Histol Histopathol (2013) 28: 531-542
DOI: 10.14670/HH-28.531

http://www.hh.um.es

Histology and Histopathology
Cellular and Molecular Biology

Immunohistochemical evidence for an impairment of autophagy in tumorigenesis of gastric carcinoids and adenocarcinomas in rodent models and patients

Reidar Alexander Vigen[1], Yosuke Kodama[1], Trond Viset[2], Reidar Fossmark[1,3], Helge Waldum[1,3], Mark Kidd[4], Timothy C. Wang[5], Irvin M. Modlin[4], Duan Chen[1] and Chun-Mei Zhao[1]

[1]Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology, Trondheim, Norway, Departments of [2]Pathology and [3]Medical Genetics, and Gastroenterology and Hepatology, St. Olav's University Hospital, Trondheim, Norway, [4]Gastrointestinal Surgical Pathobiology, Yale University School of Medicine, New Haven, USA and [5]Department of Medicine and Irving Cancer Research Center, Columbia University Medical Center, New York, USA

Summary. Background/Aim: Autophagy has dual roles in tumorigenesis: tumor-promoting or tumor-suppressing. The aim of the present study was to examine autophagy-related markers by immunohistochemistry in gastric carcinoids and adenocarcinomas in rodent models and patients.

Methods: Gastric carcinoids in *Mastomys* were induced by loxtidine treatment. Spontaneously developed gastric adenocarcinomas in Japanese cotton rats and INS-GAS transgenic mice were included. Patient tissue samples of gastric carcinoids or adenocarcinomas were collected. Immunohistochemistry was performed against autophagy-related gene protein-6 (ATG-6, also called beclin-1), ATG-5 and ATG-16.

Results: In tumor-free *Mastomys*, ATG-5, ATG-16 and beclin-1 were immunepositive in the gastric mucosa. In tumor-bearing *Mastomys*, ATG-5 and ATG-16 were negative in the tumors, whereas beclin-1 was positive in four of five animals. In carcinoid patients, ATG-5 was negative in six of ten, ATG-16 negative in nine of ten, and beclin-1 negative in three of ten patients. In cotton rats, ATG-5 and ATG-16 were negative in all tumors. Beclin-1 was negative in three of five rats. In INS-GAS mice, ATG-5 and beclin-1 were positive in the tumor area, but the numbers of immunopositive cells per gland were reduced by about 50% in comparison with wild-type mice. In adenocarcinoma patients, ATG-5 and ATG-16 were negative in eight of ten, and beclin-1 positive in all ten patients.

Conclusions: An impaired autophagy took place at the stage of formation of ATG-5-ATG-12-ATG-16 complex in both gastric carcinoids and adenocarcinoma of both rodent models and patients. ATG-5 and ATG-16 might be better markers than beclin-1 in assessing autophagy in these lesions.

Key words: Cotton rats, *Mastomys*, INS-GAS mice, patients

Offprint requests to: Dr. Chun-Mei Zhao, Department of Cancer Research and Molecular Medicine, Norwegian University of Science and Technology (NTNU), Erling Skjalgssons Gate 1, NO-7006, Trondheim, Norway. e-mail: chun-mei.zhao@ntnu.no

Introduction

Programmed cell death plays important roles in the preservation of tissue homeostasis and elimination of damaged and harmful cells. Dysfunction of programmed cell death leads to various diseases, including cancers. There are three major cell death mechanisms: apoptosis, autophagic cell death, and necrosis (Clarke, 2002; Gozuacik and Kimchi, 2007). The role of apoptosis in tumorigenesis has been much focused, but the role of autophagy in tumorigenesis as well as its potential in anti-cancer targeted therapy has gained more attention during the last few years.

It has been shown that autophagy plays a dual role in tumorigenesis, i.e. either having tumor-promoting or tumor-suppressing properties, leading to different phenotypes of the tumor cells, which are dependent on genetic composition of the tumor cells, tumor types and stages, and tumor microenvironment (Apel et al., 2009; Eisenberg-Lerner and Kimchi, 2009; Wilkinson and Ryan, 2010). Very recently, we reported an ultrastructural study of *Mastomys* model of gastric carcinoid (ECL cell tumor, or ECLoma), and suggested that a combination of genetic predisposition and long-term hypergastrinemia leads to the initiation of gastric

carcinoids and then impaired formation of vacuoles and lipofuscin bodies (markers of the autophagic pathway), and loss of gastrin control advances the tumorigenesis of these lesions (Vigen et al., 2012). In the present study, we further tested this hypothesis by means of immunohistochemistry using well-recognized biomarkers of autophagy to examine the tumorigenesis of the carcinoids in the *Mastomys* model and patients with gastric carcinoids and chronic atrophic gastritis. Autophagy-related gene protein-6 (ATG-6, also called beclin-1) was chosen as it is a widely referred to initiator of autophagy, and ATG-5 and ATG-16 were included as later downstream regulatory proteins.

Another hypergastrinemia-related model of gastric tumorigenesis is Japanese cotton rats, which display an adenocarcinoma phenotype (Martinsen et al., 2003; Fossmark et al., 2004a,b, 2005). Previously, we reported that ECL cells in cotton rats progressively underwent transformation with loss of secretory organelles, and dedifferentiation (Fossmark et al., 2005). In the present study, we further tested the hypothesis of impaired autophagy in these cotton rats as well as in patients diagnosed with gastric adenocarcinomas. In addition, we also included the "INS-GAS" mouse model of gastric adenocarcinoma in the present study. This is a transgenic mouse with a human gastrin minigene slicing onto the insulin promoter, and the gastric tumor can occur spontaneously (Wang et al., 2000).

Materials and methods

Mastomys

Twelve *Mastomys* (female, 15 months of age) were included; 6 animals receiving loxtidine (an irreversible histamine H2 blocker) at a dose of 1mg/kg/day in drinking water for 27 weeks, and 6 animals receiving tap water as controls (for details, see (Vigen et al., 2012) (Table 1). After the animals were euthanized, tissue specimens were obtained from the oxyntic gland area and subjected to a routine histological sample preparation procedure.

Cotton rats

Twenty cotton rats (female, 4-10 months of age) with 5 animals in each group were included: animals with hypergastrinemia for 2 months and age-matched normogastrinemic controls; and animals with hypergastrinemia lasting for 8 months and age-matched normogastrinemic controls (for details, see (Fossmark et al., 2005) (Table 2). The same tissue samples were further subjected to analysis by immunohistochemistry (see below).

INS-GAS mice

Six INS-GAS and three wild-type (WT) mice (male, 12 months of age) were included. The animal housing condition was SPF (specific-pathogen-free) and maintained according to the standard recommended by FELASA (Federation of European Laboratory Animal Science Association). Tap water was used throughout, and standard mouse/rat pellet food was supplied by Scanbur BK AS (Sweden). The animals received no treatment.

The study was approved by the Norwegian National Animal Research Authority (Forsøksdyrutvalget, FDU).

Patients

Twenty gastric tumors were included; ten gastric carcinoids type 1 (in patients with chronic atrophic gastritis) and 10 gastric adenocarcinomas of intestinal type (Table 3). Tissue sections were obtained from the Department of Pathology and Medical Genetics at St.

Table 1. Results of immunostaining for ATG-5, ATG-16 and beclin-1 in *Mastomys* that were treated with loxtidine for 27 weeks.

Mastomys	Age(month)	Sex	Diagnosis	ATG-5	ATG-16	Beclin-1
1	15	F	Normal	Positive	Positive	Positive
2	15	F	Normal	Positive	Positive	Positive
3	15	F	Normal	Positive	Positive	Positive
4	15	F	Normal	Positive	Positive	Positive
5	15	F	Normal	Positive	Positive	Positive
6	15	F	Normal	Positive	Positive	Positive
7	15	F	Carcinoid	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor
8	15	F	Carcinoid	Negative in tumor	Negative in tumor	Positive in small area of tumor
9	15	F	Carcinoid	Negative in tumor	Negative in tumor	Positive in small area of tumor
10	15	F	Carcinoid	Negative in tumor	Negative in tumor	Positive in small area of tumor
11	15	F	Carcinoid	Positive in normal tissue Negative in tumor	Negative in tumor	Positive in tumor
12	15	F	Carcinoid	Negative in tumor	Negative in tumor	Positive in small area of tumor

Olavs' University Hospital (Trondheim, Norway) and subjected to analysis by immunohistochemistry (see below). Approval for the use of human samples and obtaining data from patient journals was given by the Regional Ethical Committee.

Immunohistochemistry

The tissue samples were collected, fixed in 4% formaldehyde and embedded in paraffin. The 4 μm thick sections for immunohistochemistry were deparaffinised, rehydrated with water and treated with 3% H_2O_2 to quench endogenous peroxidase, washed several times with buffer, and then incubated with primary antisera for 24 h at 4°C after antigen retrival, i.e., ATG-5 (at working dilution of 1:200) (code AP1812a, Nordic BioSite, Norway), ATG-16 (1:75) (code AP1817b, Nordic BioSite), and beclin-1 (0.5 μg/ml) (code APS-3613, Nordic BioSite). Afterwards, the sections were incubated in biotinylated anti-rabbit IgG for 1 h (Envision Kit K4007, Dako, Denmark). Following several washes in Tris-buffered saline, sections were incubated in 0.05% diaminobenzidine/0.001% H_2O_2 solution and washed at least 2 times with Tris-buffered saline. Sections were counterstained, dehydrated with absolute alcohol followed by xylene, cover-slipped with Dako Mounting Medium, and examined under a microscope (Nikon Eclipse E600). Evaluation was performed together with a consultant pathologist (TV) who was blinded to the samples. Positive staining was defined as most cells

Table 2. Results of immunostaining for ATG-5, ATG-16 and beclin-1 in cotton rats with or without hypergastrinemia which was defined as the serum gastrin concentration being more than 400 pg/ml in freely fed cotton rats.

Cotton rats	Age	Sex	Hyper-gastrinemia	Diagnosis	ATG-5	ATG-16	Beclin-1
1	2M	F	No	Normal	Positive	Positive	Positive
2	2M	F	No	Normal	Positive	Positive	Positive
3	2M	F	No	Normal	Positive	Positive	Positive
4	2M	F	No	Normal	Positive	Positive	Positive
5	2M	F	No	Normal	Positive	Positive	Positive
6	2M	F	Yes	Hyperplasia	Positive	Positive	Positive
7	2M	F	Yes	Hyperplasia Dysplasia	Positive Negative	Positive Negative	Positive Negative
8	2M	F	Yes	Hyperplasia	Positive	Positive	Positive
9	2M	F	Yes	Hyperplasia	Positive	Positive	Positive
10	2M	F	Yes	Hyperplasia	Positive	Positive	Positive
11	8M	F	No	Normal	Positive	Positive	Positive
12	8M	F	No	Normal	Positive	Positive	Positive
13	8M	F	No	Normal	Positive	Positive	Positive
14	8M	F	No	Normal	Positive	Positive	Positive
15	8M	F	No	Normal	Positive	Positive	Positive
16	8M	F	Yes	Adenocarcinoma	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor
17	8M	F	Yes	Adenocarcinoma	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor
18	8M	F	Yes	Adenocarcinoma	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue and small area of tumor Negative in most area of tumor tissue
19	8M	F	Yes	Adenocarcinoma	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue and weak positive in small area of tumor Negative in most areas of tumor
20	8M	F	Yes	Adenocarcinoma	Negative in tumor Positive in small area of tumor	Positive in normal tissue Negative in tumor	Positive in normal tissue Negative in tumor

Table 3. Results of immunostaining for ATG-5 and beclin-1 in wild-type and INS-GAS mice.

	ATG-5 (number/gland)	Beclin-1 (number/gland)
Wild-type mice (3 mice, 8-12 gland/mouse)	22.1±1.0	21.8±0.9
INS-GAS mice (6 mice, 8-17 gland/mouse)	11.2±1.3**	13.7±1.6**

Means±SEM. ** $p<0.01$ (Student's t test, two tailed)

being immunoreactive, whereas negative staining was defined as no immunoreactive cells, and was evaluated both in the tumor area and normal tissue. In INS-GAS and wild-type (WT) mice, 8-17 glands/mouse and 8-12 glands/mouse were randomly selected, respectively, for counting the numbers of immunoreactive cells per gland.

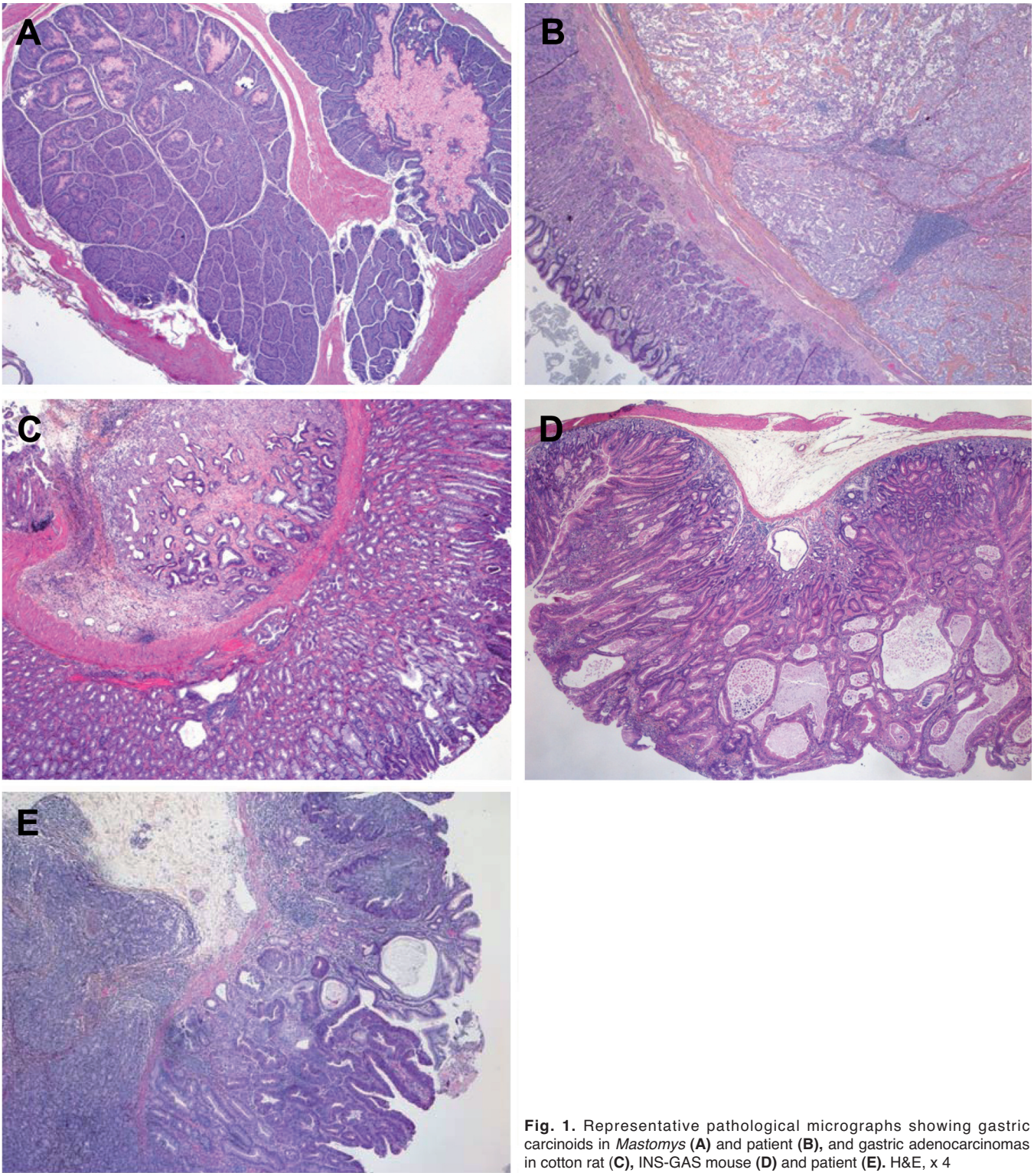

Fig. 1. Representative pathological micrographs showing gastric carcinoids in *Mastomys* **(A)** and patient **(B),** and gastric adenocarcinomas in cotton rat **(C),** INS-GAS mouse **(D)** and patient **(E).** H&E, x 4

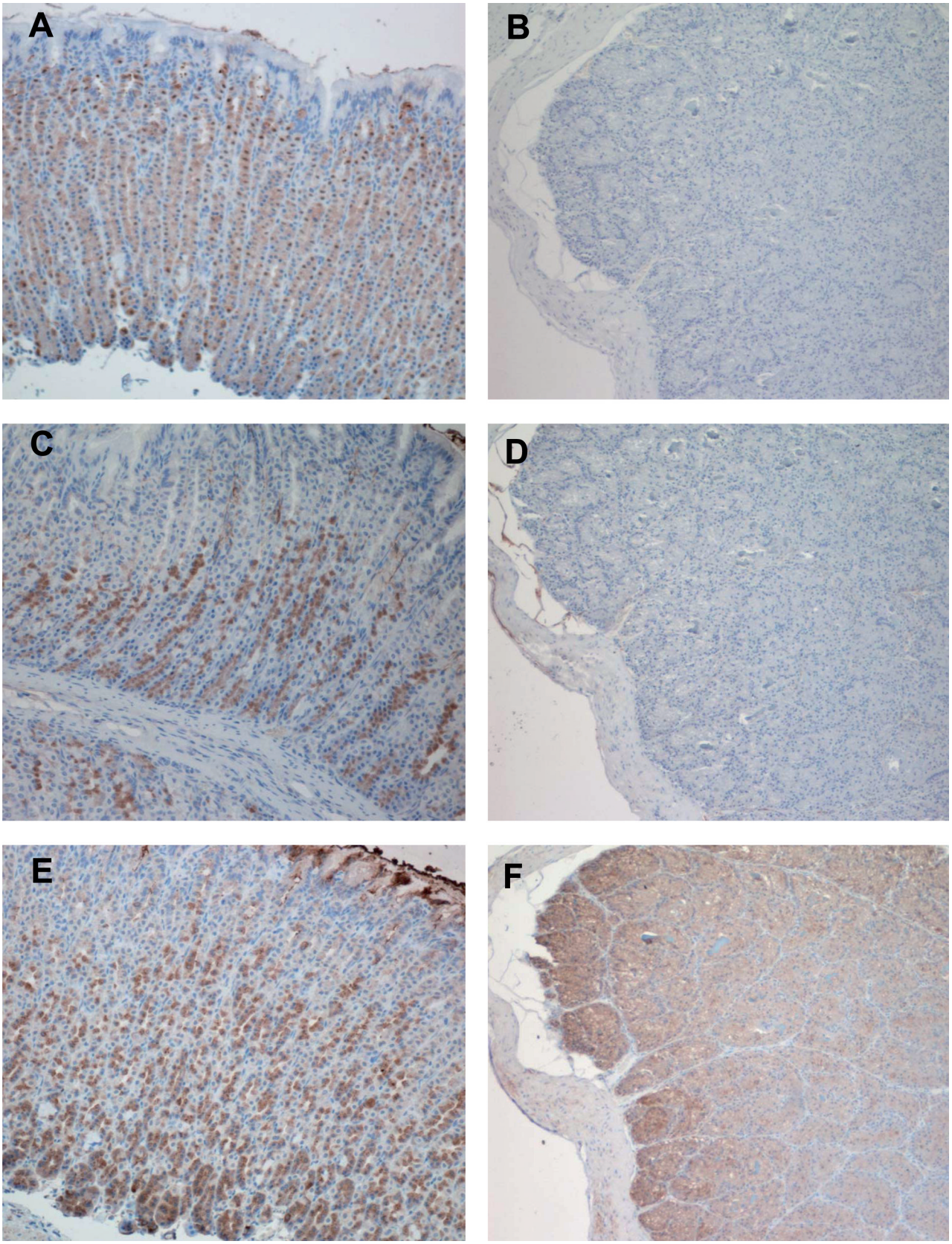

Fig. 2. Representative immunohistochemical photomicrographs showing ATG-5 **(A, B),** ATG-16 **(C, D)** and beclin-1 **(E, F)** in *Mastomys* stomachs. Note: positive in normal tissue from controls **(A, C, E)**, and negative in tumor from carcinoid-bearing *Mastomys* **(B, D)** with an exception **(F)**. x 10

Statistical analysis

The values are expressed as Means ± SEM. Student's t test was performed in SPSS version 15.0 and p-value of <0.05 (two-tailed) was considered statistically significant.

Results

Pathology of carcinoids and adenocarcinomas

General histological appearances were similar with respect to carcinoids between the *Mastomys* and patients and with respect to adenocarcinoma between the cotton rats, INS-GAS mice and patients (Fig. 1).

Autophagy of carcinoids

Immunohistochemistry showed positive ATG-5, ATG-16 and beclin-1 in all tumor-free *Mastomys* (control group), but negative ATG-5 and ATG-16 in all tumor-bearing *Mastomys*. Beclin-1 immunostaining was positive in 4 of 5 tumor-bearing animals (Fig. 2) (Table 1). The locations of the immunoreactivity for ATG-5, ATG-19 and beclin-1 appeared to be the epithelial cells in the normal fundic gland (Fig. 2A,C,E).

In the patients, ATG-5 immunostaining was negative in 6 of 10, and ATG-16 negative in nine of ten. Beclin-1 immunostaining was negative in 3 of 10 patients (Fig. 3) (Table 3).

Autophagy of adenocarcinomas

Immunostainings of ATG-5, ATG-16 and beclin-1 were all positive in normogastrinemic cotton rats in both age groups, as well as in cotton rats with 2-month-hypergastrinemia (data not shown), except one with dysplasia of the mucosa, where all the three immunostainings were positive in normal mucosa but negative in the area displaying dysplasia (Fig. 4) (Table 2).

The cotton rats with 8-month-hypergastrinemia showed negative ATG-5 and ATG-16 in the tumor area and adjacent tissue in all samples, but positive in the normal area of the same stomach. Beclin-1 immunostaining was negative in 3 of 5 and positive in small area of tumor in 2 of 5 rats (Fig. 5) (Table 2).

The INS-GAS mice showed positive immunostaining of ATG-5 and beclin-1 in the tumor area, but the numbers of immunoreactive cells per gland were reduced by about 50% ($p<0.01$) in comparison with wild-type mice (Fig. 6) (Table 3). ATG-16 antibody, which worked in rats and humans, failed to work in mice.

In the patients, both ATG-5 and ATG-16 immunostainings were negative in the tumor area in 8 of 10 patients. Beclin-1 immunostaining was sometimes negative in the tumor area but occasionally positive in adjacent area in the 10 patients (Fig. 7) (Table 4). It was

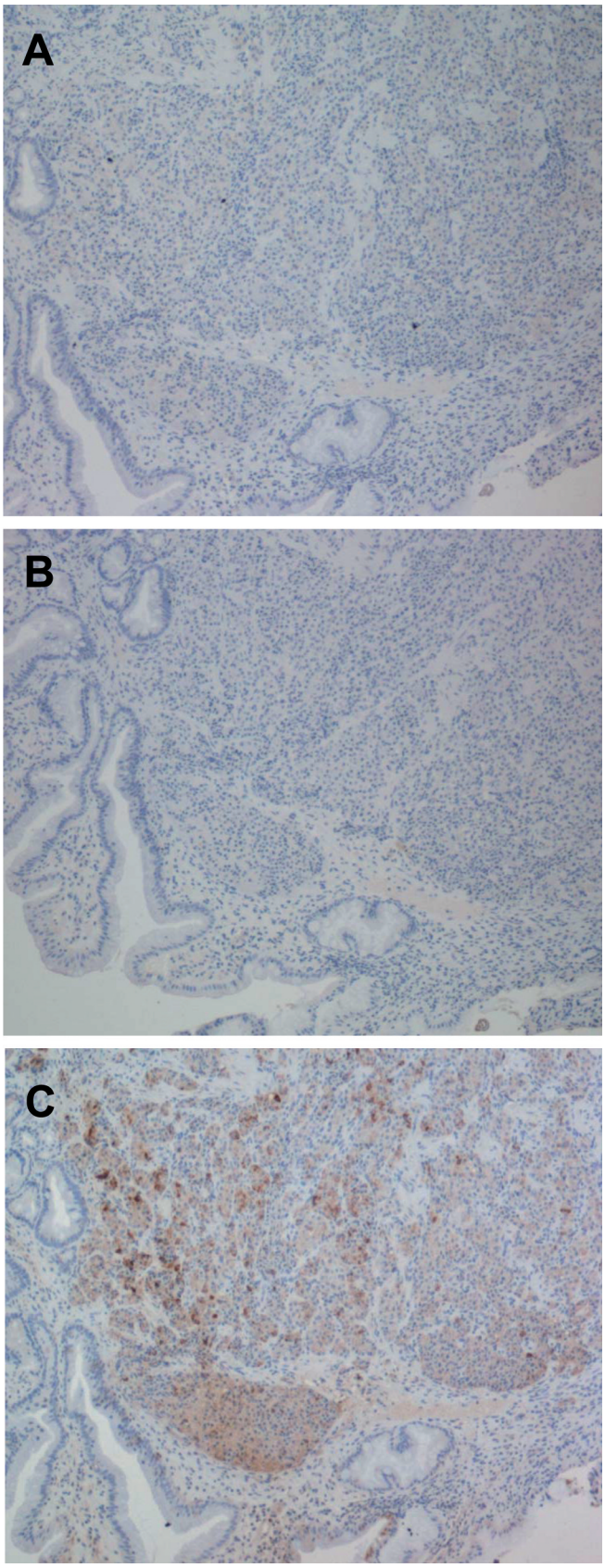

Fig. 3. Representative immunohistochemical photomicrographs showing ATG-5 **(A)**, ATG-16 **(B)** and beclin-1 **(C)** in a carcinoid patient stomach. Note: negative in A, B, but positive in C. x 10

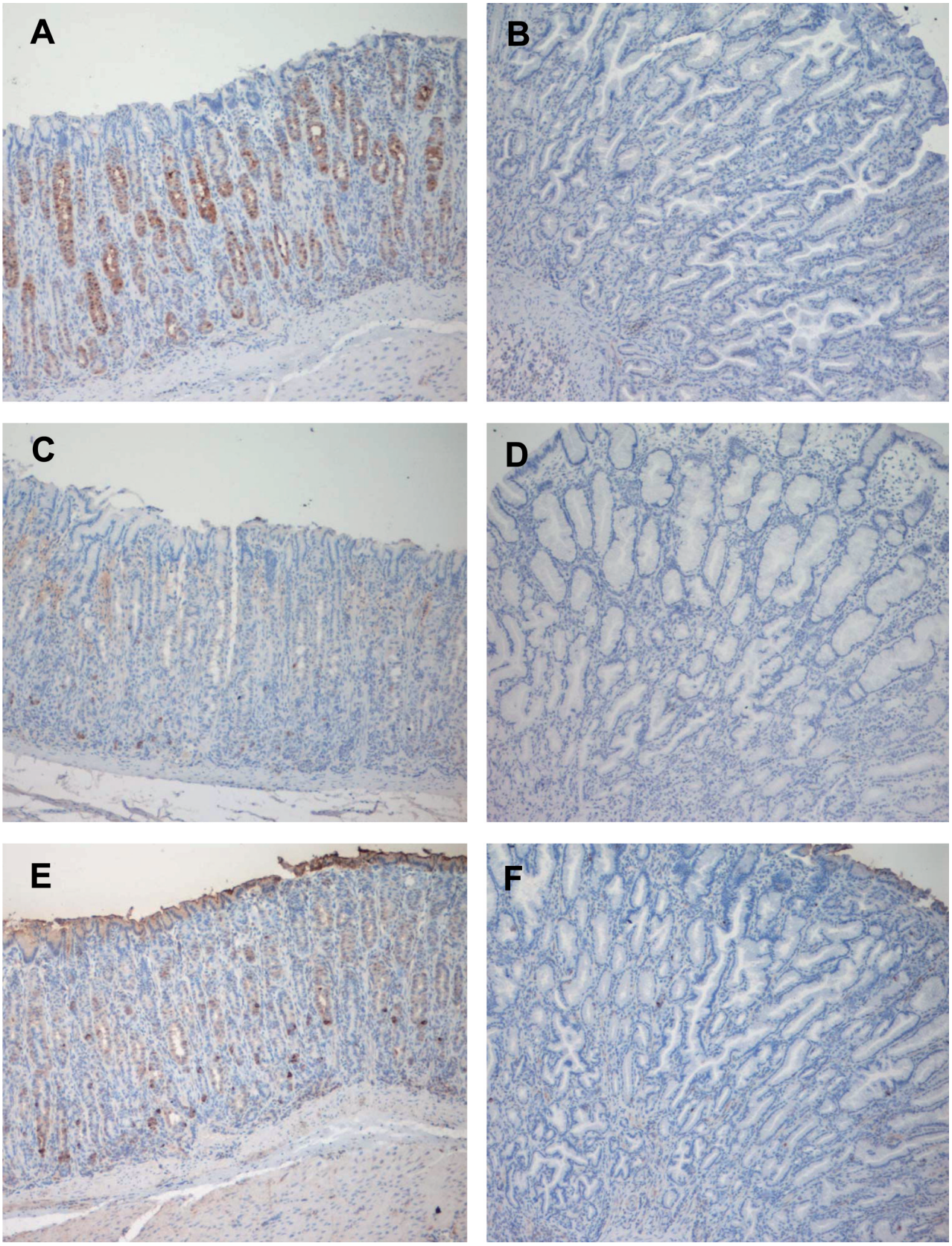

Fig. 4. Representative immunohistochemical photomicrographs showing ATG-5 **(A, B)**, ATG-16 **(C, D)** and beclin-1 **(E, F)** in a cotton rat stomach with 2-month-hypergastrinemia. Note: positive in normal area **(A, C, E)**, but negative in area displaying dysplasia **(B, D, F).** x 10

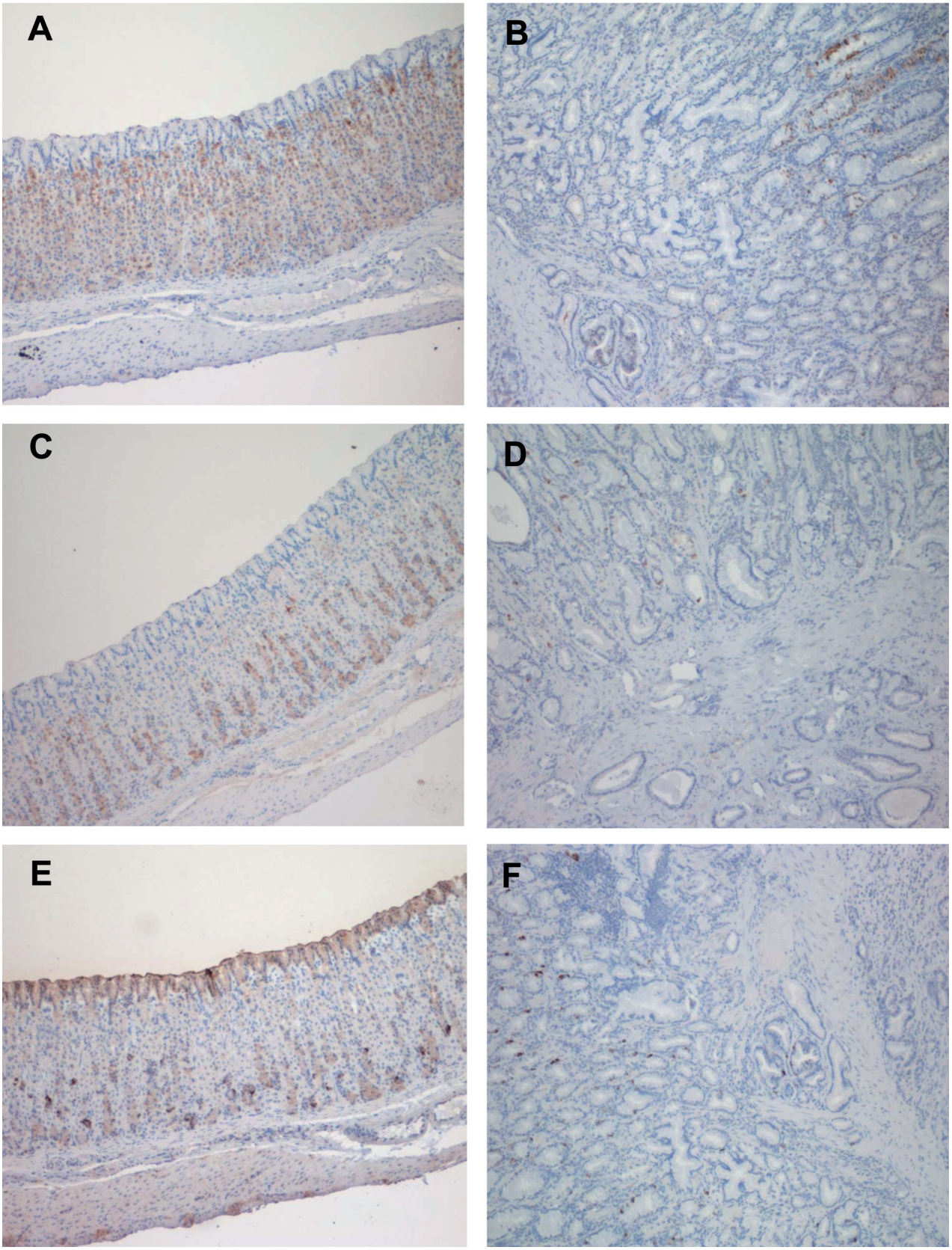

Fig. 5. Representative immunohistochemical photomicrographs showing ATG-5 **(A, B),** ATG-16 **(C, D)** and beclin-1 **(E, F)** in cotton rat stomachs with normogastrinemia **(A, C, E),** or with 8-months-hypergastrinemia and adenocarcinoma **(B, D, F)**. Note: positive in the normal area **(A, C, E),** but negative in the tumor area **(B, D, F)**. x 10

Table 4. Clinical data and results of immunostaining for ATG-5. ATG-16 and beclin-1. MI-1 (MIB-1 % or degree of differentiation).

Patient no.	Age	Sex	CgA (nM)	Gastrin (pM)	Size (cm)	TNM	MIB-1	ATG-5	ATG-16	Beclin-1
Carcinoid (type 1)										
1	67	M	8,5	365	0,3	T1N0M0	<5%	Positive	Negative	Positive
2	87	F	nd	752	0,4	T1N0M0	5%	Positive	Negative	Positive
3	56	F	7,0	1376	1,5	T3N1M0	<2%	Negative	Positive	Negative
4	64	M	7,3	528	0,4	T1N0M0	nd	Positive	Negative	Negative
5	73	F	14,8	925	0,4	T1N0M0	<2%	Positive	Negative	Negative
6	54	M	3,3	nd	1,5	T1N0M0	10%	Negative	Negative	Positive
7	41	M	18,5	400	0,4	T1N0M0	<2%	Negative	Negative	Positive
8	57	F	6,5	481	0,3	T1N0M0	<1%	Negative	Negative	Positive
9	68	M	nd	nd	0,3	T1N0M0	nd	Negative	Negative	Positive
10	69	M	nd	nd	0,3	T1N0M0	nd	Negative	Negative	Positive
Adenocarcinoma (intestinal type)										
11	82	F	nd	nd	8,5	T3N0M0	Moderate	Positive	Negative	Positive
12	51	M	nd	nd	2	T3N0M0	Poor	Negative	Negative	Positive
13	82	M	nd	nd	4	T3N0M0	Moderate	Negative	Negative	Positive
14	82	M	nd	nd	6	T3N3M1	Moderate	Negative	Positive	Positive
15	49	M	nd	nd	10	T3N1M0	Moderate	Positive	Negative	Positive
16	66	F	nd	nd	2	T3N1M0	Moderate	Negative	Negative	Positive
17	62	M	nd	nd	1,5	T3N1M0	Well	Negative	Positive	Positive
18	77	F	nd	nd	9,5	T3N1M0	Poor	Negative	Negative	Positive
19	39	M	nd	nd	4	T3N1M0	Poor	Negative	Negative	Positive
20	58	F	nd	nd	2	T1N0M0	Moderate	Negative	Negative	Positive

* nd: not determined

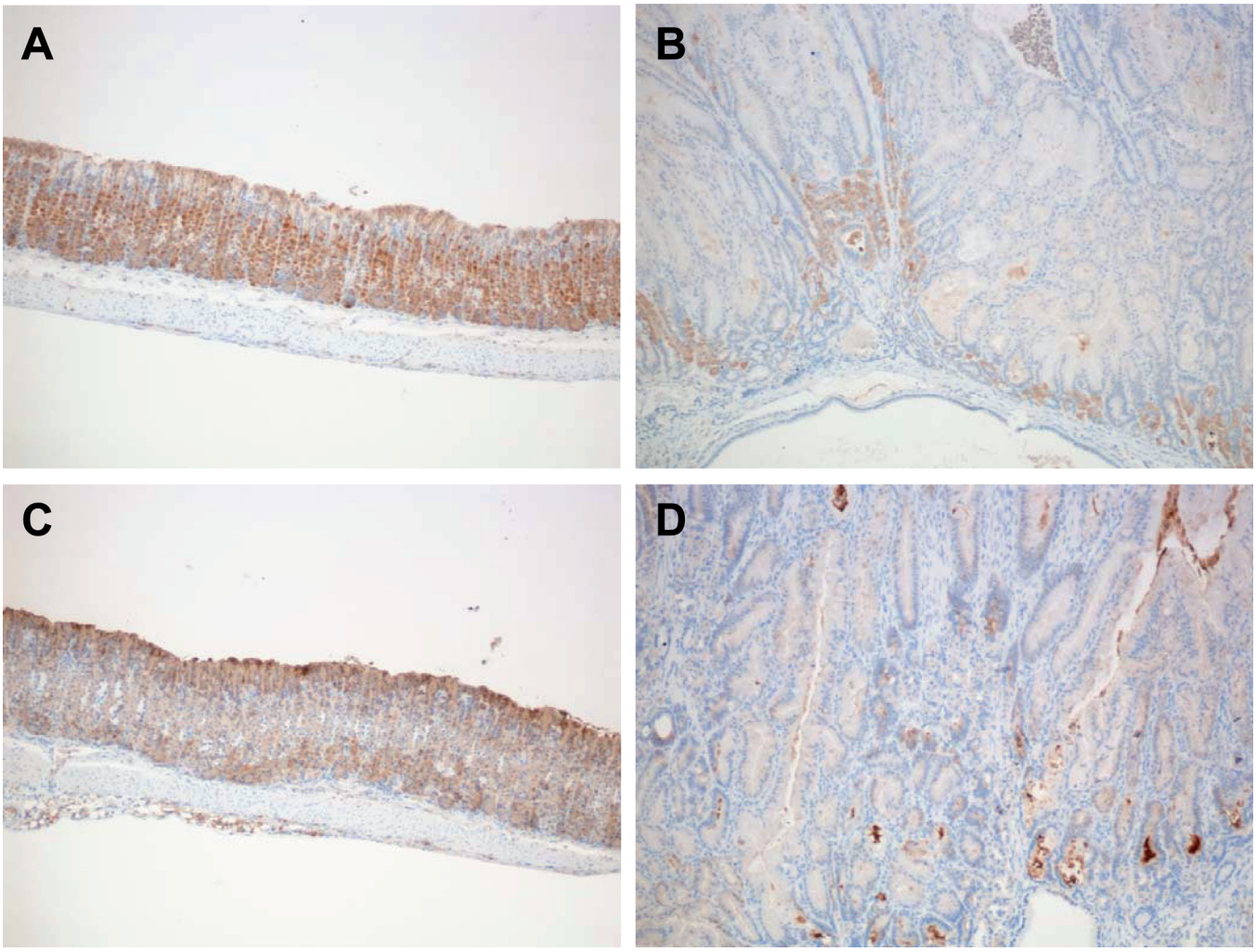

Fig. 6. Representative immunohistochemical photomicrographs showing ATG-5 **(A, B)**, and beclin-1 **(C, D)** in the stomachs of wild-type **(A, C)** and INS-GAS **(B, D)** mice. Note: more positive wild-type than INS-GAS mice. x 10

noticed that normal tissue away from the tumor was positive (data not known).

Discussion

The cell death pathways of gastric carcinoids and/or gastric adenocarcinomas have not been fully defined. Recently, and for the first time, a report of immunohistochemical analysis of cell death pathways in gastric vs. colorectal adenocarcinoma showed that there was no correlation between the expression of any markers, i.e., apoptotic markers cleaved caspase-8, -9, or autophagic marker microtubule-associated protein 1 light chain 3 (LC3), and any of the clinicopathological parameters, including age, gender, differentiation, location, depth of tumor invasion, lymph node metastasis, lymphatic invation, vascular invasion and pathological stage (Shintani et al., 2011). Unfortunately, no comparison was made between the normal vs. tumor tissues either of gastric or colorectal adenocarcinomas in that report. In the present study, we have attempted to compare the normal/control vs. tumor tissues of gastric carcinoids and adenocarcinomas in three rodent models and patients with respect to the autophagic markers.

Autophagy is orchestrated by a subset of genes that are called autophagy-related genes (ATG). The ATG signaling pathway consists of activation of a serine/threonine kinase complex to initiate autophagy, formations of ULK-ATG13-FIP200 and beclin-1-hVps34-p150 complexes to mediate early nucleation events, and then formation of ATG-5-ATG-12-ATG-16 complex and cleavage of LC3-I to form LC3-II (ATG-6), leading to the formation of autophagosome and the fusion between the autophagosome and the lysozyme (Rosenfeldt and Ryan, 2009). The role of autophagy has been described as a "double-edged sword" as it functions as a suppressor of neoplasia, but also helps cancer cells to survive (Marx, 2006; White and DiPaola, 2009; Chen and Debnath, 2010; Pavlides et al., 2010; Rosenfeldt and Ryan, 2011). Recently, it was reported that gastric carginogenesis could be inhibited by IFN-γ, probably through induction of autophagy and apoptosis in mice (Tu et al., 2011). We also reported that the formation of vacuoles and lipofuscin bodies was impaired in the carcinoids of these *Mastomys*, suggesting an impaired autophagic pathway (Fossmark et al., 2005; Vigen et al., 2012). The results of the present study confirmed that an impaired autophagy took place mainly at the later stage of the tumorigenesis (i.e. formation of ATG-5-ATG-12-ATG-16 complex), rather than at the initiating stage (formation of beclin-1-hVps34-p150 complex) in gastric carcinoids of animals and humans, and further showed that there was a similar impaired autophagy in gastric adenocarcinomas of rodent models and patients.

There have been few reports on ATG-5 and ATG-16 with regard to their roles in tumorigenesis (Kang et al., 2009), but relatively many reports on beclin-1, which are diverse. For example, mice lacking one copy of beclin-1 suffered from a high incidence of spontaneous tumors,

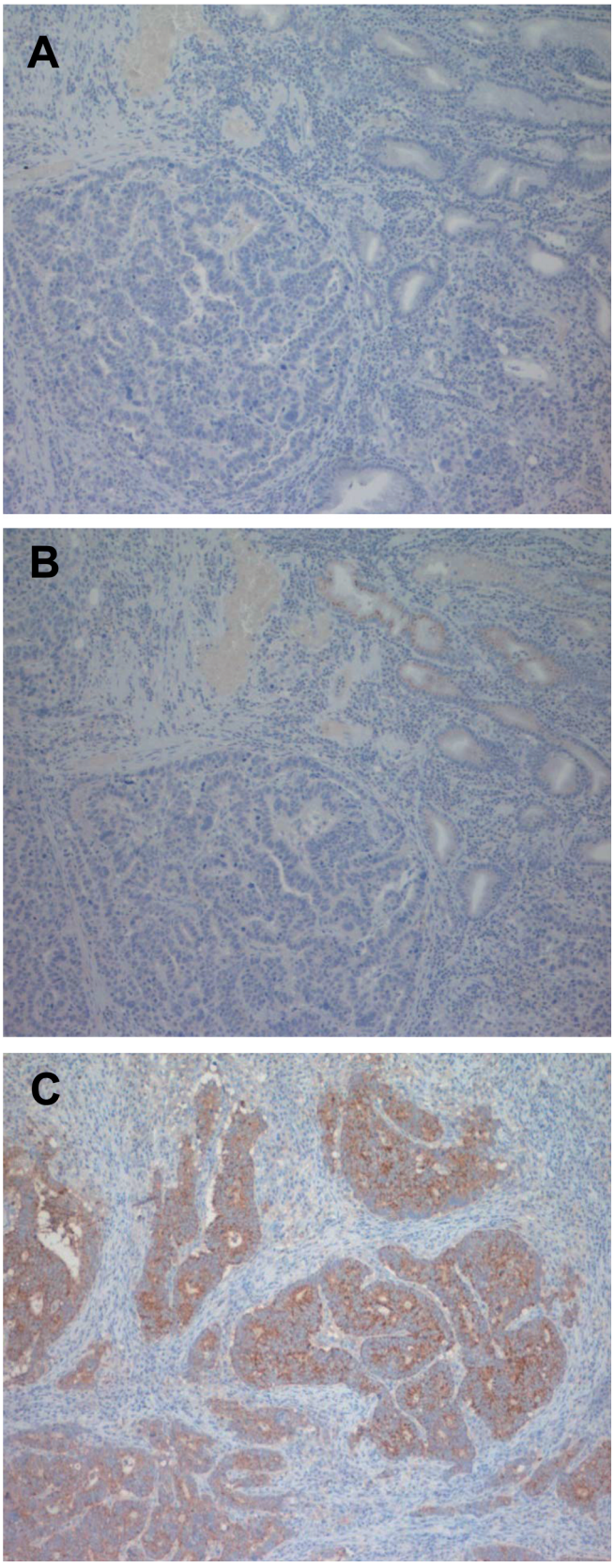

Fig. 7. Representative immunohistochemical photomicrographs showing ATG-5 **(A)**, ATG-16 **(B)** and beclin-1 **(C)** in an adenocarcinoma patient stomach. Note: negative in **A**, **B**, and positive in **C**, with an exception of occasionally positive in adjacent area **(B)**. x 10

including B cell lymphoma, hepatocellular carcinoma and lung adenocarcinoma (Qu et al., 2003; Yue et al., 2003). In humans, the beclin-1 gene is monoallelically deleted in 40-75% of cases of sporadic breast, ovarian, and prostate cancer (Qu et al., 2003). The expression of beclin-1 and LC13 was associated with FIGO stage and histological grade and the decrease of autophagic capacity might be related to tumorigenesis and development of epithelial ovarian cancer (Shen et al., 2008). However, it was reported that beclin-1 was positively expressed in most gastric carcinomas, and had no significant association with invasion, metastasis or stage (Ahn et al., 2007). Overexpression of beclin-1 was also found in MKN28 human gastric cancer cells (Furuya et al., 2005). These results might indicate that there is difference in expression between different cancer forms. In the present study, beclin-1 was expressed in all patients with adenocarcinomas of intestinal type but inconsistent in patients with carcinoids. A larger sampling study will be worthwhile in the future.

The results of the present study provided further evidence for an impairment of autophagy, particularly in the later stage of the pathway, in the tumorigenesis of gastric carcinoids as well as adenocarcinomas in rodents and patients. We may also suggest that ATG-5 and ATG-16 are better markers than beclin-1 for autophagy, at least in gastric carcinoids and adenocarcinomas, and that stimulation of ATG-5-ATG-12-ATG-16 complex formation might be a novel target strategy for gastric anticancer therapy.

Acknowledgements. This study was supported by grants from the Research Council of Norway and the Joint Programme of the Medical Faculty and St. Olavs' University Hospital, and the Liaison Committee between the Central Norway Regional Health Authority (RHA). Dr. Vigen has received a fellowship from the Faculty of Medicine of NTNU, and Kodama received a fellowship from Central Norway Regional FUGE programme. The histology was performed partly at the Cellular and Molecular Imaging Core Facility, NTNU.

References

Ahn C.H., Jeong E.G., Lee J.W., Kim M.S., Kim S.H., Kim S.S., Yoo N.J. and Lee S.H. (2007). Expression of beclin-1, an autophagy-related protein, in gastric and colorectal cancers. APMIS 115, 1344-1349.

Apel A., Zentgraf H., Buchler M.W. and Herr I. (2009). Autophagy- A double-edged sword in oncology. Int. J. Cancer. 125, 991-995.

Chen N. and Debnath J. (2010). Autophagy and tumorigenesis. FEBS lett. 584, 1427-1435.

Clarke P.G. (2002) Apoptosis: from morphological types of cell death to interacting pathways. Trends Pharmacol. Sci. 23, 308-309.

Eisenberg-Lerner A. and Kimchi A. (2009). The paradox of autophagy and its implication in cancer etiology and therapy. Apoptosis 14, 376-391.

Fossmark R., Martinsen T.C., Bakkelund K.E., Kawase S. and Waldum H.L. (2004a). ECL-cell derived gastric cancer in male cotton rats dosed with the H2-blocker loxtidine. Cancer Res. 64, 3687-3693.

Fossmark R., Martinsen T.C., Torp S.H., Kawase S., Sandvik A.K. and Waldum H.L. (2004b). Spontaneous enterochromaffin-like cell carcinomas in cotton rats (Sigmodon hispidus) are prevented by a somatostatin analogue. Endocr. Relat. Cancer 11, 149-160.

Fossmark R., Zhao C.M., Martinsen T.C., Kawase S., Chen D. and Waldum H.L. (2005). Dedifferentiation of enterochromaffin-like cells in gastric cancer of hypergastrinemic cotton rats. APMIS 113, 436-449.

Furuya D., Tsuji N., Yagihashi A. and Watanabe N. (2005). Beclin 1 augmented cis-diamminedichloroplatinum induced apoptosis via enhancing caspase-9 activity. Exp. Cell Res. 307, 26-40.

Gozuacik D. and Kimchi A. (2007). Autophagy and cell death. Curr. Top. Dev. Biol. 78, 217-245.

Kang M.R., Kim M.S., Oh J.E., Kim Y.R., Song S.Y., Kim S.S., Ahn C.H., Yoo N.J. and Lee S.H. (2009). Frameshift mutations of autophagy-related genes ATG2B, ATG5, ATG9B and ATG12 in gastric and colorectal cancers with microsatellite instability. J. Pathol. 217, 702-706.

Martinsen T.C., Kawase S., Håkanson R., Torp S.H., Fossmark R., Qvigstad G., Sandvik A.K. and Waldum H.L. (2003). Spontaneous ECL cell carcinomas in cotton rats: natural course and prevention by a gastrin receptor antagonist. Carcinogenesis 24, 1887-1896.

Marx J. (2006). Autophagy: is it cancer's friend or foe? Science 312, 1160-1161.

Pavlides S., Tsirigos A., Migneco G., Whitaker-Menezes D., Chiavarina B., Flomenberg N., Frank P.G., Casimiro M.C., Wang C., Pestell R.G., Martinez-Outschoorn U.E., Howell A., Sotgia F. and Lisanti M.P. (2010). The autophagic tumor stroma model of cancer: Role of oxidative stress and ketone production in fueling tumor cell metabolism. Cell Cycle 9, 3485-3505.

Qu X., Yu J., Bhagat G., Furuya N., Hibshoosh H., Troxel A., Rosen J., Eskelinen E.L., Mizushima N., Ohsumi Y., Cattoretti G. and Levine B. (2003). Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J. Clin. Invest. 112, 1809-1820.

Rosenfeldt M.T. and Ryan K.M. (2009). The role of autophagy in tumour development and cancer therapy. Expert Rev. Mol. Med.11, e36.

Rosenfeldt M.T. and Ryan K.M. (2011). The multiple roles of autophagy in cancer. Carcinogenesis 32, 955-963.

Shen Y., Li D.D., Wang L.L., Deng R. and Zhu X.F. (2008). Decreased expression of autophagy-related proteins in malignant epithelial ovarian cancer. Autophagy 4, 1067-1068.

Shintani M., Sangawa A., Yamao N., Miyake T. and Kamoshida S. (2011). Immunohistochemical analysis of cell death pathways in gastrointestinal adenocarcinoma. Biomed. Res. 32, 379-386.

Tu S.P., Quante M., Bhagat G., Takaishi S., Cui G., Yang X.D., Muthuplani S., Shibata W., Fox J.G., Pritchard D.M. and Wang T.C. (2011). IFN-gamma inhibits gastric carcinogenesis by inducing epithelial cell autophagy and T-cell apoptosis. Cancer Res. 71, 4247-4259.

Vigen R.A., Kidd M., Modlin I.M., Chen D. and Zhao C-M. (2012). Ultrastructure of ECL cells in Mastomys after long-term treatment with H(2) receptor antagonist loxtidine. Med. Mol. Morphol. 45, 80-85.

Wang T.C., Dangler C.A., Chen D., Goldenring J.R., Koh T., Raychowdhury R., Coffey R.J., Ito S., Varro A., Dockray G.J. and Fox J.G. (2000). Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. Gastroenterology 118, 36-47.

White E. and DiPaola R.S. (2009). The double-edged sword of

autophagy modulation in cancer. Clin. Cancer Res. 15, 5308-5316.

Wilkinson S. and Ryan K.M. (2010). Autophagy: an adaptable modifier of tumourigenesis. Curr. Opin. Genet. Dev. 20, 57-64.

Yue Z., Jin S., Yang C., Levine A.J. and Heintz N. (2003). Beclin 1, an autophagy gene essential for early embryonic development, is a haploinsufficient tumor suppressor. Proc. Natl. Acad. Sci. USA 100, 15077-15082.

Accepted October 26, 2012