levels was not found (Fig. 6G–6I). Additionally, no

PGC I was detectable in human and bovine milk, but a comparatively high level was found in a healthy volunteer, a patient with atrophic gastritis, and in gastric cancer (Fig. 6J).

Table 5
The age and gender distribution of spontaneous tumors in PGC-Cre$^{+/-}$; JCV T antigen$^{+/-}$ mice

Number	Gender	Pancreatic cancer(months)	Gastric cancer (months)	Colorectal cancer (months)
1		-	-	-
2		-	-	-
3		-	-	-
4		-	-	-
5		-	-	-
6		-	-	-
7		-	-	-
8		12	-	-
9		11.5	-	-
10		12.5	-	-
11		12	-	-
12		15	-	-
13		15	-	-
14		-	17	-
15		-	17	-
16		-	-	17

Discussion

Cellular malignant transformation requires a series of genetic or epigenetic accumulations, including oncogene activation or overexpression. Although JCV is a highly neurotropic virus that induces brain tumors, JCV DNA was found in respiratory and gastrointestinal tracts, and even in tonsil B lymphocytes and renal tubules due to JCV persistence [6, 7]. Therefore, establishing spontaneous tumors in transgenic mice expressing JCV T antigen in a tissue-specific manner is essential and helpful for understanding the oncogenic role of JCV in epithelial carcinogenesis. In the present study, we generated CAG-LacZ-T antigen

loxp/loxp mice to cross with various cre tool mice, in which cre is expressed in gastric pit, chief, parietal, intestinal and pancreatic cells, islet β cells, or hepatocytes respectively.

The K19 promoter was used to overexpress cyclooxygenase-2 and mPGES-1 in transgenic mice, which had gastric dysplasia [13]. SV40 T antigen was expressed under CEA promoter guidance to induce gastric pyloric multi-focal cancers,14 and under the control of the Atp4b promoter to induce gastric neuroendocrine-like cancer due to the transdifferentiation of parietal cell progenitors [15]. Synergistic abrogation of both E-cadherin and p53 in gastric parietal cells resulted in a metastatic diffuse-type gastric cancer using Atp4b-cre mice [16], while rapid tumorigenesis and progression from adenoma to invasive intestinal-type gastric carcinoma was observed in the conditional knockout of Smad4 and phosphatase and tensin homolog (PTEN) in gastric Lgr5-positive stem cells [17]. A villin promoter was also employed to conditionally knock out PPARD, and 38% of mice developed spontaneous invasive gastric adenocarcinomas [18]. Here, we found no gastric carcinogenesis in transgenic mice expressing T antigen in pit and parietal cells, which was intriguing but confusing, while we observed gastric adenocarcinomas in K19- or PGC-cre/T antigen mice, and gastric adenoma in Pdx1-cre/T antigen mice. However, we cannot elucidate the cell population responsible for gastric carcinogenesis among Pdx1-positive cells. Strangely, lung adenoma and adenocarcinoma, but not gastric neoplasms, were detected in K19-T antigen mice in our previous report.11 Additionally, K19-cre/PTEN f/f mice developed breast cancer, but not gastric cancer [19]. We also found no difference in K19 mRNA expression between gastric mucosa and cancer tissues, but higher expression in breast cancer than in normal tissue. As a result, a change in K19 promoter activity during carcinogenesis might account for its tissue specificity and for its genetically spontaneous carcinogenesis.

Colorectal adenomas were present in conditional Fbxw7fl(R482Q)/+ mice using villin-cre mice [20]. Spontaneous duodenal dysplasia and intestinal tumors were developed in ApcMin/+TLR4-/- mice [21]. Intestinal adenoma and adenocarcinoma was detectable in Apc(1638N)/ Rb(tm2brn)/ villin-cre [22] and Apc(1638N/wt); villin-cre; Tgfbr2 f/f mice [23]. Kane et al. [24] observed the transition of colorectal sessile serrated lesions to cancer in BRAFV600E/villin-cre mice. Czéh et al. [25] found that vill-cre×LoxP-SV40 T antigen mice developed intestinal and colorectal adenocarcinomas at 6 months of age. Intestinal adenomas or adenocarcinomas were generated in villin-cre+/JNK1 f/f, Prss8 f/f, PTEN f/f, and Msh2 f/f mice, but spontaneous serrated lesions, goblet cell hyperplasia, low-grade and high-grade dysplasia, and colorectal mucinous adenocarcinomas were seen in villin-cre/Notch-1 f/f mice [26–30]. Meta-analysis indicated that the presence of JCV in colorectal tissues increased the odds of colorectal cancer 4.70 times as much as for normal mucosa [31]. Here, we first established spontaneous intestinal adenocarcinomas in villin-cre/T antigen and PGC-cre/T antigen mice, indicating the oncogenic role of T antigen in colorectal carcinogenesis.

Ochiai et al [32]. found spontaneous hepatocellular carcinogenesis in Alb-cre/LSL- KrasG12D mice. Visible liver tumor nodules in miR-122a-/-; PTEN+/- and Mir122a-/-; Alb-cre; PTEN f/+ mice at 6 months of age [33]. Tsc1f/f; Alb-cre, PTEN f/f; Alb-cre, and Tsc1f/f; PTEN f/f; Alb-cre mice developed liver tumors [34]. In Tsc1f/f; Alb-cre mice, the onset of liver tumors was later than in the other strains and these were

predominantly hepatocellular carcinomas (HCC). PTEN f/f; Alb-cre mice suffered from intrahepatic cholangiocarcinomas. Livers of Tsc1f/f; PTEN f/f; Alb-cre mice had a large number of tumors with histologically-mixed architecture. Sekine et al. [35] found that Alb-cre; Ctnnb1f/f mice gained efficient deletions of β-catenin in hepatocytes at 2 months, but the reappearance and expansion of β-catenin-positive hepatocytes were seen with aging. In 12-month-old mice, pericentral hepatocytes were proportionally replaced with β-catenin–expressing hepatocytes, whereas most periportal hepatocytes appeared negative. Most 1-year-old mice spontaneously developed β-catenin–positive hepatocellular adenomas and carcinomas. In Alb-cre/T antigen mice, we found HCC and peritoneal spreading as evidenced by ascites, CT scanning and HE staining. Strong T antigen expression was found in HCC, supporting the hypothesis that T antigen protein played an oncogenic role in hepatocellular carcinogenesis. Although T antigen expression was gradually decreased, the speed of HCC onset was faster than the weakening speed of Alb promoter activity during hepatocellular carcinogenesis.

Feldmann et al. [36] generated Pdx1-cre; Brca2 f/f and Pdx1-cre; Brca2 f/f; LSL-Trp53 mice, and found pancreatic intraepithelial neoplasia. Pancreas-specific KrasG12D mice sufficiently developed pancreatic intraepithelial neoplasia using a Pdx1 promoter, and active Akt1 cooperated with KrasG12D to accelerate pancreatic carcinoma onset and progression [37, 38]. Waldmann et al. [39] observed pancreatic intraepithelial neoplasia and invasive carcinoma in LsL-KrasG12D; LsL-Trp53R172H; Pdx1-cre, and LsL-KrasG12D; Pdx1-cre mice. Al et al. [40] demonstrated that activated Kras and TP53INP1 loss accelerated pancreatic intraepithelial neoplasias and intraductal papillary mucinous neoplasms in Kras-INP1 KO mice. Reportedly, pancreatic ductal adenocarcinomas in Pdx1-cre; KrasG12D; PTEN f/+ mice, Pdx1-cre; PTEN f/f mice, and aged Pdx1-cre; LSL-KrasG12D; Tif1γ f/f mice [41–43]. Qiu et al. [44] observed the progression from primary pancreatic tumors to metastases in p16 f/f; LSL- KrasG12D; Pdx1-cre mice. Here, we also employed Pdx1-cre to activate T antigen and observed pancreatic ductal carcinoma and insulinoma. Pdx1 protein was widely expressed in gastric epithelial and intestinal mucosa, so that Pdx1-cre/T antigen also developed gastric adenoma and duodenal adenocarcinoma. However, it is very difficult to speculate which cells are responsible for tumor histogenesis due to a lack of specificity of the Pdx1 promoter.

Li et al. [45] used a mouse mammary tumor virus LTR (long tandem repeats) enhancer to establish Wnt-1 transgenic mice, and found beast ductal hyperplasia and adenocarcinoma by 6 months of age. Loss of a transforming growth factor (TGF)-β response increased the risk of spontaneous breast tumorigenesis [46]. Mammary tumors arose in 30% of mouse mammary tumor virus (MMTV)-PIK3CA-H1047R mice and 13% of MMTV-PIK3CA-E545K mice [47]. Guillory et al. [48] build up a breast cancer model of a polyomavirus middle T antigen transgenic mouse model. Tzeng et al. [49] established whey acidic protein-SV-T antigen transgenic mice, which developed mammary carcinomas with high frequency. It was of interest to note that the activation of T antigen in PGC-positive cells will cause breast triple-negative lobular carcinoma in female transgenic mice, in line with PGC-cre/PTEN f/f (unpublished) and K19-cre/PTEN f/f mice [25], but gastric cancer in male mice. Therefore, we speculated that the serum estrogen level, or the proliferation of lobular glands during breeding might contribute to breast carcinogenesis in PGC-cre/T antigen transgenic mice.

Hachana et al. [50] detected JCV T antigen DNA in invasive ductal carcinomas (28/112) but not in five invasive lobular carcinomas or six medullary carcinomas. However, we found breast triple-negative lobular adenocarcinoma in PGC-cre/T antigen mice although PGC was mainly expressed in gastric chief cells. Therefore, we believe that the PGC promoter is specific for the breast lobule, and any genetic alteration in the breast lobule might result in breast cancer. According to our knowledge, it is reasonable to speculate that a mother's breast might functionally substitute for an infant's stomach due to the following causes: (1) breast milk and gastric digestion supply nutrients for the human body; (2) no PGC production and similar pH value (6.84) in breast milk and infant serum; (3) similar anatomic structure between breast and stomach: myocytes are present around glands in spite of different germ layer origins (ectoderm vs. endoderm); (4) both sucking and digestive secreting reflexes include neuroendocrine regulation; (5) hereditary diffuse gastric cancer carries a *CDH1* mutation, and displays gastric and breast cancer [51].

If a virus has an oncogenic role, it must infect cells and subsequently encode oncogenic proteins to replicate DNA, disrupt cell function, and finally to induce carcinogenesis. According to our findings, we found JCV copies that differed according to tissue type (stomach < lung < breast < liver < pancreas) since the distinct distribution of its receptors (α 2, 6-linked sialic acid, non-sialylated glycosaminoglycans and serotonin) determined its different infection capabilities [52–54]. Nukuzuma et al. [55] found a suppressive effect of the topoisomerase I inhibitors, topotecan and β-lapachone, on JCV propagation in human neuroblastoma cells. The adipocyte plasma membrane protein, phosphoinositide 4 kinase γ, and its regulatory subunit, PIK3R5, promoted JCV infection in human glial cells [56, 57]. Uleri et al. [58] have shown that splicing factor 2/alternative splicing factor (SF2/ASF), negatively regulated transcription and splicing of JCV genes via direct interaction with the viral promoter in glial cells. SF2/ASF hyperexpression induced growth and proliferation of JCV-transformed tumor cells, and either endogenous or ectopic LIP (liver-inhibitory isoform) expression mediated the degradation of T antigen in a JCV-transgenic mouse tumor cell line [59]. Transcriptionally, we found that alternative splicing of the T antigen intron did not show tissue specificity in stomach, pancreas, liver and intestine, in line with our previous study.11 Transmembrane receptors, cytoplasmic signal proteins, and T antigen protein status might result in the tissue specificity of its oncogenesis in JCV-related cancer.

In conclusion, JCV T antigen might induce gastrointestinal tumorigenesis with cell specificity, which is not linked to alternative splicing of the T antigen intron. The combination of T antigen, exposure to cyclic estrogen or the proliferation of lobular glands during breeding might initiate breast lobular adenocarcinogenesis in PGC-positive cells. These spontaneous tumor models are good cancer models that allow investigation of the oncogenic role of JCV T antigen and novel therapeutic approaches to cancer.

Declarations

Acknowledgement

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Availability of data and materials

There is no data needed to be deposited. The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval

This study was approved by the Ethics committee of Chengde Medical University Affiliated Hospital. All methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable

Consent to participate statement

Informed consent was obtained from all subjects and/or their legal guardian(s).

Author contribution

HZ designed experiments. HX and HZ analyzed the data, interpreted the results, and wrote the manuscript. HX and YE performed the experiments and analyzed the data. ZC made suggestions during the writing. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no potential conflicts of interest.

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Figures

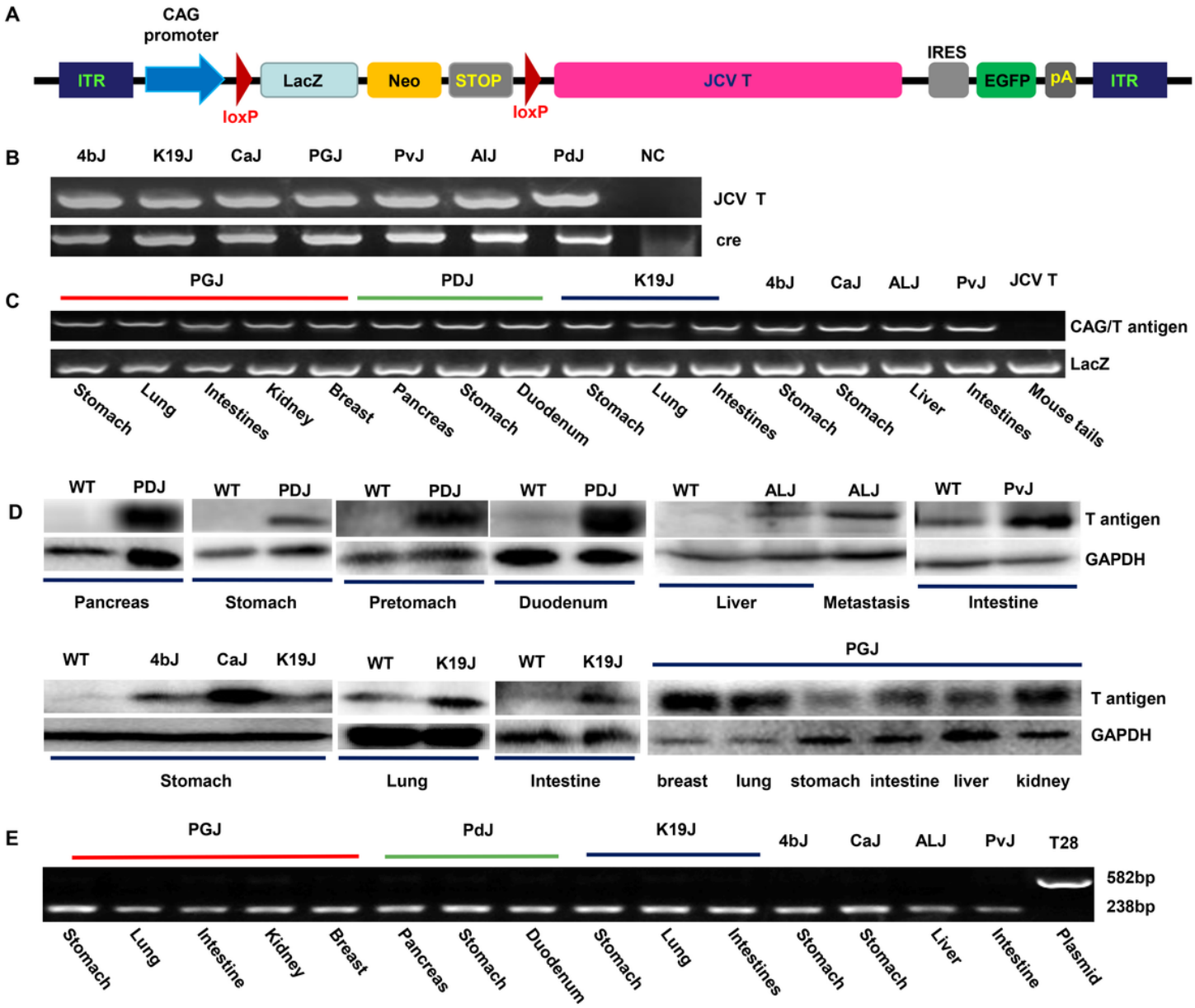
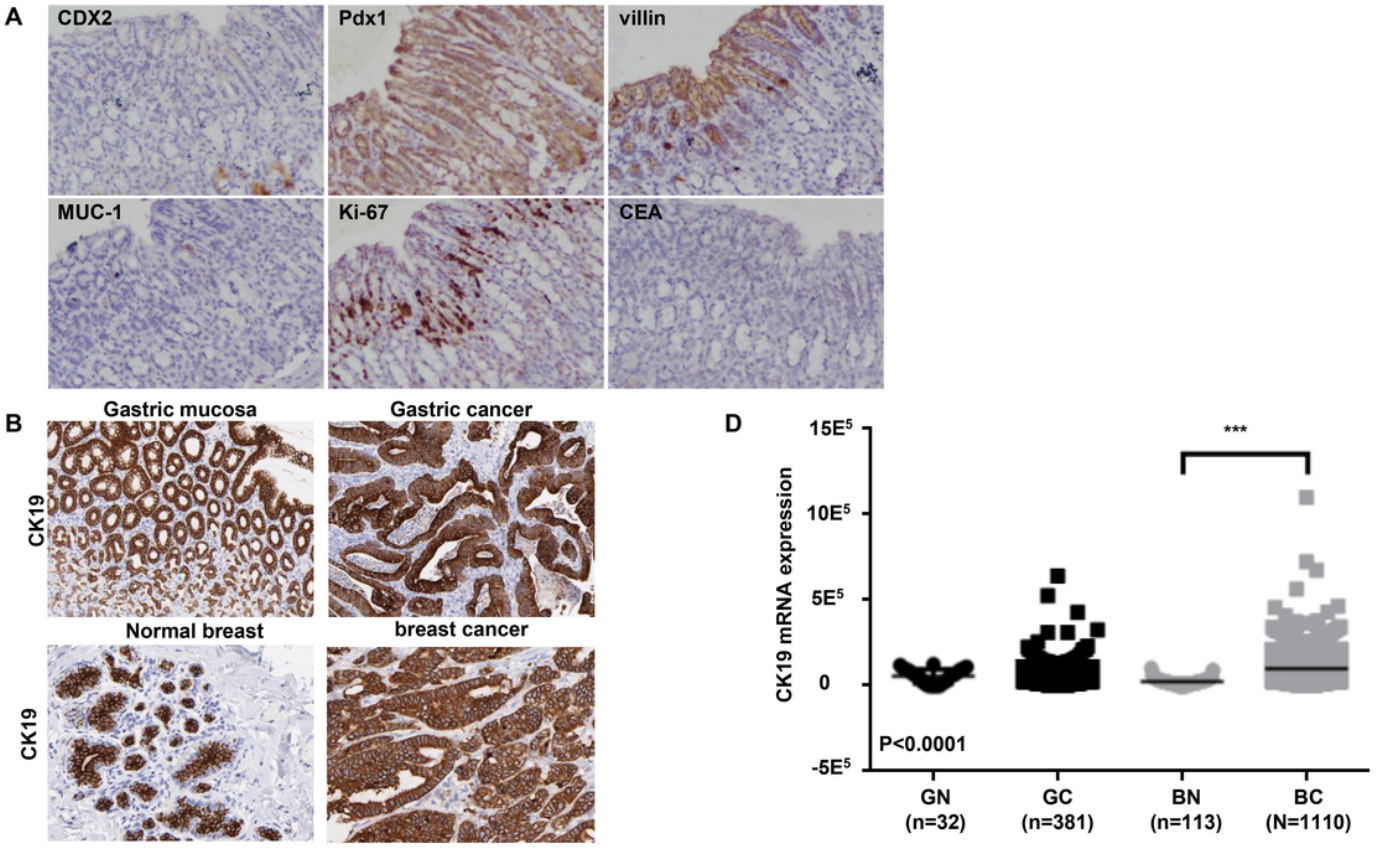


Figure 1

The generation and check of JCV T antigen transgenic mice

CAG-loxp-Laz-loxp-T antigen transgenic mice were established according to schematic diagram using CAG promoter. To activate its tissue-specific expression, the mice were crossed with Atp-4b-cre (gastric parietal cell), PGC-cre (gastric chief cell), Capn8-cre (gastric pit cell), K19-cre (stem-like cell), villin-cre (intestinal epithelial cells), Alb-cre (hepatocyte), and Pdx1-cre (pancreas and gastrointestine) mice (A). These positive mice showed cre+/T antigen+ by tail DNA PCR (B). To verify the successful knockout of LaZ, we perform PCR of objective organs' DNA targeting LacZ and CAG/T antigen (C). T antigen expression was examined in target organs of transgenic mice by Western blot (D). Finally, RT-PCR was employed to detect the alternative splicing of T antigen targeting intron using target organs' mRNA of

transgenic mice (E). ITR, inverted terminal repeat; IRES, internalribosome entrysite; 4bJ, Atp-4b-cre/ JCV T antigen; K19J, K19-cre/ JCV T antigen; CaJ, Capn8-cre/ JCV T antigen; PGJ, PGC-cre/ JCV T antigen; PvJ, pvillin-cre/ JCV T antigen; AlJ, Alb-cre/ JCV T antigen; PdJ, Pdx1-cre/ JCV T antigen; WT, wild-type; T28, PBS-T antigen plasmid; NC, negative control



C

Type	n	Stomach		n	Breast	
		negative	positive		negative	positive
Normal	48	14(29.2%)	34(70.8%)	43	0(0.0%)	43(100%)
Cancer	217	1(0.5%)	216(99.5%)**	94	1(1.1%)	93(98.9%)

Figure 2

JCV T antigen can induce gastric carcinogenesis.

Gastric poorly-differentiated carcinoma from transgenic mouse of K19-cre/JCV T antigen (,18months) was subjected to immunohistochemistry of CDX2, Pdx1, villin, MUC-1, ki-67 and CEA (A). CK19 expression was examined in gastric and breast cancers, and matched normal glands (B), which was summarized in Table (C). TCGA database was employed to analyze CK19 mRNA expression in gastric and breast cancers, and their corresponding normal tissues (D). GN, gastric normal tissue; GC, gastric cancer; BN, breast normal tissue; BC, breast cancer.

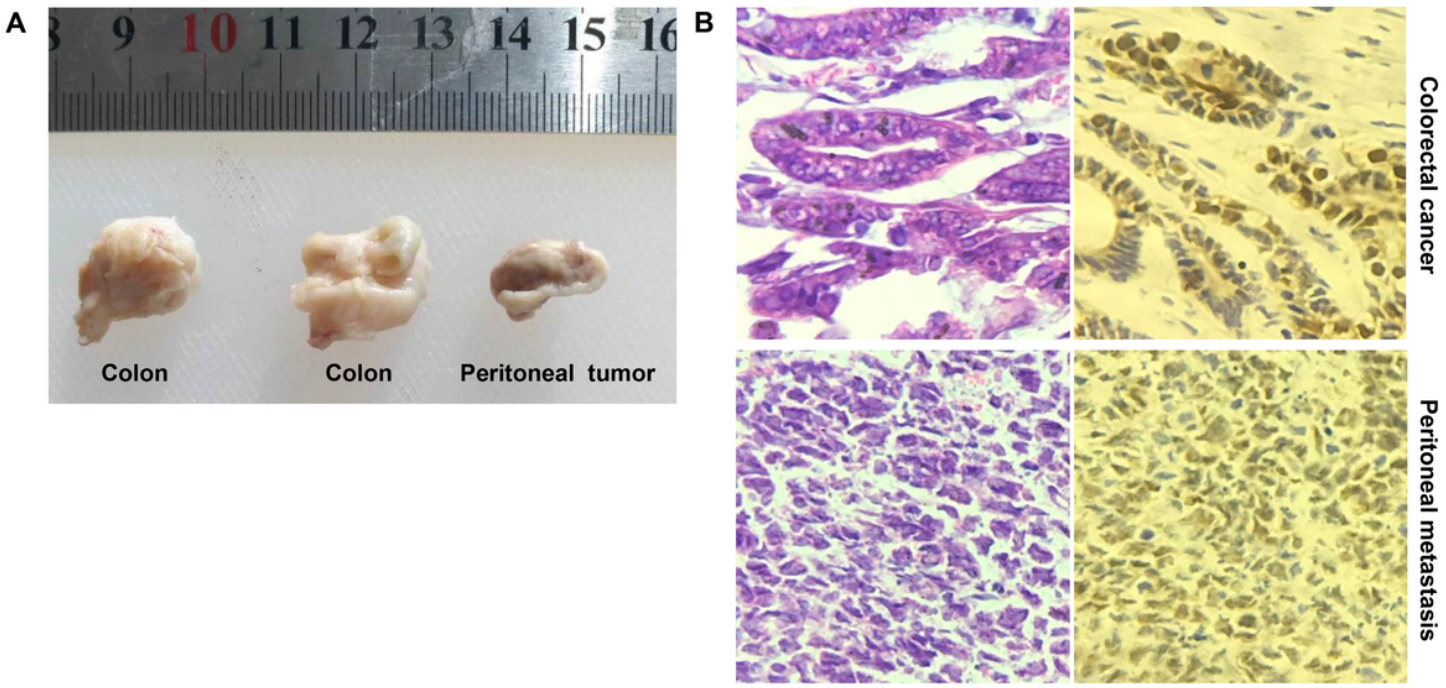


Figure 3

JCV T antigen was involved in colorectal carcinogenesis and progression.

Colorectal cancer and peritoneal metastasis was observed transgenic mouse of villin-cre/JCV t antigen(,10months) both grossly (A) and using HE staining and immunostaining of T antigen (B).

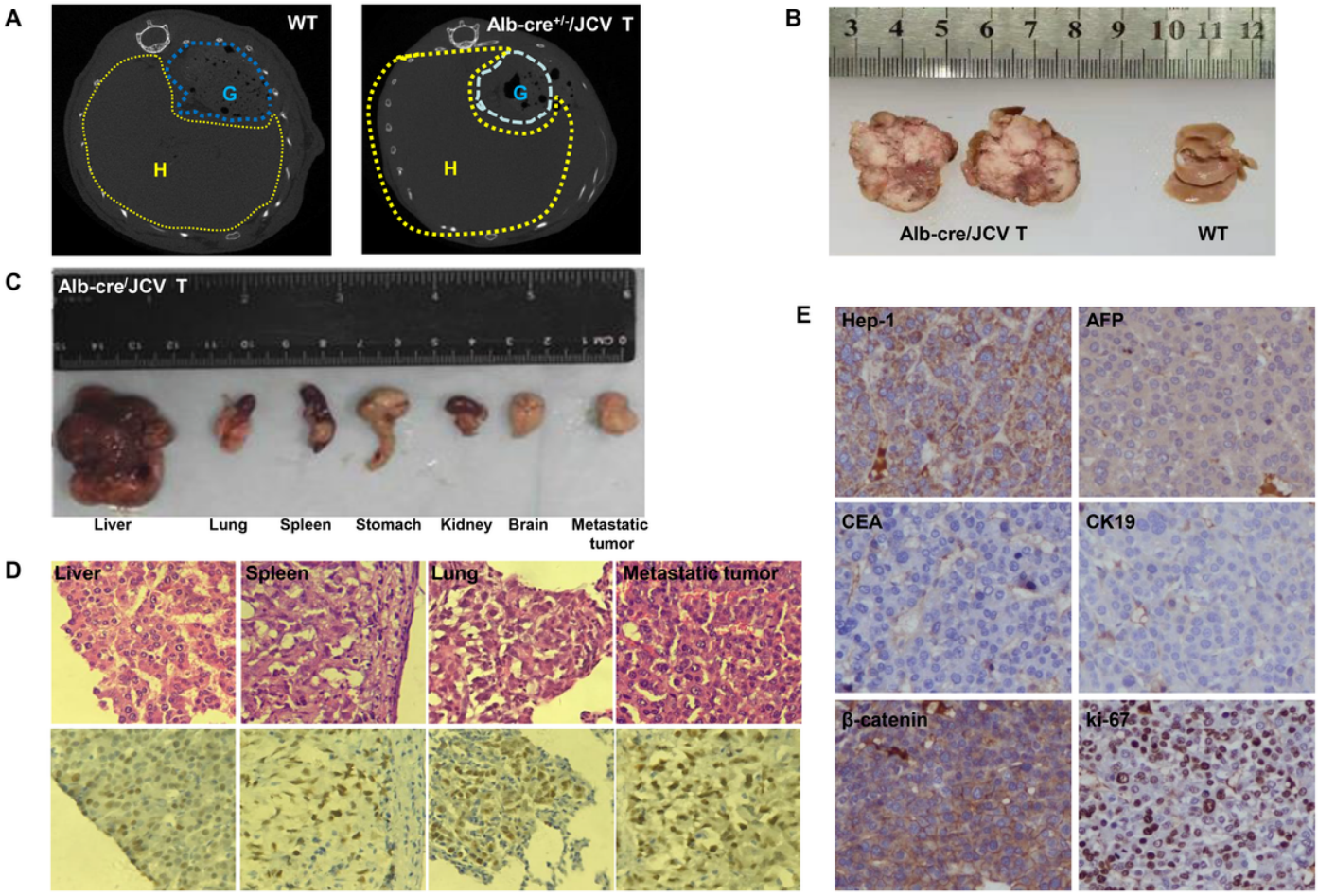


Figure 4

JCV T antigen mediates hepatocellular carcinogenesis and progression.

The livers from albumin-cre/JCV T antigen (,7months) and wild-type mice were examined by CT scanning (A) and grossly(B). The liver, lung, spleen, stomach and kidney, brain and metastatic tumor were dissected from Alb-cre/T antigen mice (C). We found primary hepatocellular carcinoma and metastatic carcinoma into spleen, lung and peritoneum according HE staining and immunostaining of T antigen (D). Hepatocellular carcinoma displayed the positive expression of Hep-1, AFP, β-catenin and ki-67, but negative expression of CEA and CK19 (E). WT, wild-type.

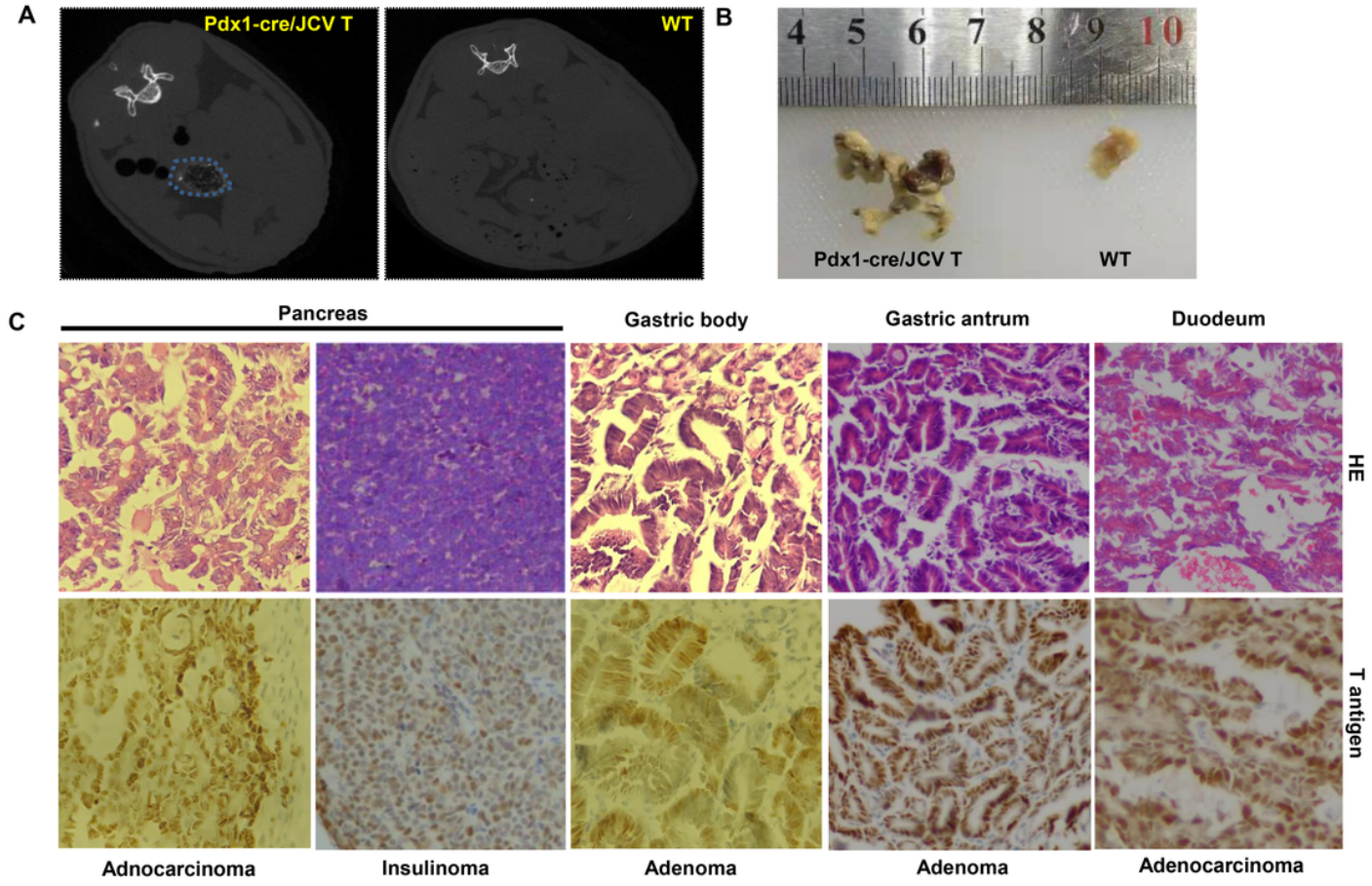
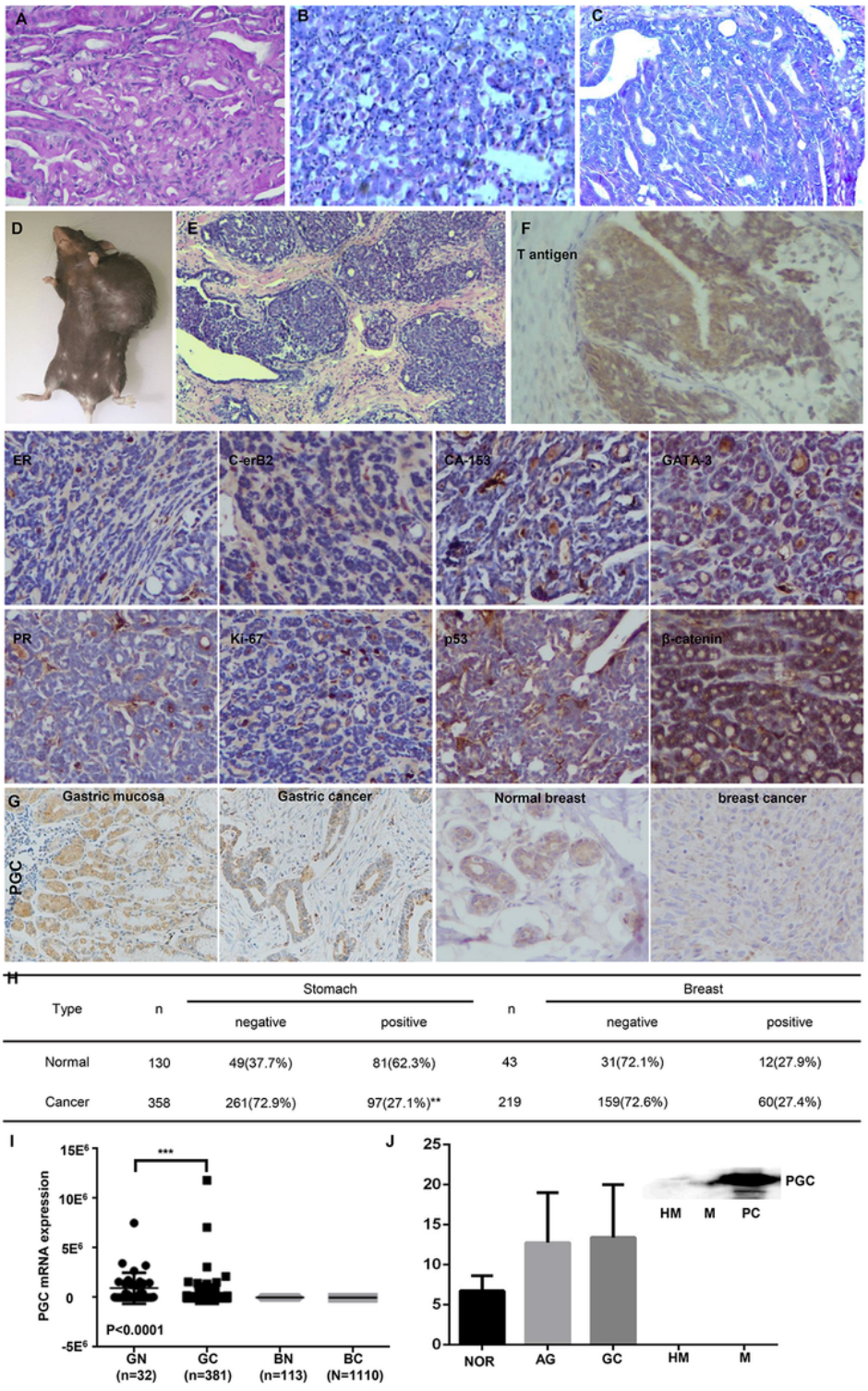


Figure 5

Multiple tumorigenesis was detectable in Pdx1-cre/JCV T antigen mice

According to CT scanning (A) and gross appearance(B), irregular pancreatic tumor was found in Pdx1-cre/T antigen transgenic mouse(,3 months), compared with wild-type mice. We found pancreatic ductal carcinoma and insulinoma, adenoma in gastric body and antrum, and duodenal adenocarcinoma in according to HE staining and immunostaining (C). WT, wild-type.



Type	n	Stomach		n	Breast	
		negative	positive		negative	positive
Normal	130	49(37.7%)	81(62.3%)	43	31(72.1%)	12(27.9%)
Cancer	358	261(72.9%)	97(27.1%)**	219	159(72.6%)	60(27.4%)

Figure 6

Multiple tumorigenesis was found in PGC-cre/JCV T antigen mice

Gastric cancer (A, ,12months; B, ,17 months) was histologically found in the PGC- cre/JCV T antigen transgenic mouse. Colorectal well-differentiated adenocarcinoma(C, ,17 months) was also seen in the kind of transgenic mouse. Breast tumor was grossly observed in the belly of PGC-cre/JCV T antigen

transgenic mouse (12months, D). According to HE staining (E), lobular carcinoma was diagnosed with the positive expression of T antigen, GATA-3, CA153 and β-catenin (F). PGC expression was immunohistochemically examined in gastric and breast cancers, and matched normal glands (G), which was summarized in Table (H). TCGA database was employed to analyze PGC mRNA expression in gastric and breast cancer, and their corresponding normal tissues (I). Finally, PGC content was determined by ELISA or Western blot in the serum of the healthy individual, atrophic gastritis, and gastric cancer, human and bovine milk (J). GN, gastric normal tissue; GC, gastric cancer; BN, breast normal tissue; BC, breast cancer; NOR, healthy individual; AG, atrophic gastritis; HM, human milk; M, milk powder; PC, positive control.