Practical Points in Gastric Pathology

Sangjeong Ahn, MD; Do Youn Park, MD, PhD

• Context.—The diagnosis of gastric epithelial lesions is difficult in clinical practice, even with the recent developments and advances in endoscopic modalities, owing to the diverse morphologic features of the lesions, lack of standardized diagnostic criteria, and the high intraobserver and interobserver variabilities in the nonneoplastic (regenerative–neoplastic) spectrum.

• Objective.—To provide an overview of the current concepts and unresolved issues surrounding the diagnosis of diseases in the nonneoplastic-neoplastic spectrum, and to discuss some noteworthy properties and histologic features of gastric epithelial lesions.

• Data Sources.—A comprehensive assessment of the medical literature on gastric epithelial lesions was performed; we also interjected our own experiences into the discussion. Sources included original studies, review articles, and textbooks related to the field.

Conclusions.—Our literature review revealed that clear cell changes and micropapillary carcinoma components in gastric carcinomas are associated with poor clinical outcomes and should hence be included in pathologic reports. Moreover, we suggest a stepwise biopsy–endoscopic resection modality for the diagnosis of borderline neoplasia–nonneoplasia cases.

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With the advancements in endoscopy as a diagnostic and therapeutic modality, we have encountered a myriad of gastric epithelial lesions in clinical practice, which has raised several challenges. First, infrequently encountered entities or histologic features might be unfamiliar and difficult to diagnose. Second, diseases on the dysplasia–carcinoma spectrum are difficult to diagnose, especially in borderline situations where determining the diagnosis using biopsy specimens is difficult.

In this review, we discuss some of the most challenging but noteworthy entities in the stomach, including those experienced at our institution. We also review current concepts and unresolved issues related to the topic and suggest stepwise biopsy–endoscopic resection as an effective diagnostic approach, especially pertaining to the decision tree in borderline neoplasia/nonneoplasia situations.

OVERVIEW OF GASTRIC EPITHELIAL LESIONS WITH NEW CONCEPTS

Definitions and Terminologies of Gastric Preneoplastic Lesions

Intraobserver and interobserver variabilities in the dysplasia–carcinoma spectrum are longstanding issues, especially between Eastern and Western pathologists. In particular, disputes regarding discrepancies in the upper spectrum have been ongoing (as will be discussed in the next section). International standardization of diagnosis and reporting is a prerequisite for allowing a comparison between studies worldwide, and unified diagnostic and treatment guidelines should eventually be developed. To that end, several attempts to reduce these discrepancies and to improve reproducibility have been described. However, a unified guideline still does not exist. Moreover, the terminology used to describe premalignant lesions in the stomach is not uniform, with dysplasia being used interchangeably with intrarepithelial neoplasia depending on the institutions or geographic locations.

Dysplasia is traditionally defined as an obvious, unambiguous cytologic and architectural alteration (particularly morphologic) without invasion into the lamina propria, whereas intrarepithelial neoplasia implies malignant potential, regardless of morphologic alteration. Adenoma, which is considered a discrete protruding lesion by Western pathologists, includes other gross types, such as flat and depressed lesions, in Japan. Most dysplasia/intrarepithelial neoplasias of the stomach can be divided into several histologic subsets, including intestinal (adenomatous) and gastric (foveolar and pyloric type) phenotypes, and pit dysplasia/crypt dysplasia. A two-tiered scheme of low-grade dysplasia (LGD) and high-grade dysplasia (HGD) is applied.

The most commonly encountered subset, the adenomatous type, shares common histologic features with colonic adenoma, whereas the foveolar type, the second most common subset, resembles normal surface-foveolar epithelium and is characterized by cuboidal or low columnar epithelium cells with round monolayered nuclei. Hyperplasia of the foveolar region with irregular glandular branching and epithelial folding is frequently observed.
with absent goblet and Paneth cells.6,10 On the other hand, the hybrid type harbors morphologic features of both these subsets. These 2 distinct morphologic subtypes have different immunohistochemical expressions; mucin (MUC) 2, cluster of differentiation 10 (CD10), and caudal type homeobox 2 (CDX2) are expressed in the adenomatous type, and MUC5AC is expressed in the foveolar type.6,11 Nuclear pseudostratification can be used as a criterion in the lower spectrum of dysplasia in the adenomatous phenotype.10 However, in the foveolar phenotype, the criterion for pseudostratification is not applicable owing to its nonstratified, monolayered arrangement.12 Other criteria favoring LGD are mild crowding, simple tubules, increased nuclear to cytoplasmic (N/C) ratio and mucin depletion, overlapping nuclei, and hyperchromasia with conspicuous nucleoli.13 The HGD histologic features include severe back-to-back crowding with mildly branching glands and higher N/C ratios, cuboidal and round (rather than columnar and elongated) nuclei, and prominent nucleoli.13 Although it is controversial, cribriform patterns, branching, and intraluminal necrotic debris are considered beyond the upper spectrum of dysplasia, indicating intramucosal carcinoma.10 As mentioned earlier, separating LGD from HGD in the lower spectrum of dysplasia in the stomach is difficult. Full-thickness mucosal atypia is a characteristic feature of the foveolar-type dysplasia that distinguishes this tumor type from regenerating atypia; histologic features favoring HGD include a nuclear size cutoff 3 to 4 times the size of a small mature lymphocyte, increased nucleoli, increasingly eosinophilic to oncocytic cytoplasm, and crowded to irregular glandular architecture.14

Pyloric Gland Adenoma With Malignant Transformation

Pyloric gland adenoma (PGA), one of the gastric-type adenoma subtypes, is a recently noted entity with distinct attributes. Pyloric gland adenomas are uncommon, occurring in only 2.7% of nonsyndrome patients,15 in contrast to its relatively high incidence in patients with familial adenomatous polyposis and Lynch syndrome (6% [4 of 66]16 and 20% [3 of 15],17 respectively). Sporadic and familial adenomatous polyposis–associated PGAs show a predilection for older female patients, and tend to be located in the fundic mucosa,16,18,19 while Lynch syndrome–associated PGAs have conversely been reported to be more commonly observed in young males.16 Microscopically, these lesions are composed of closely packed small tubules with occasional dilatation and a monolayer of cuboidal-to-short columnar epithelia showing round basal nuclei and pale-to-eosinophilic cytoplasm without an apical mucin cap.19,20 Moreover, PGAs are often observed in the background of autoimmune (metaplastic) atrophic gastritis, which is presumably associated with the observed female predominance.21 Dysplastic changes, irregular-to-complex glands, and nuclear atypia are common occurrences, observed in 52.6% (2 of 19 LGD and 8 of 19 HGD) of patients with PGA,13 with observed malignant transition ranging from 15.9% (3 of 19)19 to 45.8% (22 of 48)22 of cases. Morphologically, signs of carcinoma include intertubular fusion or lateral expansion of the glands; however, cytomorphic discrimination of HGD and carcinoma in PGA may be tricky.23 Immunohistochemically, PGAs show a peculiar graded pattern of MUC6 and MUC5AC, with strong and diffusely positive MUC6 expression observed in the whole lesion, and low (or sometimes absent) MUC5AC expression noted on the surface epithelium.22 PGA also harbors considerable chromosomal abnormalities, as has been commonly reported in gastric adenocarcinoma.24 Sporadic PGAs show some chromosomal abnormalities, including 17pq and 20q gains and 4q, 5q, and 6q losses24 along with frequent GNAS (48%, 11 of 23) and KRAS (41%, 9 of 23) mutations.25 On the other hand, Lynch syndrome–associated PGAs have been shown to be affected by mutations in various DNA mismatch repair genes, resulting in mismatch repair protein expressions.26 Because of its high potential for malignant transformation, clinicians and pathologists should be mindful of this particular entity.

Figure 1 shows a PGA transitioning to an intramucosal carcinoma. Grossly, PGA presents as a polyoid lesion in the fundic mucosa. Microscopically, PGA consists of small to medium-sized glands, with occasional cystic dilatation, showing round and basally located nuclei (Figure 1, A and C). In some areas, high-grade features, a highly increased N/C ratio, distinct nucleoli, and back-to-back and complex glands are observed (Figure 1, D and E), along with suspicious stromal invasion such as lateral expansion and intertubular fusion (Figure 1, F). Immunohistochemistry reveals a characteristic graded pattern (Figure 1, B). Interestingly, carcinomatous transformation is found to be associated with partial loss of expression of MUC6 (marker of pyloric gland mucin; Novocastra Laboratories, Newcastle, United Kingdom), while increased expression of MUC5AC (a marker of foveolar mucin; Novocastra Laboratories) is observed (Figure 1, B, inset), which, based on the association of MUC5AC expression with high-grade morphology, may be beneficial for differentiation of high-grade morphology.6 This example shows gastric adenocarcinoma arising from a background of a PGA with HGD.

Gastric Adenocarcinoma With a Micropapillary Carcinoma Component

Micropapillary carcinoma (MPC) exhibits distinct morphologic features, with small nests of tumor cells floating in clear spaces devoid of fibrovascular cores.27 Since the first identification of micropapillary carcinoma of the stomach,27 subsequent studies28–33 have demonstrated that gastric cancers (GCs) with an MPC component exhibit aggressive biological behavior, with frequent lymphatic invasion, extensive lymph node metastasis, and lower overall survival. This is similarly observed in other organs. Moreover, GCs with an MPC component have been reported to have worse prognoses, especially for early-stage GCs,31 similar to colon and lung cancers.34,35 However, the presence of an MPC component in the stomach is observed infrequently, with the reported rates in GCs generally ranging from 1% to 6% (1.5% [14 of 930],28 [17 of 1178],32 [32 of 2036],33 and 6.1% [72 of 1171]28), although it was as high as 13.4% (25 of 172) in one previous study.28 The proportion of GCs with MPC components broadly ranges from 5% to 90%.30,32 However, the extent of the MPC component does not appear to influence the overall survival of the patients, as cases with a low ratio of the MPC component (less than 5%) were also associated with worse prognoses.30,31 The MPC component is usually observed in the intestinal-type31 and papillary or tubular adenocarcinoma subtypes of adenocarcinoma.30,32 Immunohistochemically, epithelial
membrane antigen (also known as MUC1) demonstrates a characteristic “inside-out pattern” in the MPC component, while other mucin markers (MUC5AC, MUC6, MUC2, and CD10) indicate a gastric phenotype. Therefore, the MPC component, notorious for conferring a worse prognosis, should be noted in surgical pathology reports when occurring in GCs, even if its extent is very low (5%).

Figure 1. A, Low magnification of pyloric gland adenoma with carcinomatous transition (arrow). B, Diffuse expression of MUC6 (MUC5AC expression on the surface and in the carcinomatous change [inset]). C, Pyloric gland adenoma with low-grade dysplasia and (D, E) high-grade dysplasia with (F) stromal invasion (hematoxylin-eosin, original magnifications ×12.5 [A], ×200 [C, E, and F], and ×100 [D]; original magnification ×12.5 [B and inset]).
Gastric Adenocarcinoma With Clear Cell Change

Clear cell changes in tumors are thought to be caused by the cytoplasmic accumulation of glycogen, mucin, lipid, and/or water. Although it has been described in many other organs, GCs with clear cell changes have not been fully investigated, with only limited literature available on the topic.α-Fetoprotein (AFP)–producing GC with enteroblastic differentiation usually exhibits clear cytoplasm.36,39 However, gastric cancer with clear cell change is not always associated with AFP-producing GC, with 14.5% (9 of 62) of clear cell GCs showing AFP positivity.39 Clear cell changes have been reported in 74% (17 of 23) of AFP-membrane.12,42 On the other hand, Japanese pathologists have reported stromal reaction, and definite destruction of the basement cell formation, micronests or budding, desmoplastic upon definite histologic evidence of invasion, that is, single pathologists diagnose a specimen as adenocarcinoma only (9 of 62) of clear cell GCs showing AFP positivity.39 Clear cell changes in gastric epithelial lesions, especially between Eastern and Western pathologists. Therefore, different treatment ap-

Figure 2 shows a tumor with complex tubular and trabecular formations composed of polygonal-shaped tumor cells, with distinct cytoplasmic borders. A marked clear cell change in more than 90% of the total tumor volume is observed (Figure 2, A). Immunohistochemical stains for AFP, glycian-3, and/or CD10 expression, while mucin-deposited GCs with clear cell changes frequently show MUC5AC and/or MUC6 expression.39 Furthermore, all types of GCs with clear cell changes also show increased positive immunostaining of hepatocyte nuclear factor-1B.39

CHALLENGING ISSUES IN BIOPSY DIAGNOSIS

High-Grade Dysplasia or Adenocarcinoma?

Using biopsy for the diagnosis of adenocarcinoma is challenging for multiple reasons, including a lack of standardized criteria, inadequate tissue sampling, and the difficulty in distinguishing dysplasia, or even regenerating atypia, from carcinoma.10 Basically, adenocarcinoma is defined as invasion of tumor cells into the lamina propria via penetration of the basement membrane. Despite this clear definition, distinguishing between dysplasia and carcinoma appears to be problematic in biopsy specimens of gastric epithelial lesions, especially between Eastern and Western pathologists. Therefore, different treatment approaches to identical histologic profiles are adopted.4 In general, Western pathologists tend to place emphasis on identification of actual invasion, characterized by a breach of the basement membrane, whereas Eastern pathologists focus on lamina propria invasion.42 Moreover, Western pathologists diagnose a specimen as adenocarcinoma only upon definite histologic evidence of invasion, that is, single cell formation, micronests or budding, desmoplastic stromal reaction, and definite destruction of the basement membrane.12,42 On the other hand, Japanese pathologists attach more weight to the degree of cytologic and architectural atypia, such as a markedly increased N/C ratio, nuclear pleomorphism, loss of polarity, irregular chromatin, large prominent nucleoli, atypical mitosis, and gland-in-gland, back-to-back, abortive, and cribriform glands.42,43 based on 3-dimensional analyses and the potential for submucosal invasion.44,45,46 Sakurai et al44 demonstrated submucosal and venous invasions in 3.8% (3 cases) and 1.3% (1 case) of 78 HG cases, respectively, on the basis of Western criteria of HGD. Lee et al45 demonstrated that other histologic features in forceps-obtained biopsy specimens, such as intraluminal necrotic debris, a cribriform tumor pattern, and papillary architecture (which are not ultimate evidence of stromal invasion), were associated with submucosal invasion. Furthermore, additional features implicating carcinoma have been described, including intertubular fusion, glandular lateral extension, erosion or ulceration, and intraglandular apoptotic cell debris.50

Regenerative Atypia or Adenocarcinoma?

Well-differentiated adenocarcinoma is difficult to distin-

Figure 3 shows HGD in the biopsy specimen, while subsequent endoscopic submucosal dissection shows sub-

mucosal invasion. Esophagogastroduodenoscopy shows an elevated and hyperemic mucosa with minute erosion in the angle of the lesser curvature (Figure 3, A). Endoscopic biopsy reveals irregular branching and complex glands, showing greater than 50% nuclear stratification, hyperchromatic nuclei, and irregular chromatin, but no definite evidence of stromal invasion (Figure 3, B and C). Therefore, the biopsy diagnosis is HGD. Endoscopic submucosal dissection was subsequently performed. In the resected specimen, small tumor cell nests with obvious stromal invasion are observed beneath the overlying HGD lesion, mainly in the submucosa (Figure 3, D through F), and lymphovascular invasion is also detected. Gastrectomy and lymph node dissection were performed. This demonstrates the possibility of invasive adenocarcinoma (including with submucosal invasion) occurring in cases diagnosed as HGD as based on biopsy specimens.

Regarding biopsy samples obtained by forceps, this method is not a satisfactory modality for the accurate diagnosis of neoplastic or nonneoplastic lesions, as a high discrepancy rate between biopsy and endoscopic resec-

tion has been reported (27%–60%; 27.1% [23 of 85], 51 31.7% [387 of 1258].52 39.2% [105 of 268],53 44.5% [177 of 397],24 and 60.5% [69 of 114]). The diagnosis of HGD based on a biopsy specimen can predict a high-grade pathologic result (such as carcinomatous transformation) in the subsequently resected specimens,52 as we have experienced. Furthermore, we saw a limitation in the dependability of biopsy specimens. In the resected specimen, stromal invasion was only observed under the overlying HGD, which cannot be obtained by regular biopsy. The strict adherence to the criteria for stromal invasion in biopsy specimens (which is supported by Western pathologists) might prevent negative conversion in a resected specimen, while the descriptive criteria supported by Eastern pathologists, which suffer from low specificity, might increase discrepancies related to nega-

Therefore, attaining agreed-upon diagnostic guidelines in a proper point is important and remains to be achieved.
As mentioned previously, difficulty in the recognition of foveolar-type dysplasia stems from its morphologic features, which resemble normal foveolar epithelium and show inconspicuous pseudostratification. Reactive changes in the epithelium due to inflammation produce features similar to dysplasia, such as mucin depletion and nuclear hyperchromasia. However, some useful clues for identifying reactive atypia include vascular congestion, stromal edema, lack of pseudostratification, and gradual transition of the atypical glands to the adjacent normal epithelium. Abrupt transition of the atypical glands can create doubt regarding whether neoplastic rather than regenerative changes are being observed. Moreover, it is important to note the balance between the degree of atypia and inflammation, and this requires stepwise serial biopsy. If the inflammation is similar to, or higher than, the degree of atypia, we suggest follow-up biopsy rather than conclusive diagnosis. Conversely, if the level of atypia exceeds the degree of inflammation, we would conclude that this is indicative of dysplasia.

Figure 4 shows upper gastrointestinal endoscopy with slightly elevated and hyperemic mucosa and erosion in the antrum of the greater curvature (Figure 4, A). Examination of the biopsy specimens obtained from this lesion showed focal proliferation of irregular glands with hyperchromatic nuclei and abundant inflammatory cells (Figure 4, B); the lesion was diagnosed as “regenerative atypia or well-differentiated adenocarcinoma.” Follow-up examination 3 months later revealed no endoscopic alleviation (Figure 4, C); subsequently, a biopsy from the lesion showed prominent abortive and angulated glands with nuclear hyperchromasia, while the inflammation subsided (Figure 4, D). Abrupt transition between the atypical and adjacent normal glands was also noticed, and the diagnosis was “atypical foveolar epithelium, suggestive of well-differentiation adenocarcinoma.” Endoscopic submucosal dissection showed extremely well-differentiated adenocarcinoma (also reported in the Japanese literature as “WHYX lesions”), with invasion into the muscularis mucosa (Figure 4, E through F), measuring 1.8 × 1.4 cm.

**Stepwise Serial Biopsy With Diagnostic Endoscopic Submucosal Dissection: A Method to Solve Current Problems**

As mentioned above, it is challenging to obtain a precise diagnosis with biopsy samples obtained with forceps in cases of differentiation between regenerative atypia and dysplasia or well-differentiated adenocarcinoma. From therapeutic and diagnostic viewpoints, discrimin-
inauguration between dysplasia and carcinomatous transformation may not be crucial, because in each of these cases, local endoscopic modalities would be advisable. Therefore, we propose a stepwise diagnostic process for accurate diagnosis, focusing on the regenerative changes caused by inflammation. If the biopsy specimen is indeterminable and exhibits inflammation, we recommend repeated biopsy after considering the balance between the atypia and inflammation. Further endoscopic resection is indicated for diagnostic purposes if the atypia still exists after follow-up biopsy. If such a borderline situation is sustained even after repeated biopsy, endoscopic submucosal dissection may be a more reliable diagnostic approach.

CONCLUSIONS

Accurate diagnosis of tumors in the stomach is challenging. Based on the diverse morphologies and the clinical
Figure 4. A, Initial endoscopy finding the lesion. B, Biopsy specimen showing atypical glands; however, marked inflammation made the diagnosis difficult. C and D, Follow-up endoscopy and biopsy showed a sustained lesion. E, Subsequent resection for diagnosis revealed (F, H) well-differentiated adenocarcinoma with invasion into muscularis mucosa (circle). G, The lesion was mapped (red dotted line) (hematoxylin-eosin, original magnifications ×200 [B and D], ×12.5 [F], and ×400 [H]).
implications thereof, special attention should be paid to several infrequent but noteworthy histologic features; these should be included in pathologic reports. With respect to borderline situations between nonneoplastic and neoplastic lesions, we recommend stepwise serial biopsy with diagnostic endoscopic submucosal dissection after considering the balance between the degree of dysplasia and inflammation.

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References

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