Original article (revised)

Expression of adipophilin in gastric epithelial neoplasia is associated with intestinal differentiation and discriminates between adenoma and adenocarcinoma

Ryosuke Gushima [1,2], Takashi Yao [1], Aiko Kurisaki-Arakawa [1], Kieko Hara [1], Takuo Hayashi [1], Yuki Fukumura [1], Tsuyoshi Saito [1], Atsushi Arakawa [1], Kenshi Yao [3], Yutaka Sasaki [2]

[1]Department of Human Pathology, Juntendo University School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan;

[2]Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-0811, Japan;

[3]Department of Endoscopy, Fukuoka University Chikushi Hospital, 1-1-1 Zokumyoin, Chikushino, Fukuoka 818-8502, Japan;

Correspondence: Takashi Yao, MD. Ph.D.

Department of Human Pathology, Juntendo University School of Medicine, 1-1-19 Hongo, Bunkyo-Ku, Tokyo 113-0033, Japan. Phone: 81-3-5802-1037; Fax: 81-3-3812-1056

E-mail: tyao@juntendo.ac.jp

Abstract:

Adipophilin, a lipid droplet-associated protein that regulates lipid droplet structure and formation, is expressed in a wide variety of tumors. The aim of this study was to evaluate the frequency and distribution pattern of adipophilin expression in gastric epithelial neoplasia, and to correlate these variables with clinicopathological features and the mucin phenotype. We retrospectively examined 159 cases of gastric epithelial neoplasia, which were classified according to the Vienna classification system as 52 non-invasive low grade adenoma (Category 3), 65 non-invasive high-grade neoplasia (Category 4), and 42 invasive neoplasia (Category 5). Immunohistochemistry for adipophilin was performed, and phenotypic marker expression was determined by immunohistostaining with MUC2, MUC5AC, CD10, MUC6, and Villin. Adipophilin was expressed in 41 of 52 (79%) Category 3 cases, in 42 of 65 (65%) Category 4 cases, and in 23 of 42 (55%) Category 5 cases. Expression of adipophilin was only present in lesions with complete or incomplete intestinal phenotypes. Adipophilin was expressed more frequently in surface epithelium in Category 3, whereas there was stepwise increase in cryptal staining of adipophilin from Category 3 to Category 5. In conclusion, adipophilin expression is closely related to the intestinal differentiation of the tumor. The pattern of immunostaining for adipophilin might be a useful new marker for discriminating adenomas from adenocarcinomas.

Keywords: Adipophilin ・lipid droplet ・gastric epithelial neoplasia ・mucin phenotype

Introduction

Gastric adenocarcinoma is one of the most common malignant tumors worldwide. It has been classified as intestinal-type and diffuse-type by Lauren et al [1] and as differentiated-type and undifferentiated-type by Nakamura et al [2]. Gastric adenoma/dysplasia is classified as a premalignant lesion, and the risk of adenocarcinoma formation increases with the histological grade of dysplasia [3, 4]. Differences between Japanese and Western pathologists exist regarding the diagnosis of gastric epithelial neoplasia [4, 5]. The Vienna classification is useful for resolving these discrepancies between pathologists in the diagnosis of gastric epithelial neoplasia [6]. Previous reports suggested that high-grade adenoma (Category 4.1 in the Vienna classification) was highly predictive of invasive neoplasia (Category 5) [7-11]. In contrast, Yamada et al. reported that low-grade adenoma (Category 3) had a low risk of progression into high-grade adenoma or non-invasive carcinoma (Category 4), and no risk of progressing to an invasive neoplasia (Category 5) [12].

Yao et al. reported that the white opaque substance (WOS) within the superficial part of the gastric intraepithelial neoplasia can be visualized using magnifying endoscopy with narrow-band imaging [13]. The morphology of the WOS could be a new indication for discriminating gastric adenoma from gastric adenocarcinoma [13]. More recently, Yao et al. demonstrated that the WOS consisted of lipid droplets that can be visualized with histology using the Oil red O stain [14]. Ueo et al. showed that WOS in gastric epithelial neoplasia represents an intracytoplasmic accumulation of lipid droplets as seen on adipophilin staining and immunoelectron microscopy [15].

Lipid droplet-associated proteins, such as perilipin, adipophilin, and TIP47 play important roles in the formation, maintenance, modification, and involution of lipid droplets. The expression of adipophilin was reported in cells of the lactating mammary epithelium, adrenal cortex, male reproductive system (Sertoli and Leydig cells), and in steatosis or

fatty changes in hepatocytes in alcoholic liver cirrhosis. In addition, adipophilin expression was reported in a wide variety of tumors such as colorectal carcinomas, renal cell carcinomas, hepatocellular carcinomas, and lung carcinomas [16, 17]. Lipid droplet accumulation is a frequent feature of neoplastic cells. Using immunohistochemistry and immunoelectron microscopy, Ueo et al. reported that intraepithelial distribution and morphology of the lipid droplets differ between gastric adenomas and adenocarcinomas [15]. Based on these findings, adipophilin expression could become a specific marker for discriminating adenomas from adenocarcinomas. However, little is known about the expression of adipophilin in gastric epithelial neoplasia.

In this study, we aimed to evaluate the frequency and distribution pattern of adipophilin in gastric epithelial neoplasia and to elucidate whether adipophilin expression correlates with clinicopathological features and the mucin phenotype.

Materials and methods

Patients and tissue samples

For this study, we randomly examined 159 samples of gastric epithelial neoplasia diagnosed between 2008 and 2013 from the pathologic files of endoscopically resected gastric specimens at Juntendo University Hospital. All lesions were classified according to the Vienna classification of gastrointestinal epithelial neoplasia: adenoma with low grade dysplasia was classified as non invasive low-grade neoplasia (Category 3), adenoma with high grade dysplasia (Category 4.1) or non-invasive carcinoma (Category 4.2) was classified as Category 4 (Non invasive high-grade neoplasia), and intramucosal carcinoma (Category 5.1) or submucosal carcinoma (Category 5.2) was classified as Category 5 (Invasive neoplasia). Adenoma with high grade dysplasia (Category 4.1) was diagnosed that the nuclei were spindle-shaped and basally located with a high nucleus to cytoplasm ratio. The reasons for the random collection of cases were that the

collection of the consecutive series of cases would result in the simple increase in Category 4 cases. The lesions analyzed in this study included of 52 Category 3, 65 Category 4, and 42 Category 5 lesions, matched for age and sex.

All specimens were fixed in 10% formalin solution, embedded in paraffin, and cut into 4 μm thick slices. Subsequently, one section from each patient was stained with hematoxylin and eosin for histological diagnosis. Histological and immunohistochemical evaluations were performed by 2 pathologists (R.G and T.Y), who were unaware of the clinical characteristics of the patients. This retrospective study was approved by the Institutional Review Board and ethical committee of our hospital (registration #2012008). Written informed consents were obtained from all patients.

Immunohistochemical staining and evaluation

The monoclonal antibodies used in this study were MUC5AC (CLH2, Novocastra, Newcastle-upon-Tyne, UK, dilution 1:200) as a marker for gastric foveolar cells, MUC6 (CLH5, Novocastra, Newcastleupon-Tyne, UK, dilution 1:200) as a marker for gastric mucous neck cells and pyloric glands, MUC2 (Ccp58, Novocastra, Newcastle-upon-Tyne, UK, dilution 1:200) as a marker for intestinal goblet cells, CD10 (56C6, Novocastra, Newcastle-upon-Tyne, UK, dilution 1:200) as a marker for the small intestinal brush border, Villin (1D2 C3, DAKO, Glostrup, Denmark, dilution 1:1600) as a marker for the small intestinal brush border and adipophilin (AP125, Acris Antibodies, Hiddenhausen, dilution 1:200).

In brief, sections were cut to a thickness of 4 μm, deparaffinized in xylene, and dehydrated in descending dilutions of ethanol. For antigen retrieval, specimens were autoclaved in 0.01 M citrate buffer (pH 6.0) at 121°C for 10 min. Then, endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxidase in absolute methanol for 30 minutes. Sections were incubated overnight at 4°C with primary antibodies, and then incubated with secondary antibodies (EnVision; DAKO, Glostrup, Denmark) for 30 minutes at room temperature. Diaminobenzidine

tetrahydrochloride was used as the chromogen. Finally, the sections were counterstained with hematoxylin.

The expressions of MUC2, MUC5AC, MUC6, CD10, and Villin were classified as significantly positive when more than 10% of the neoplastic cells or gastric epitheliums in the background mucosa were stained. The classification of the phenotypes of the carcinomas and background non-neoplastic mucosa was determined by these immunohistochemical findings. The phenotypes were classified into 4 categories according to the combination of the expression of MUC2, MUC5AC, MUC6, CD10, and Villin. The gastric type (G-type) exhibited expression of MUC5AC or MUC6, but not of MUC2, CD10, or Villin. Regardless of the MUC2 expression status, the complete intestinal type (C-type) was characterized by either CD10 or Villin expression and by the absence of MUC5AC and MUC6 expression. Specimens of the incomplete intestinal type (I-type) demonstrated positive expression for CD10, MUC2, or Villin, in addition to the expression of either MUC5AC or MUC6. Specimens of the unclassified type (U-type) exhibited no expression of CD10, MUC2, MUC5AC, MUC6, or Villin [18]. The scheme of the phenotypic classification is shown in Table 1.

Staining intensity of adipophilin was graded as follows: 0, negative stain; 1+, focally or diffusely weakly positive stain; 2+, focally strongly positive stain; 3+, diffusely strongly positive stain. Positive staining of adipophilin was determined grade 2+ or 3+ (Figure 1). The distribution pattern of adipophilin expression was classified into the following 3 categories: surface accumulation, surface plus cryptal accumulation, and cryptal accumulation. We defined surface accumulation as adipophilin expression within the superficial epithelium of the intervening part, cryptal accumulation as adipophilin expression in the cryptal epithelium, and surface plus cryptal accumulation as adipophilin expression in both the surface epithelium of the intervening apical epithelium and the cryptal epithelium (Figure 2) [16].

Determination of Helicobacter pylori infection status in background non-neoplastic mucosa

To determine Helicobacter pylori (*H.pylori*) infection status, serum anti-*H.pylori* antibodies, rapid urease test, urea breath test, or histologic evaluation by modified Giemsa stain were used. If any of these tests were positive, we considered as *H.pylori* positive. In case of *H.pylori* test negative, we checked for the presence of the atrophic gastritis by background mucosa. If we detected atrophic gastritis, we considered post *H.pylori* infection. In other cases, we considered as *H.pylori* negative.

Statistical analysis

All statistical analyses were carried out using StatView for Windows Version 5.0 (SAS Institute, Inc, Cary, NC). Continuous data were compared using the Mann-Whitney U test. Categorical variables were analyzed using either the $\chi 2$ test (with Yates's correction) or Fisher's exact test, as appropriate. A *p*-value of less than 0.05 was considered as statistically significant.

Results

Expression of adipophilin and clinicopathological features

Among the 159 lesions with gastric epithelial neoplasia, 106 (67%) were positive for adipophilin expression, whereas adipophilin was expressed only in 26 of 159 (16%) cases of background non-neoplastic gastric mucosa. Compared with non-neoplastic gastric mucosa, the expression of adipophilin in gastric epithelial neoplasia was significantly increased ($P<0.001$). With respect to the Vienna classification, adipophilin was expressed in 41 of 52 (79%) Category 3, 42 of 65 (65%) Category 4, and 23 of 42 (55%) Category 5 cases.

The clinicopathological features of adipophilin-positive and adipophiln-negative gastric epithelial neoplasia cases are summarized in Table 2. There were significant differences in macroscopic type and Vienna classification, however, no

significant differences were detected with respect to patient age, sex, the mean tumor size, and tumor location. In addition, there was no correlation between adipophilin expression and the depth of invasion in submucosal cancer (Category 5.2).

Mucin phenotype and phenotypic classification

Mucin phenotypes of all cases are summarized in Table 3. Incomplete intestinal phenotype tumors were significantly associated with higher levels of invasion compared with complete intestinal phenotype (Table 3).

Of 106 cases of adipophilin-positive gastric epithelial neoplasia, MUC2, MUC5AC, MUC6, CD10, and Villin were expressed in 75 (71%), 34 (32%), 44 (42%), 69 (65%), and 105 (99%) cases, respectively. Expression of adipophilin was only present in lesions with either complete or incomplete intestinal type component, while it was absent in lesions of the gastric type (Table 4). With regard to the Vienna classification, among adipophilin positive neoplasias, the complete intestinal phenotype was more frequent in Category 3 cases (29/41, 71%) than in Category 5 cases (7/23, 30%). In contrast, the incomplete intestinal phenotype was more frequently observed in Category 5 cases (16/23, 70%) than in Category 3 cases (12/41, 29%). For Category 4 lesions, the complete intestinal type was detected in 19 cases (45%) and the incomplete intestinal type in 23 cases (55%). A significant difference in phenotypic classification was found between Category 3 and Category 4 lesions (P=0.033), and between Category 3 and Category 5 lesions (P=0.004) (Table 4). In the background normal gastric mucosa, adipophilin was expressed in 3 cases (12%) with complete intestinal metaplasia and in 23 lesions (88%) with incomplete intestinal metaplasia.

Distribution pattern of adipophilin expression

Surface accumulation was more frequently observed for Category 3 and Category 4.1 lesions. In contrast, surface plus

cryptal accumulation was more frequently observed in Category 5.1 and Category 5.2 lesions, while cryptal accumulation was only observed in Category 4.2 lesions. Expression of surface epithelium decreased according to the increased grade of Vienne classification (Table 5). In the background non-neoplastic gastric mucosa, surface accumulation was more frequent where adipophilin expression was observed (23/26, 88%) (Figure 2). Regarding *H.pylori* infection status, there were no significant differences in adipophilin expression according to the *H.pylori* infection status (Table 5).

Discussion

Adipophilin was recently reported to be a marker of lipid accumulation that can be used on formalin-fixed paraffin-embedded tissue sections. It is an adipose differentiation-related protein located on the surface of lipid droplets and contacts between lipid droplets and intermediate filaments [17]. In addition, the expression of adipophilin was reported in a wide variety of tumors, and lipid droplet formation is a very common phenomenon in cancer cells [16]. In the case of gastric epithelial neoplasia, Ueo et al. reported that expression of adipophilin was observed in neoplastic cells of the surface epithelium of the intervening apical and cryptal epithelium. Adipophilin expression was divided into 2 categories: surface accumulation and surface plus cryptal accumulation. Surface accumulation was observed in most adenomas, while surface plus cryptal accumulation was only observed in half of all adenocarcinomas restricted to the mucosa [15]. In our study, surface accumulation was more frequently observed in adenoma with low grade dysplasia (Category 3 lesions) and adenoma with high grade dysplasia (Category 4.1 lesions). Conversely, surface plus cryptal accumulation was observed for most intramucosal or submucosal carcinoma (Category 5 lesions), being consistent with the findings reported by Ueo et al [15]. We considered that the progression of adenoma into carcinomas was associated with unique expression pattern of adipophilin. In brief, adipophilin was expressed more frequently in surface epithelium

in tumors with adenoma with low grade dysplasia (Category 3), whereas there was stepwise increase in cryptal staining of adipophilin from adenoma with low grade dysplasia (Category 3) to invasive neoplasia (Category 5).

Regarding the relationship between adipophilin expression and differentiation, Matsubara et al demonstrated that adipophilin was expressed in well- or moderately differentiated adenocarcinoma, but not in poorly differentiated adenocarcinoma in a report pf colorectal cancer [19]. In this study, we investigated the expression of adipophilin only in gastric adenomas and well-differentiated adenocarcinomas. However, we observed the loss of expression of adipophilin in poorly differentiated gastric adenocarcinoma (unpublished data). Therefore, adipophilin expression might also associate with tumor differentiation in gastric adenocarcinoma.

The mechanism of lipid droplet accumulation in gastric neoplasia remains unclear. Previous reports revealed that intestinal metaplasia of the stomach functions to absorb lipid droplets similar to the normal intestine [20]. In the case of duodenal neoplasia, Tanaka et al. hypothesized that lipid droplets accumulate in absorptive cells resulting in abnormalities in the synthesis and discharge of chylomicrons [21]. Yao et al. proposed 2 mechanisms of accumulation of lipid droplets in the gastric epithelium; absorption and production [14]. Our findings suggested a possible role to absorb lipid in intestinal phenotype of gastric epithelial neoplasia, because adipophilin expression was observed only in either complete or incomplete intestinal phenotype. When we reviewed the histological findings, we noticed that the size and density of lipid droplets were different and well-correlates with endoscopic findings. Yao et al. suggested that lipid droplets could be resynthesized from externally absorbed lipids [14] as possible phenomenon to absorb lipid in gastric epithelial neoplasia with intestinal phenotype. In contrast, Matsubara et al. suggested that expression of adipophilin is induced during the early process of colorectal carcinogenesis [19].

There have been no reports regarding the relationship between the mucin phenotype and the expression of adipophilin in gastric epithelial neoplasia. In this report, adipophilin expression was observed in gastric epithelial neoplasia of complete and incomplete intestinal phenotypes, but not in the gastric phenotype. Yao et al. reported that WOS-positive gastric neoplasia was present only in either gastrointestinal or the intestinal phenotype. Our results are compatible with these findings [14]. We found that the intestinal phenotype, especially complete intestinal phenotype, was closely associated with the expression of adipophilin. In addition, the expression of Villin was more frequently observed compared to that of CD10 in adipophilin-positive gastric epithelial neoplasia. Villin is an actin-binding cytoskeletal protein of the epithelial cell brush border in normal epithelial cells of the intestine [22], while CD10 is a human membrane-associated neutral peptidase [23]. In the small intestine of fetal mice, Villin is expressed on the brush border prior to CD10. Niwa et al. assumed that Villin-positive but CD10-negative cells were functionally immature absorptive cells [24]. In this study, Villin was expressed in 99% of all cases of adipophilin-positive gastric epithelial neoplasia, whereas CD10 was expressed in only 65% of cases of adipophilin-positive gastric epithelial neoplasia. Moreover, among adipophilin-positive gastric epithelial neoplasia, Villin(+)/CD10(-) neoplasia was observed in 11 of 41 (27%) Category 3, in 16 of 42 (48%) Category 4, and in 9 of 23 (49%) Category 5. These results suggest that the tumor cells of gastric epithelial neoplasia have the ability to absorb lipid droplets, however, it might be immature especially in Category5. On the other hand, incomplete intestinal phenotype was more frequent in Category 5 lesions than Category 3 lesions in this study. It has been previously reported that tumors with incomplete intestinal phenotype were associated with a higher rate of infiltration as compared with complete intestinal phenotype tumors [25-27], being consistent with our findings.

Finally, the diagnostic utility of adipophilin needs to be described. Part of the diagnostic utility of adipophilin in

gastric epithelial neoplasia has been already described [15]. Surface accumulation was observed in most adenomas, whereas surface plus cryptal accumulation was associated with adenocarcinomas [15]. In this study, this trend was also observed. In addition, it is of value to note that all of the neoplasias with surface plus cryptal expression of adipophilin in adenoma with low grade dysplasia (Category 3) and adenoma with high grade dysplasia (Category 4.1) showed complete intestinal phenotype. This finding suggests that the combination of adipophilin expression pattern and mucin phenotype would be useful to differentiate adenomas from well-differentiated adenocarcinoma.

In conclusion, we demonstrated the correlation between the expression pattern of adipophilin and the Vienna classification in gastric epithelial neoplasia. Moreover, our results indicated that the intestinal phenotype, especially complete intestinal phenotype, was closely associated with adipophilin expression. The pattern of immunostaining for adipophilin might be a useful new marker for discriminating adenomas from adenocarcinomas.

Acknowledgments

We thank Dr. Tetsuya Ueo (Oita Red Cross Hospital) for the advice on immunohistochemical staining methods, and Mrs. Noriko Sasahara for her technical assistance. This work was partially supported by Central Research Institute for Endoscopy, Fukuoka University. This work was supported in part by a Grant-in-Aid for General Scientific Research from the Minstry of Education, Science, Sports, and Culture (#26460428 to T. Yao), Tokyo, Japan.

Conflict of interest

The authors declare no conflict of interest.

References

1. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 64: 31-49.

2. Nakamura K, Sugano H, Takagi K (1968) Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gan 59: 251-258.

3. Oehlert W, Keller P, Henke M, Strauch M (1979) Gastric mucosal dysplasia: what is its clinical significance? Front Gastrointest Res 4: 173-182.

4. Ming SC, Bajtai A, Correa P, Elster K, Jarvi OH, Munoz N, Nagayo T, Stemmerman GN (1984) Gastric dysplasia. Significance and pathologic criteria. Cancer 54: 1794-1801.

5. Schlemper RJ, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, Sipponen P, Stolte M, Watanabe H, Takahashi H, Fujita R (1997) Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. Lancet 349: 1725-1729.

6. Schlemper RJ, Riddell RH, Kato Y et al (2000) The Vienna classification of gastrointestinal epithelial neoplasia. Gut 47: 251-255.

7. Fertitta AM, Comin U, Terruzzi V et al. Clinical significance of gastric dysplasia: a multicenter follow-up study. Gastrointestinal Endoscopic Pathology Study Group. Endoscopy 1993; 25: 265-268.

8. Farinati F, Rugge M, Di Mario F, Valiante F, Baffa R (1993) Early and advanced gastric cancer in the follow-up of moderate and severe gastric dysplasia patients. A prospective study. I.G.G.E.D.--Interdisciplinary Group on Gastric Epithelial Dysplasia. Endoscopy 25: 261-264.

9. Di Gregorio C, Morandi P, Fante R, De Gaetani C (1993) Gastric dysplasia. A follow-up study. Am J Gastroenterol 88: 1714-1719.

10. Rugge M, Farinati F, Di Mario F, Baffa R, Valiante F, Cardin F (1991) Gastric epithelial dysplasia: a prospective multicenter follow-up study from the Interdisciplinary Group on Gastric Epithelial Dysplasia. Hum Pathol 22: 1002-1008.

11. Rugge M, Farinati F, Baffa R, Sonego F, Di Mario F, Leandro G, Valiante F (1994) Gastric epithelial dysplasia in the natural history of gastric cancer: a multicenter prospective follow-up study. Interdisciplinary Group on Gastric Epithelial Dysplasia. Gastroenterology 107: 1288-1296.

12. Yamada H, Ikegami M, Shimoda T, Takagi N, Maruyama M (2004) Long-term follow-up study of gastric adenoma/dysplasia. Endoscopy 36: 390-396.

13. Yao K, Iwashita A, Tanabe H, Nishimata N, Nagahama T, Maki S, Takaki Y, Hirai F, Hisabe T, Nishimura T, Matsui T (2008) White opaque substance within superficial elevated gastric neoplasia as visualized by magnification endoscopy with narrow-band imaging: a new optical sign for differentiating between adenoma and carcinoma. Gastrointest. Endosc 68: 574–580.

14. Yao K, Iwashita A, Nambu M, Tanabe H, Nagahama T, Maki S, Ishikawa H, Matsui T, Enjoji M (2012) Nature of white opaque substance in gastric epithelial neoplasia as visualized by magnifying endoscopy with narrow-band imaging. Dig. Endosc 24: 419-425.

15. Ueo T, Yonemasu H, Yada N et al (2013) White opaque substance represents an intracytoplasmic accumulation of lipid droplets: immunohistochemical and immunoelectron microscopic investigation of 26 cases. Dig. Endosc 25:

147-155.

16. Straub BK, Herpel E, Singer S, Zimbelmann R, Breuhahn K, Macher-Goeppinger S, Warth A, Lehmann-Koch J, Longerich T, Heid H, Schirmacher P (2010) Lipid droplet-associated PAT-proteins show frequent and differential expression in neoplastic steatogenesis. Mod. Pathol 23: 480-492.

17. Heid HW, Moll R, Schwetlick I, Rackwitz HR, Keenan TW (1998) Adipophilin is a specific marker of lipid accumulation in diverse cell types and disease. Cell Tissue Res 294: 309-321.

18. Kabashima A, Yao T, Sugimachi K, Tsuneyoshi M (2000) Gastric or intestinal phenotypic expression in the carcinomas and background mucosa of multiple early gastric carcinomas. Histopathology 37: 513-522.

19. Matsubara J, Honda K, Ono M et al (2011) Identification of adipophilin as a potential plasma biomarker for colorectal cancer using label-free quantitative mass spectrometry and protein microarray. Cancer Epidemiol Biomarkers Prev 20: 2195-2203.

20. Siurala M, Tarpila S (1968) Absorptive function of intestinal metaplasia of the stomach. Scand J Gastroenterol 3: 76–79.

21. Tanaka M, Usuda K, Ookura Y et al. Significance of magnifying endoscopy in diagnosis of duodenal elevated lesions [in Japanese with English abstract]. Stomach and Intestine 2003; 38: 1709-1720.

22. West AB, Isaac CA, Carboni JM, Morrow JS, Mooseker MS, Barwick KW (1988) Localization of villin, a cytoskeletal protein specific to microvilli, in human ileum and colon and in colonic neoplasms. Gastroenterology 94: 343-352.

23. Groisman GM, Amar M, Livne E (2002) CD10: a valuable tool for the light microscopic diagnosis of microvillous

inclusion disease (familial microvillous atrophy). Am J Surg Pathol 26: 902-907.

24. Niwa T, Ikehara Y, Nakanishi H, Tanaka H, Inaba K, Tsukamoto T, Ichinose M, Tatematsu M (2005) Mixed gastric- and intestinal-type metaplasia is formed by cells with dual intestinal and gastric differentiation. J Histochem Cytochem 53: 75-85.

25. Tajima Y, Shimoda T, Nakanishi Y, Yokoyama N, Tanaka T, Shimizu K, Saito T, Kawamura K, Kusano M, Kumagai K (2001) Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: Immunohistochemical analysis of 136 lesions. Oncology 61: 212-220.

26. Yamachika T, Inada K, Fijimitsu Y, Nakamura S, Yamamura Y, Kitou T, Itzkowitz SH, Werther JL, Miki K, Tatematsu M (1997) Intestinalization of gastric signet ring cell carcinomas with progression. Virchows Arch 431: 103-110.

27. Tsukashita S, Kushima R, Bamba M, Sugihara H, Hattori T (2001) MUC gene expression and histogenesis of adenocarcinoma of the stomach. Int J Cancer 94: 166-170.

Table 1. Phenotypic classification by immunohistochemical stains

<table>
<tr><td colspan="2"></td><td>MUC5AC (-)
and
MUC6 (-)</td><td>MUC5AC (+)
or
MUC6 (+)</td></tr>
<tr><td colspan="2">CD10 (+) or Villin (+)</td><td>Complete intestinal type
(C-type)</td><td rowspan="2"></td></tr>
<tr><td rowspan="2">CD10 (-)
and
Villin (-)</td><td>MUC2 (+)</td><td colspan="2">Incomplete intestinal type
(I-Type)</td></tr>
<tr><td>MUC (-)</td><td>Unclassified type
(U-type)</td><td>Gastric type
(G-type)</td></tr>
</table>

Table 2. Correlation between clinicopathological features and adipophilin expression in gastric epithelial neoplasias

	Adipophilin		
	Positive	Negative	*P* -value
Number (n)	106	53	
Age (years)	70.6±7.8	72.2±7.5	0.207
Sex			0.739
Male	80	42	
Female	26	11	
Tumor size (mm)	16.9±11.3	15.3±12.6	0.43
Macroscopic type			<0.001
elavated	74	20	
flat	0	1	
depressed	32	32	
Location			0.101
Upper	10	10	
Middle	52	18	
Lower	44	25	
Vienna classification			0.043
adenoma with low grade dysplasia (Category 3)	41	11	
Noninvasive high-grade neoplasia (Category 4)	42	23	
Invasive neoplasia (Category 5)	23	19	
Mucin phenotype			<0.001
C-type	55	13	
I-type	51	34	
G-type	0	6	

Table 3. Mucin phenotype in gastric epithelial neoplasia.

	C-type	I-type	G-type	U-type
Adenoma with low grade dysplasia [Category 3] (n=52)	36 (69%)	15 (29%)	1 (2%)	0 (0%)
Noninvasive high-grade neoplasia [Category 4] (n=65)	22 (34%)	41 (63%)	2 (3%)	0 (0%)
Adenoma with high grade dysplasia [Category 4.1] (n=5)	2 (40%)	3 (60%)	0 (0%)	0 (0%)
Noninvasive carcinoma [Category 4.2] (n=60)	20 (33%)	38 (63%)	2 (4%)	0 (0%)
Invasive neoplasia [Category 5] (n=42)	10 (24%)	29 (69%)	3 (7%)	0 (0%)
Intramucosal carcinoma [Category 5.1] (n=17)	4 (24%)	13 (76%)	0 (0%)	0 (0%)
Submucoal carcinoma [Category 5.2] (n=25)	6 (24%)	16 (64%)	3 (12%)	0 (0%)

P=0.001: Category 3 versus Category 4. P<0.001: Category 3 versus Category 5. P=0.387: Category 4 versus Category 5.

Table 4. Mucin phenotypic characterization in adipophilin-positive gastric epithelial neoplasia.

	C-type	I-type	G-type	U-type
Adenoma with low grade dysplasia [Category 3] (n=41)	29 (71%)	12 (29%)	0 (0%)	0 (0%)
Noninvasive high-grade neoplasia [Category 4] (n=42)	19 (45%)	23 (55%)	0 (0%)	0 (0%)
Adenoma with high grade dysplasia [Category 4.1] (n=5)	2 (40%)	3 (60%)	0 (0%)	0 (0%)
Noninvasive carcinoma [Category 4.2] (n=37)	17 (46%)	20 (54%)	0 (0%)	0 (0%)
Invasive neoplasia [Category 5] (n=23)	7 (30%)	16 (70%)	0 (0%)	0 (0%)
Intramucosal carcinoma [Category 5.1] (n=9)	2 (22%)	7 (78%)	0 (0%)	0 (0%)
Submucoal carcinoma [Category 5.2] (n=14)	5 (36%)	9 (64%)	0 (0%)	0 (0%)

P=0.033: Category 3 versus Category 4. P=0.004: Category 3 versus Category 5. P=0.366: Category 4 versus Category 5.

Table 5. Distribution pattern of adipophilin expression in gastric epithelial neoplasia and background non-neoplastic mucosa

	Distribution			
	Surface	Surface plus crypt	Crypt	None
Adenoma with low grade dysplasia [Category 3] (n=52)	64% (33/52)	15% (8/52)	0% (0/52)	21% (11/52)
C-type	21	8	0	7
I-type	12	0	0	3
G-type	0	0	0	1
Noninvasive high-grade neoplasia [Category 4] (n=65)	34% (22/65)	29% (19/65)	2% (1/65)	35% (23/65)
C-type	6	13	0	3
I-type	16	6	1	18
G-type	0	0	0	2
Adenoma with high grade dysplasia [Category 4.1] (n=5)	60% (3/5)	40% (2/5)	0% (0/5)	0% (0/5)
C-type	0	2	0	0
I-type	3	0	0	0
G-type	0	0	0	0
Noninvasive carcinoma [Category 4.2] (n=60)	32% (19/60)	28% (17/60)	2% (1/60)	38% (23/60)
C-type	6	11	0	3
I-type	13	6	1	18
G-type	0	0	0	2
Invasive neoplasia [Category 5] (n=42)	7% (3/42)	48% (20/42)	0% (0/42)	45% (19/42)
C-type	1	6	0	3
I-type	2	14	0	13
G-type	0	0	0	3
Intramucosal carcinoma [Category 5.1] (n=17)	6% (1/17)	47% (8/17)	0% (0/17)	47% (8/17)
C-type	0	2	0	2
I-type	1	6	0	6
G-type	0	0	0	0
Submucoal carcinoma [Category 5.2] (n=25)	8% (2/25)	48% (12/25)	0% (0/25)	44% (11/25)
C-type	1	4	0	1
I-type	1	8	0	7
G-type	0	0	0	3
Background non-neoplastic mucosa (n=159)	14% (23/159)	2% (3/159)	0% (0/159)	84% (133/159)
C-type	3	0	0	10
I-type	20	3	0	101
G-type	0	0	0	22
H.pylori infection status				
H.pylori positive (n=77)	7	1	0	69
Post *H.pylori* infection (n=80)	16	2	0	52
H.pylori negative (n=2)	0	0	0	2

P=0.014: Category 3 versus Category 4. P<0.001: Category 3 versus Category 5. P=0.010: Category 4 versus Category 5.

Figure 1

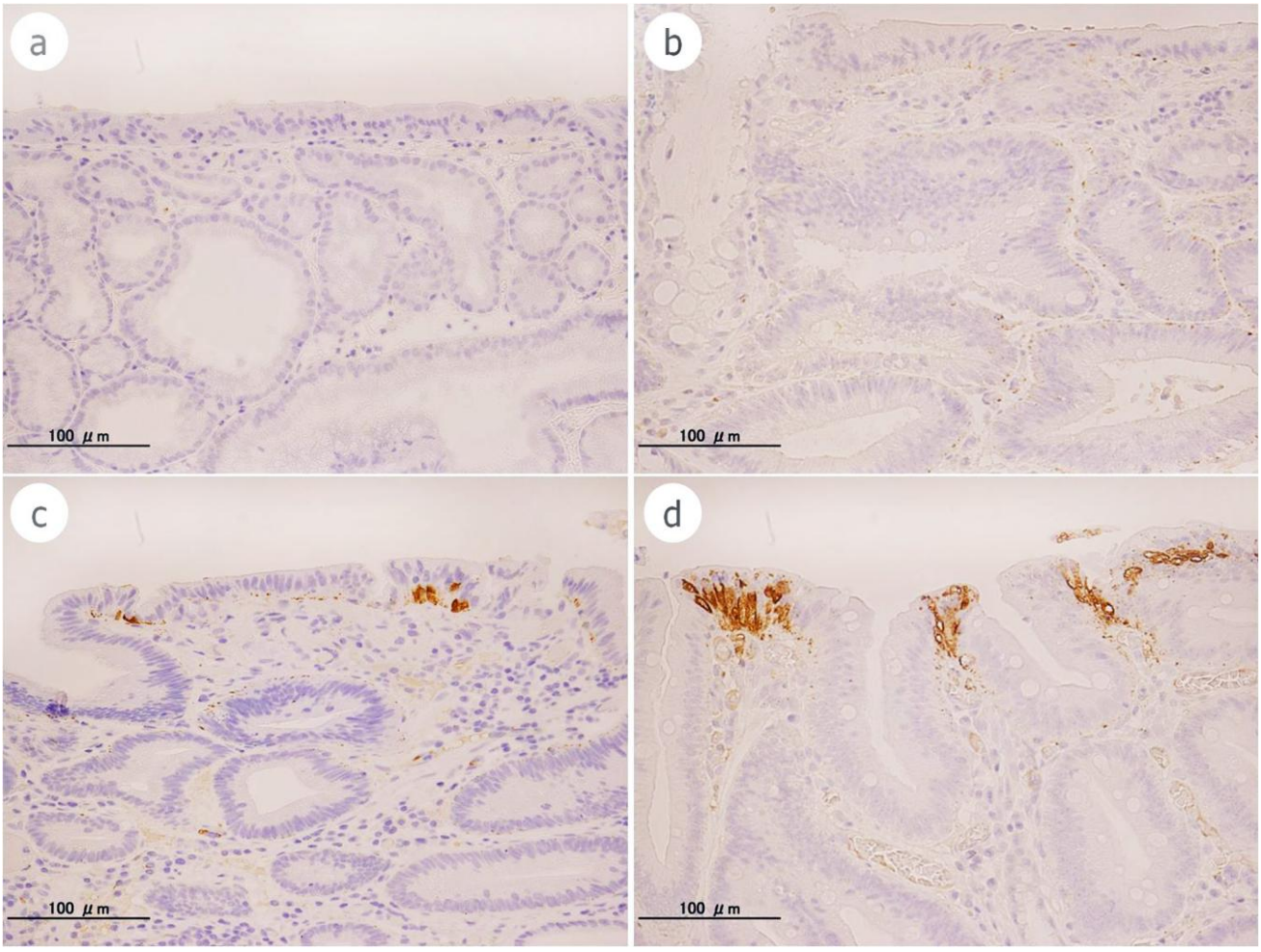

Figure 2

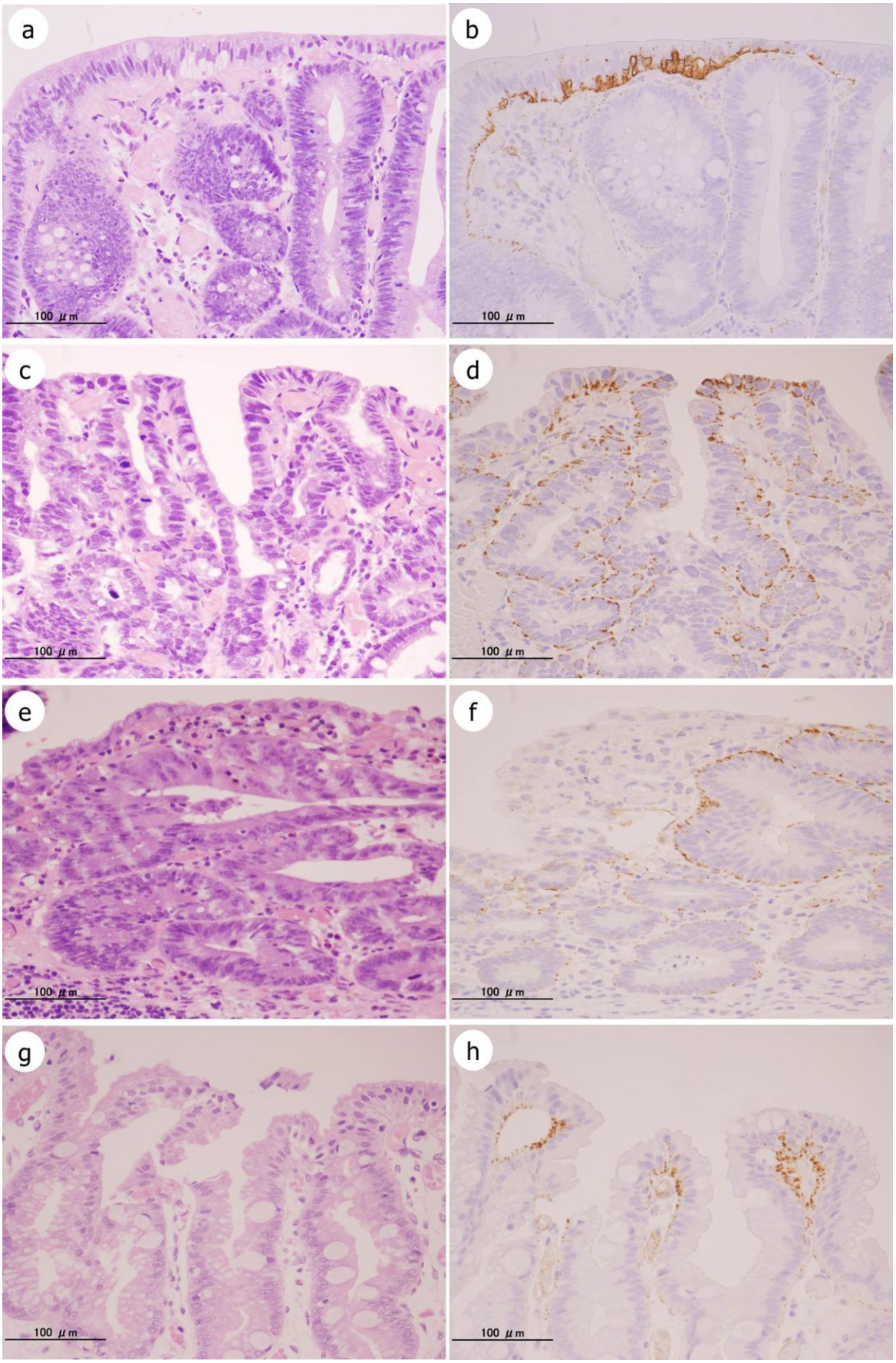

Figure Captions

Fig. 1 Immunohistochemical staining of adipophilin. Staining intensity of adipophilin was scored as 0 (no stain, a), 1+ (weakly stain, b), 2+ (focally strong stain, c), and 3+ (diffusely strong stain, d). (b) Small dot-like stainings were detected at the basal part of epithelial cells. (c, d) Adipophilin was expressed at the basal side of intracytoplasmic vacuoles.

Fig. 2 Adipophilin expression in gastric epithelial neoplasia and background non-neoplastic gastric muocsa. (a, b) Category 3 lesions, (c, d) Category 5 lesions, (e, f) Category 4 lesions, and (g, h) background non-neoplastic gastric mucosa. (b, f) Adipophilin was detected in the surface epithelium. (d) Adipophilin was detected in the surface and cryptal epithelium. (f) Adipophilin was detected in cryptal epithelium.