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ORIGINAL ARTICLE

Nature of white opaque substance in gastric epithelial neoplasia as visualized by magnifying endoscopy with narrow-band imaging

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Background and Aims: Magnifying endoscopy (ME) with narrow-band imaging (NBI) revealed a white opaque substance (WOS) within the superficial part of the gastric neoplasia; however, its nature has remained obscure. A WOS noted within the duodenum was reported to comprise lipid droplets (LD) absorbed by the duodenal epithelium. We attempted to ascertain whether the WOS within gastric neoplasia could also comprise LD and whether the presence of this WOS could be correlated with a specific phenotype.

Methods: Forty-three patients with early gastric epithelial neoplasia underwent ME with NBI. The presence or absence of WOS in the neoplasias was recorded based on the findings of ME with NBI. One biopsy specimen was taken from each of the neoplasias. Cryostat sections underwent oil red O staining for LD. Serial sections were immunostained using the first antibody of CD10, MUC2, CDX2, human gastric mucin, MUC5AC and MUC6. The tissue phenotype was classified as intestinal (I), gastric (G) and gastrointestinal (GI) type based on the results of immunostaining. In total, 49 gastric neoplasias from 43 patients were investigated.

Results: Prevalence of LD in WOS-positive versus WOS-negative lesions was 96.2% (25/26) and 4.3% (1/23), respectively ($P < 0.001$, Fisher's exact test). WOS was present in GI- and I-type lesions, but not in G-type lesions.

Conclusions: WOS may be LD that have been accumulated in the superficial part of the gastric neoplasia of a certain intestinal phenotype.

Key words: fat droplet, gastric cancer, gastric epithelial neoplasia, lipid droplet, white opaque substance.

INTRODUCTION

The subepithelial microvascular architecture as visualized by high-resolution magnifying endoscopy (ME) is a reliable marker for making a precise diagnosis of gastrointestinal pathology.[1–4] When we incorporate narrow-band imaging (NBI)[5] with ME, we can clearly visualize both the subepithelial microvascular architecture and the microsurface structure.[6,7] However, we sometimes encounter difficulties when attempting to observe the subepithelial microvascular architecture in neoplastic lesions within the stomach, even by ME with NBI, because a white opaque substance (WOS) within the superficial part of the early gastric neoplasia obscures the microvessels that are just beneath the neoplastic epithelium.[8–11] We have already reported that the morphology of the WOS as visualized by ME is a new optical marker for differentiating between low-grade dysplasia and high-grade dysplasia/early carcinoma, making it an alternative to microvascular architecture.[8–11] However, the precise nature of this WOS is still unknown.

It has been reported that the endoscopic findings of a whitish duodenal non-neoplastic mucosa in both normal subjects[12] and patients with chylomicron retention disease[13,14] is due to lipid droplets (LD) that have substantially accumulated within the enterocytes. In addition, it has been reported that a WOS is also present in sporadic epithelial neoplasias within the duodenum and that the nature of this WOS could be LD that have accumulated within the duodenal neoplastic epithelium.[15] Accordingly, we assumed that the WOS within the gastric neoplasia could also be made up of LD, as is the case within the duodenum, if the gastric neoplasia acquired a certain phenotype similar to the small intestine. We thus attempted to ascertain first whether the WOS is made up of LD accumulated in gastric neoplasia and second whether the presence of this WOS could be correlated with a specific gastric neoplasia phenotype.

METHODS

Patients and endoscopic procedures

A total of 185 patients were referred to our endoscopic department for further endoscopic examination of known

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early gastric epithelial neoplasia between December 2009 and November 2010. Of these, we included consecutive patients who fulfilled the following inclusion criteria: (i) patients who gave written informed consent; (ii) patients who were assigned to the endoscopy lists of two experienced endoscopists, KY and TN; and (iii) patients who underwent ME with NBI. Patients who were receiving warfarin or any other anticoagulant treatment and patients who did not give informed consent were excluded from this study. Neoplasia of less than 10 mm in size was excluded in order to ensure that an adequate biopsy sample could be obtained from the neoplasia. This study was approved by the Medical Ethics Committee of Fukuoka University Chikushi Hospital.

Written informed consent was obtained from 43 patients. After fasting for 12 h before the endoscopic examination, they underwent ME with NBI which was carried out by two experienced endoscopists (KY and TN) who are familiar with this procedure and who understand the findings of ME with NBI, using a high-resolution magnifying upper GI endoscope (GIF-Q240Z; Olympus, Tokyo, Japan) or a high-definition magnifying upper GI endoscope (GIF-H260Z; Olympus), and an electronic endoscopy system (EVIS LUCERA SPECTRUM; Olympus), as described previously.[9]

When an epithelial neoplastic lesion was found during non-magnifying observation with white light imaging, visualization of the lesion was immediately zoomed up to maximal magnification with NBI. The presence or absence of a WOS was recorded for each of the neoplasias based on the findings of ME with NBI.

One targeted biopsy specimen was taken from each of the neoplasias and all the specimens were embedded in Tissue-Tek OCT compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands) and immediately frozen in liquid nitrogen-cooled isopentane (Sigma-Aldrich, Dorset, UK). Serial cryostat sections (5-μm thick) were cut at –20°C and the sections were mounted on glass slides for oil red O staining. The remainder of each specimen was fixed with 20% formalin overnight and embedded in paraffin. Seven serial sections (5-μm thick) were cut. One was stained with hematoxylin and eosin (HE) for standard histological investigations and the others were prepared for immunohistochemistry.

Oil red O staining

In order to investigate lipid accumulation in the gastric neoplasias, LD were observed using the oil red O staining method as described elsewhere.[16] First, the dried frozen sections were fixed with 20% formalin for 15 min and then the sections were washed with running tap water for 5 min. After the sections were rinsed with 60% isopropanol, they were stained with freshly prepared oil red O working solution for 15 min. Then the sections were again rinsed with 60% isopropanol. Counterstaining with Mayer's hematoxylin was carried out for 5 min and then the sections were rinsed with distilled water. Finally, they were mounted in glycergel mounting medium (Dako North America Inc., Carpinteria, CA, USA).

We prepared the reagent according to the following method.

1. Oil red O stock stain:

Dye (0.5 g oil red O (color index: 26125)) was dissolved in 100 mL isopropanol, using the very gentle heat of a water bath.

2. Oil red O working solution:

For use, the stock stain was diluted in 20 mL distilled water and was allowed to stand for 10 min. After the diluted solution was filtered into a Coplin staining jar, it was covered immediately. In each instance, the working solution was freshly prepared according to the above procedure, as the stain can easily become aggregated.

Immunohistochemistry and classification of phenotypes

After deparaffinization and rehydration, the sections were incubated with Super Block (ScyTek, Logan, UT, USA). To classify phenotypic expression, the presence of CD10, MUC2, CDX2, human gastric mucin (HGM), MUC5AC or MUC6 was investigated by immunohistochemical methods, according to previous studies.[17,18] We used monoclonal antibodies as the first antibodies as follows: CD10 (Leica Biosystems, Newcastle, UK), MUC2 (Leica Biosystems), CDX2 (BioGenex Laboratories, San Ramon, CA, USA), HGM (Leica Biosystems), MUC5AC (Leica Biosystems), and MUC6 (Leica Biosystems). Immunostaining was carried out using the labeled streptavidin biotin method that consists of a secondary antibody of biotinylated horse antimouse IgG (H + L) (Vector Laboratories, Burlingame, CA, USA) and alkaline phosphatase-conjugated avidin (Vector Laboratories).[19] When more than 5% of the neoplastic cells in the neoplastic areas were stained, it was classified as positive expression. When fewer than 5% of the neoplastic cells in the neoplastic areas were stained, it was classified as negative expression.

Histopathological assessment

Standard histological diagnosis was made by a single experienced pathologist (AI) who was blinded to the endoscopic findings. Diagnosis was based on either biopsied specimens or resected specimens according to the revised Vienna classification.[20]

Histological investigation of LD and the phenotypic classification of neoplasia were made by an experienced pathologist (HT) who was blinded to the endoscopic findings as follows. By histological investigation of the section stained by oil red O at 100× magnification rate, the presence or absence of LD was determined. If LD were evident, the histological distribution of the droplets was further recorded according to the localization of the droplets; that is: (i) they were present at a relatively elevated apical part between the crypts, at a cryptal part or at both an apical part and a cryptal part; and (ii) they showed intraepithelial, subepithelial or both intraepithelial and subepithelial distribution. The neoplastic phenotype was classified as intestinal (I) type if the neoplastic epithelium was positive for either CD10, MUC2 or CDX2; gastric (G) type if it was positive for either HGM, MUC5AC or MUC6; and gastrointestinal (GI) type if it showed both gastric and intestinal phenotypes.[17,18]

Statistical analysis

Comparison of the prevalence between the two groups was made by chi-squared test or Fisher's exact test. Statistical significance was taken as a P-value <0.05. SPSS 10.5J for Windows was used for statistical processing.

RESULTS

A total of 49 gastric epithelial neoplasias from 43 patients were included in this study. The average age (range) of the patients was 71 years (48–85 years). The male : female ratio was 30:13. Regarding the macroscopic findings of neoplasias according to the Paris classification,[21] the number of protruded (0-I), superficial-elevated (0-IIa), superficial-flat (0-IIb), and superficial-depressed (0-IIc) types was 1, 30, 3 and 15, respectively. When we divided the stomach into upper third (U), middle third (M) and lower third (L), four, 29 and 16 lesions were located in the U, M and L parts, respectively. Eleven neoplasias were followed up after a biopsy was taken. Twenty-seven lesions were completely resected by endoscopic submucosal dissection (ESD). The remaining 11 lesions were treated by surgical resection because the lesions were preoperatively diagnosed as carcinomas that had invaded the submucosa. The final histological diagnosis according to the revised Vienna classification was 19 low-grade neoplasias (Category 3), 13 high-grade neoplasias (Category 4) and 17 invasive carcinomas (Category 5).

Regarding the prevalence of the WOS based on the findings of ME with NBI, 26 (53.1%) of the 49 neoplasias demonstrated a WOS in the superficial part of the neoplasias. However, 23 (46.9%) of the 49 neoplasias did not show any WOS. Instead, the subepithelial microvascular architecture was clearly visualized in all of these 23 neoplasias.

The presence of the WOS as visualized by ME with NBI was dependent upon the presence of LD as histologically visualized by oil red O staining (Table 1, Figs 1–3). With regard to the 26 neoplasias that showed a WOS, 25 (96.2%) of 26 lesions were positive for LD, with only one (3.8%) lesion showing no LD. In contrast, with regard to the 23 neoplasias that did not demonstrate a WOS, only one (4.3%) of the 23 lesions was positive for LD, whereas 22 (95.7%) lesions did not show any LD. This strong correlation between the presence of a WOS and the presence of LD was statistically significant ($P < 0.001$, Fisher's exact test).

When we investigated the histological localization of LD for the 26 lesions that showed LD, the LD were not distributed within the crypt epithelium, but rather were found only at a relatively apical part between the crypts (Fig. 4). With regard to the vertical distribution of LD, in 10 (36.5%) of the 26 lesions, they were located within the surface epithelial cells (intraepithelial distribution) (Fig. 3) and in 16 (61.5%) of the lesions, they were located both in the epithelial cells (intraepithelial distribution) and in the superficial part of the lamina propria just beneath the surface epithelium (subepithelial distribution) (Table 2, Fig. 2).

Table 1. Histological prevalence of LD by oil red O staining according to the presence of WOS by ME with NBI

		WOS			
		Positive ($n = 26$)		Negative ($n = 23$)	
LD	Positive	25	(96.2%)	1	(4.3%)
	Negative	1	(3.8%)	22	(95.7%)

ME, magnifying endoscopy; LD, lipid droplets; NBI, narrow-band imaging; WOS, white opaque substance.

According to the results obtained by immunohistochemistry, 11, 17 and 21 of the 49 lesions were classified as G, GI and I type, respectively. The WOS was only present in either GI or I type; in contrast, the WOS was absent in G type (Table 3). The prevalence of the WOS was more evident in GI and I type than in G type ($P < 0.001$, Fisher's exact test).

DISCUSSION

In the present study, we demonstrated a strong correlation between the WOS as visualized by ME with NBI and lipid micro-droplets accumulated in the superficial (intraepithelial and subepithelial) part of gastric epithelial neoplasias (adenoma and cancer).

The reason why accumulated LD contribute to visualization of WOS was elucidated in bio-optical studies.[22,23] As LD have a higher reflective index than those of intracellular organelles and organic components of tissue,[22,23] and LD are categorized as Mie's scattering particles,[23] it follows that projected light is strongly scattered and reflected by LD. Accordingly, when LD accumulated in the epithelial and the subepithelial part of the mucosa strongly scatter and reflect projected light, the projected light cannot reach hemoglobin in microvessels located underneath the epithelium. Therefore, the opacity is increased. In addition, such strong backward scattering and reflection of light is recognized as white coloration by the human eye. Accordingly, accumulated LD may be the cause of WOS. In other words, this is the nature of the WOS that we previously reported in our clinical observation.[8–10] Prior to this study, this phenomenon had not been reported in gastric neoplasias.

The mechanism of accumulation of LD in the epithelium and subepithelium is still unknown. There are two possible mechanisms, one being that the LD are derived from external lipid absorbed by the epithelial surface (absorption hypothesis). The other is that neoplastic cells themselves synthesize LD from glucose or lipid supplied by capillaries (production hypothesis).[24] We are uncertain which hypothesis is correct.

In the case of intestinal epithelium and intestinal metaplasia, accumulated lipids are thought to be derived from digested micellar lipid.[25–28] In order to absorb lipid, lipolysis and micellar formation are essential. Up to 20% of dietary triglycerides can be hydrolyzed within the normal stomach.[26] Furthermore, bile and pancreatic juice that has been regurgitated into the atrophic stomach, whose luminal content is not acid, may have been able to hydrolyze triglycerides within the lumen and even to form micelles. If the amount of lipid accumulation increased after we loaded micellar lipids on the surface of the neoplasias, it would suggest that LD are resynthesized from external digested lipid (fatty acids and mono- or diglycerides). In fact, according to our preliminary observations, when we loaded patients with micellar lipid, WOS positivity and density increased (Yao K., unpubl. data, 2011). Accordingly, we speculate that resynthesis of triglycerides from absorbed external lipids may at least be one of the mechanisms for accumulation of LD. However, these observations need to be tested in further well-designed studies. In addition, further intensive laboratory work is needed to investigate absorbed lipid transfer and metabolism to clarify the actual mechanisms.

It has been proposed that the metabolism of cancer cells, and all proliferating cells, adapts to facilitate the uptake and

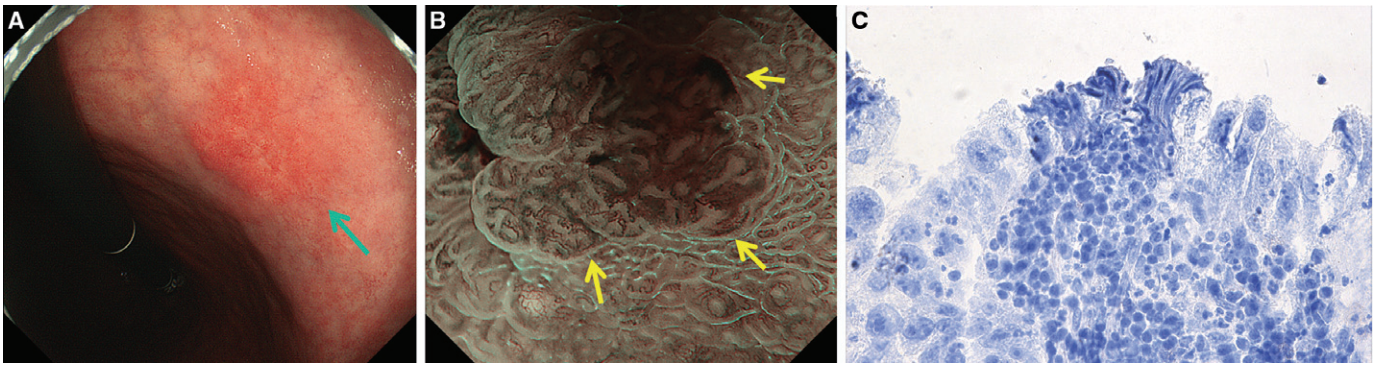

Fig. 1. (a) Standard endoscopic findings with white light of high-grade neoplasia of 0-IIa type. Slightly elevated reddened lesion (arrow) is present at the posterior wall of the gastric body. (b) Magnifying endoscopy (ME) with narrow-band imaging (NBI) findings. When we observed the margin of the lesion by ME with NBI, there was a clear demarcation line (arrow) between the background mucosa and the lesion. At that demarcation line, both the microvascular architecture and the microsurface structure in the background mucosa disappeared. Instead, dense brownish microvessels in the superficial part of the neoplasia became clearly visualized by ME with NBI. (c) Histopathological findings (oil red O staining, 100×). In the superficial part of the mucosa, no LD were detected by oil red O staining.

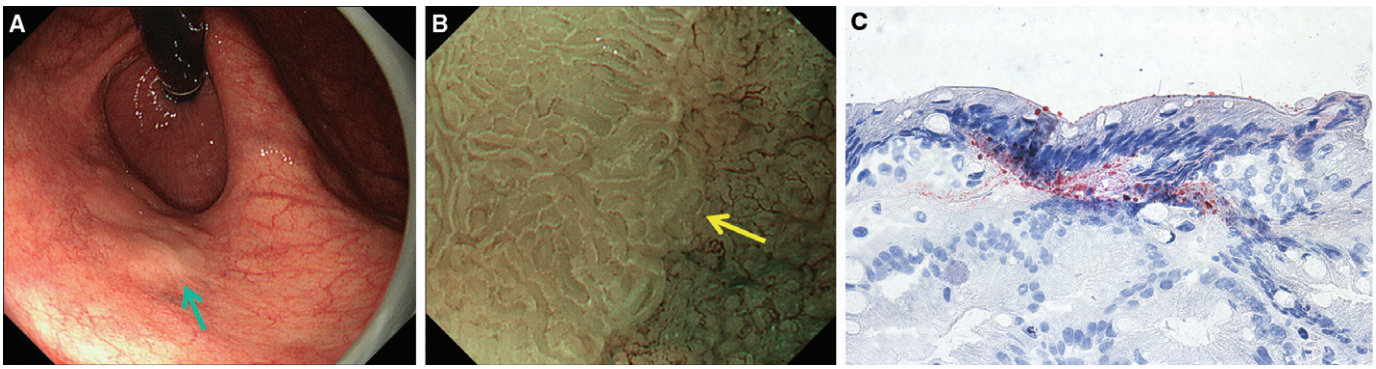

Fig. 2. (a) Standard endoscopic findings with white light of low-grade neoplasia (adenoma) of 0-IIa type. Superficial pale elevated lesion (arrow) can be seen at the lesser curvature of the gastric cardia. (b) Magnifying endoscopy (ME) with narrow-band imaging (NBI) findings. When we magnified the marginal part of the neoplasias, distinct brownish subepithelial capillaries were seen in the background mucosa. However, in the neoplastic mucosa within the margin of the lesion (arrow), the microvascular pattern could not be visualized because some WOS obscured the subepithelial microvascular architecture beneath the neoplastic epithelium. (c) Histopathological findings of the biopsied specimen from the neoplasia (oil red O staining, 100×). Oval amorphous LD in various sizes have accumulated within both the epithelium and the subepithelial part of the neoplastic tissue.

incorporation of nutrients into the biomass (nucleotides, amino acids, lipids etc.) needed to produce new cells. This is supported by the observation that certain cancer-associated mutations enable cancer cells to acquire and metabolize nutrients in a manner conducive to proliferation rather than to adenosine 5′-triphosphate (ATP) production.[24] We should also take this mechanism into consideration. Further intensive basic laboratory studies are required to investigate whether neoplastic cells synthesize LD.

The absorbed LD were located only at the surface of the mucosa of the relatively apical part between the crypts and there were no LD in the cryptal epithelium. This localization of LD to the apical part correlates well with previously reported endoscopic findings as visualized by ME with NBI (i.e. WOS is invariably detected within the epithelium of the intervening (apical) part between the cryptal epithelium).[7,9,11] The cause of this localization is unclear. We speculate that this might be because the surface epithelium at the apical parts has a greater chance to expose lipid emulsion than the cryptal epithelium, which is usually filled with rich mucus, or because the surface epithelial cells are more differentiated than the cryptal epithelium within the neoplasias. Further study is needed in order to clarify this characteristic localization of LD.

It has been suggested that oil red O, which detects lipid largely by its solubility in lipid materials, may have been unable to detect micellar lipid because micelle itself has a hydrophobic nature.[26] The intraepithelial LD as visualized by oil red O staining may be composed of triglycerides which have been resynthesized by neoplastic cells with an intestinal phenotype from the micellar fatty acid and monoglycerides, or may simply be chylomicron.

Even after the patients had fasted for more than 12 h, LD were retained within the epithelium and in the subepithelial part. In the case of the normal small intestine, chylomicron is drained into the lymphatics within the lamina propria at the tip of the intestinal villi. It is possible that this retention is not due to the lack of beta-lipoprotein, but rather is due to the

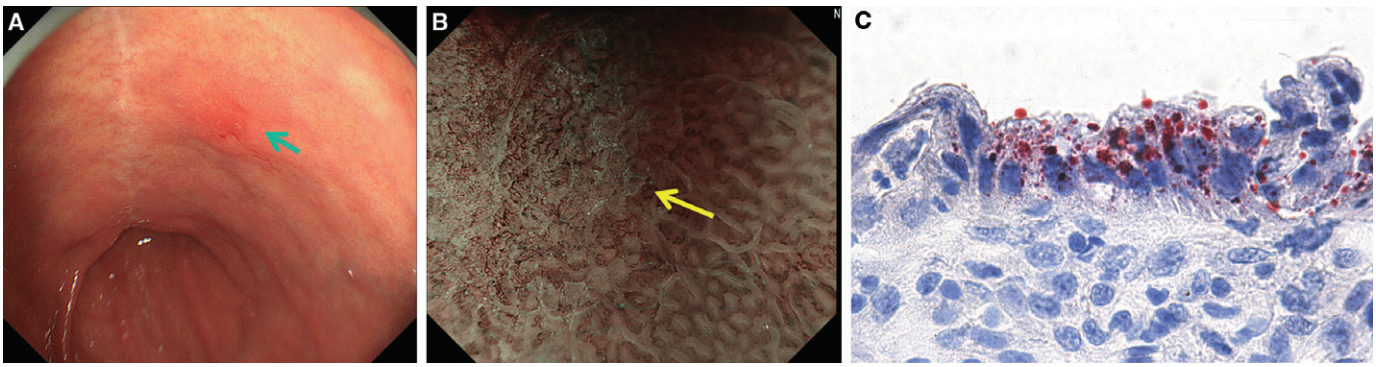

Fig. 3. (a) Standard endoscopic findings with white light of high-grade neoplasia of 0-IIc type. Slightly depressed reddened lesion (arrow) can be seen at the posterior wall of the gastric antrum. (b) Magnifying endoscopy (ME) with narrow-band imaging (NBI) findings. There is a clear demarcation line (arrow) between the background mucosa and the neoplastic lesion. Brownish subepithelial capillaries can be clearly visualized in the background mucosa. In contrast, as a fine speckled WOS is present in the neoplasia within the demarcation line, the subepithelial microvascular architecture cannot be visualized clearly. (c) Histopathological findings of the biopsied specimen from the neoplasia (oil red O staining, 200×). By oil red O staining, numerous round or oval amorphous LD are demonstrated within the neoplastic epithelium alone.

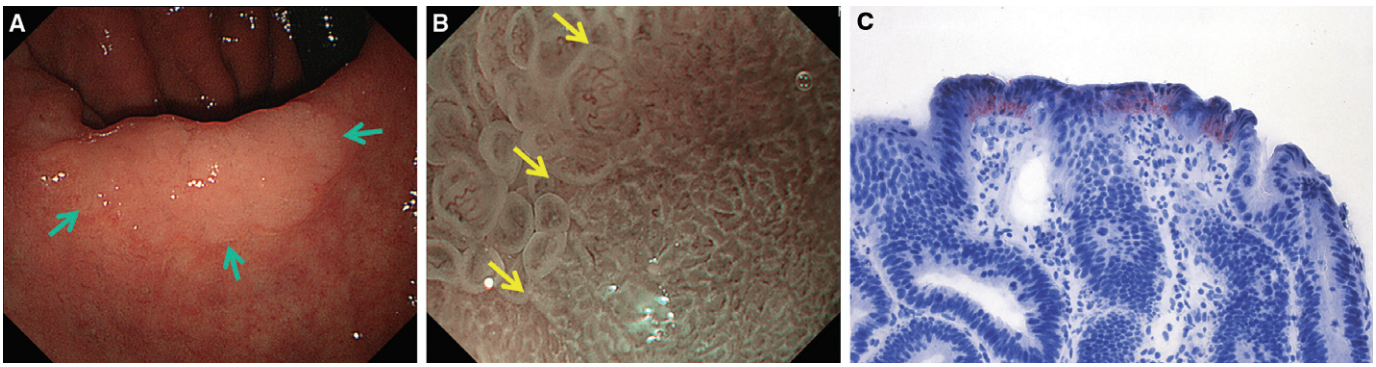

Fig. 4. (a) Standard endoscopic findings with white light of high-grade neoplasia of 0 IIa type. Slightly elevated pale lesion (arrows) is present at the lesser curvature of the gastric angulus. (b) Magnifying endoscopy (ME) with narrow-band imaging (NBI) findings. At the margin of the lesion, there is a clear demarcation line (arrows) between the background mucosa and the lesion. In the background mucosa, brownish subepithelial capillaries are clearly visualized by ME with NBI, but as the WOS in reticular morphology was present in the neoplasia within the demarcation line, the subepithelial microvascular pattern of the neoplasia is totally obscured. (c) Histopathological findings of the biopsied specimen from the neoplasia (oil red O staining, 50×). The accumulated LD can be detected only in the epithelium of the relatively elevated apical part between the crypts; they are absent in the cryptal part of the epithelium.

anatomical structure of the lamina propria in the stomach which is different from that of the small intestine. In the case of chylomicron retention disease, the accumulation of LD is limited to the epithelium. However, in our study, 61.5% of the lipid droplet-positive neoplasias showed both intraepithelial and subepithelial accumulation of LD. Therefore, it is speculated that the accumulated LD had been transported to the subepithelial part after forming chylomicron in the neoplastic cells with the intestinal phenotype. Nevertheless, as the mucosal lymphatics in the stomach are anatomically present only in the deepest level of the lamina propria,[29] LD cannot be easily transported into the lymphatics. Consequently, they may be retained within the superficial part of the mucosa for a longer period.

Although the presence of the WOS was dependent upon the presence of LD, we encountered two exceptions. In one case, WOS was detected by endoscopic observation using ME with NBI, but LD were not present on histological examination. To determine the reason, we reviewed the endoscopic findings using ME with NBI of that case, finding the density of the WOS was sparse and the distribution patchy. Accordingly, we speculated that a very thin section of the biopsy specimen failed to contain LD. The other case showed that although WOS was not identified using ME with NBI, LD were detected in the histological specimens. When we reviewed the histological findings carefully, we noticed that the size and the density of the LD were very small and remarkably low, respectively, compared with those in other LD-positive cases. Accordingly, we speculated that small low-density LD in the cells may not cause strong backward scattering of the light. However, we need to conduct further studies to clarify whether visualization of WOS depends upon the size and density of LD.

In conclusion, we have reported a novel and unique bio-optical finding, that is, the nature of WOS is the visualization

Table 2. Histological distribution of LD ($n = 26$)

Distribution		No. lesions
Intraepithelial	10	38.5%
Intraepithelial + subepithelial	16	61.5%
Subepithelial	0	0%

LD, lipid droplets.

Table 3. Phenotypic characterization according to the presence of the WOS

		WOS			
		Positive ($n = 26$)		Negative ($n = 23$)	
Phenotype	G	0	(0%)	11	(47.8%)
	GI	13	(50%)	4	(17.4%)
	I	13	(50%)	8	(34.8%)

G, gastric phenotype; GI, gastrointestinal phenotype; I, intestinal phenotype; WOS, white opaque substance.

of LD accumulated in the superficial part of epithelial neoplasias within the stomach.

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REFERENCES

1. Yao K, Oishi T, Matsui T *et al*. Novel magnified endoscopic findings of microvascular architecture in intramucosal gastric cancer. *Gastrointest. Endosc.* 2002; **56**: 279–84.
2. Yao K, Iwashita A, Tanabe H *et al*. Novel zoom endoscopy technique for diagnosis of small flat gastric cancer, a prospective, blind study. *Clin. Gastroenterol. Hepatol.* 2007; **5**: 869–78.
3. Muto M, Minashi K, Yano T *et al*. Early detection of superficial squamous cell carcinoma in the head and neck region and esophagus by narrow band imaging: A multicenter randomized controlled trial. *J. Clin. Oncol.* 2010; **28**: 1566–72.
4. Higashi R, Uraoka T, Kato J *et al*. Diagnostic accuracy of narrow-band imaging and pit pattern analysis significantly improved for less-experienced endoscopists after an expanded training program. *Gastrointest. Endosc.* 2010; **72**: 127–35.
5. Gono K, Yamazaki K, Doguchi N *et al*. Endoscopic observation of tissue by narrowband illumination. *Opt. Rev.* 2003; **10**: 1–5.
6. Yao K, Takaki Y, Matsui T *et al*. Clinical application of magnification endoscopy and narrow-band imaging in the upper gastrointestinal tract: New imaging techniques for detecting and characterizing gastrointestinal neoplasia. *Gastrointest. Endosc. Clin. N. Am.* 2008; **18**: 415–33.
7. Yao K, Anagnostopoulos GK, Ragunath K. Magnifying endoscopy for diagnosing and delineating early gastric cancer. *Endoscopy* 2009; **41**: 462–7.
8. Yao K, Iwashita A, Matsui T *et al*. White opaque substance within superficial-elevated gastric neoplasia as visualized by magnification endoscopy (ME) with narrow-band imaging (NBI): A new useful marker for discriminating adenoma from carcinoma. *Endoscopy* 2007; **39** (Suppl):A16.
9. Yao K, Iwashita A, Tanabe H *et al*. 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.* 2008; **68**: 574–80.
10. Yao K, Iwashita A, Nagahama T *et al*. White opaque substance as visualized by magnifying endoscopy with narrow-band imaging: A new useful sign for differentiating high-grade dysplasia/early gastric carcinoma from low-grade dysplasia in gastric neoplastic lesions. *Endoscopy* 2008; **40** (Suppl):A61.
11. Yao K, Iwashita A, Tanabe H *et al*. Author's reply to Letter to the Editor, 'White opaque substance' and 'light blue crest' within gastric flat tumors or intestinal metaplasia: Same or different signs? *Gastrointest. Endosc.* 2009; **70**: 402–3.
12. Tanaka M. Endoscopic study of intestinal absorption [in Japanese with English abstract]. *Gastroenterol. Endosc.* 1976; **18**: 711–20.
13. Mitchell CJ, Scott BB, Bullen AW, Losowsky MS. Snow-white duodenum: A new endoscopic sign in a patient with hypobetalipoproteinemia. *Gastrointest. Endosc.* 1978; **24**: 123–4.
14. Peretti N, Sassolas A, Roy CC *et al*. Guidelines for the diagnosis and management of chylomicron retention disease based on a review of the literature and the experience of two centers. *Orphanet J. Rare Dis.* 2010; **5**: 24. doi: 10.1186/1750-1172-5-24
15. 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**: 1709–20.
16. Ljungberg O, Tibblin S. Peroperative fat staining of frozen sections in primary hyperparathyroidism. *Am. J. Pathol.* 1979; **95**: 633–41.
17. Kabashima A, Yao T, Maehara Y *et al*. Relationship between biological behavior and phenotypic expression in undifferentiated-type gastric carcinomas. *Gastric. Cancer* 2005; **8**: 220–7.
18. Ueyama H, Yao T, Nakashima Y *et al*. Gastric adenocarcinoma of fundic gland type (chief cell predominant type): Proposal for a new entity of gastric adenocarcinoma. *Am. J. Surg. Pathol.* 2010; **34**: 609–19.
19. Yao K, Yao T, Iwashita A *et al*. Microaggregate of immunostained macrophages in noninflamed gastroduodenal mucosa: A new useful histological marker for differentiating Crohn's colitis from ulcerative colitis. *Am. J. Gastroenterol.* 2000; **95**: 1967–73.
20. Schlemper RJ, Kato Y, Stolte M. Diagnostic criteria for gastrointestinal carcinoma in Japan and Western countries: Proposal for a new classification system of gastrointestinal epithelial neoplasia. *J. Gastroenterol. Hepatol.* 2000; **15** (Suppl):C52–60.
21. Participants in the Paris Workshop. The Paris endoscopic classification of superficial neoplastic lesions: Esophagus, stomach, and colon. *Gastrointest. Endosc.* 2003; **58** (6 Suppl): S44–5.

22. Beckman M. Great balls of fat. *Science* 2006; **311**: 1232–4.
23. Johnsen S, Widder E. The physical basis of transparency in biological tissue: Ultrastructure and minimization of light scattering. *J. Theor. Biol.* 1999; **199**: 181–98.
24. Heiden MGV, Cantly LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science* 2009; **324**: 1029–33.
25. Siurala M, Tarpila S. Absorptive function of intestinal metaplasia of the stomach. *Scand. J. Gastroenterol.* 1968; **3**: 76–9.
26. Rubin W, Ross LL, Jeffries GH *et al*. Some physiologic properties of heterotopic intestinal epithelium: Its role in transporting lipid into the gastric mucosa. *Lab. Invest.* 1967; **16**: 813–27.
27. Tanaka M, Takase Y, Suzuki S *et al*. Some problems of intestinal metaplasia: (IV) Functional changes of metaplastic intestinal epithelium [in Japanese with English abstract]. *J. Tokyo Wom. Med. Univ.* 1975; **45**: 767–81.
28. Borgstrom B, Dahlqvista A, Lundh G, Sjovall J. Studies of intestinal digestion and absorption in the human. *J. Clin. Invest.* 1957; **36**: 1521–36.
29. Gannon B. The vasculature and lymphatic drainage. In: Whitehead R (ed.). *Gastrointestinal and Oesophageal Pathology*. Edinburgh: Churchill Livingstone, 1995; 129–99.