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Original Article

Unique immunohistochemical profiles of MUC5AC, MUC6, P53, and Ki67 in gastric atypical hyperplasia and dysplasia

Lanfang Miao[1], Yuanyuan Sun[1], Mei Guo[1], Haijun Yang[1], Xianjuan Du[1], Junkuo Li[1], Jingwei Shen[1], Xiaomin Wang[2], Ruixue Lei[1]

[1]Department of Pathology, Anyang Tumor Hospital/The Affiliated Anyang Tumor Hospital of Henan University of Science and Technology, Anyang 455000, Henan, China; [2]Department of Radiotherapy, Anyang Tumor Hospital/The Affiliated Anyang Tumor Hospital of Henan University of Science and Technology, Anyang 455000, Henan, China

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Abstract: Objectives: Differentiating gastric atypical hyperplasia (AH) from dysplasia, including low-grade dysplasia (LGD) and high-grade dysplasia (HGD), poses significant challenges in small biopsies and specimens with technical artifacts. This study aims to establish objective diagnostic criteria for these conditions through combined morphologic and immunohistochemical (IHC) analyses. Methods: Between January 2018 and September 2020, a total of 123 gastric mucosa biopsy specimens were collected at Anyang Tumor Hospital. According to the WHO Classification of Digestive System Tumors (5th edition), specimens were categorized into three groups: AH (n=48), LGD (n=30), and HGD (n=45). Morphologic characteristics were assessed, and IHC staining for MUC5AC, MUC6, MUC2, CD10, P53, and Ki67 was performed, followed by statistical analysis. Results: Histologically, AH was predominantly marked by a pronounced inflammatory background (60.42%), intestinal metaplasia (64.58%), indistinct boundaries (83.33%), and a distinct maturation gradient (97.72%). AH nuclei were typically circular (97.92%), with a high nucleus-to-cytoplasm ratio (64.58%), prominent nucleoli (47.92%), and preserved polarity (89.58%). In contrast, LGD and HGD typically exhibited well-defined boundaries with an absent maturation gradient. LGD nuclei were rod-shaped (96.67%), with a low nucleus-to-cytoplasm ratio (96.67%) and preserved polarity (100%), whereas HGD demonstrated a loss of cellular polarity (77.78%). IHC findings revealed a consistent maturation gradient in AH, with polarized MUC5AC and MUC6 expression, significantly reduced in LGD (86.67%), and absent in HGD. P53 expression in HGD showed a predominant 'mutation-type pattern' (66.67%), contrasting with 'wild-type pattern' expression in AH and LGD (100%, 93.33%). Ki67 expression patterns varied from a 'pit neck pattern' in AH (95.83%) to a 'polarity pattern' in LGD (76.67%) and a 'diffuse pattern' in HGD (57.78%). The expression patterns of MUC5AC, MUC6, CD10, P53, and Ki67 varied significantly across the three groups (P<0.001). Conclusions: The integration of histomorphological features and expression profiles of MUC5AC, MUC6, P53, and Ki67 is instrumental in diagnosing gastric atypical hyperplasia and dysplasia.

Keywords: Gastric mucosal biopsy, pathological diagnosis, atypical hyperplasia, dysplasia, histomorphology, immunohistochemistry

Introduction

Atypical hyperplasia (AH) is an ambiguous diagnostic term used when a clear diagnosis of dysplasia cannot be established. This category often includes patterns of 'hyperplastic', 'hyperproliferative', or 'deep metaplasia' atypia, marked by densely packed glands lined with irregular cells. These cells exhibit large, hyperchromatic, and pseudostratified nuclei with frequent mitoses [1]. Notably, a gradient of cytoarchitectural normalization towards the mucosal surface often indicates surface maturation, suggesting a benign nature [1, 2]. This feature helps distinguish AH from indefinite dysplasia, which, despite its ambiguous nature, has been found to progress to dysplasia in 6%-15.8% of cases [3, 4].

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Dysplasia, in contrast, is characterized by unequivocal neoplastic changes in the gastric epithelium, without evidence of invasion into the lamina propria. It is categorized as low-grade (LGD) or high-grade (HGD) based on the extent of architectural distortion, nuclear and cytoplasmic cell alterations, and mitotic activity [5]. The clinical management of AH, LGD, and HGD varies substantially. AH generally warrants conservative management with regular monitoring. In contrast, LGD may require localized treatment, re-biopsy, or follow-up [6, 7], while HGD often necessitates more aggressive interventions such as endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) [8]. Therefore, the accurate pathologic discrimination of these three proliferative conditions is vital for appropriate clinical diagnosis and treatment.

However, differentiating AH from dysplasia histologically poses significant challenges, especially in small biopsies or specimens with technical artifacts. Moreover, the current WHO classification may oversimplify the complex nature of these lesions. Thus, this study aims to investigate the morphologic and immunohistochemical characteristics of gastric mucosal biopsy specimens to refine the diagnostic accuracy for dysplasia.

Materials and methods

Subjects

From January 2018 to September 2020, a cohort of 123 patients with endoscopy- and histology-confirmed gastric mucosal lesions was selected at Anyang Tumor Hospital, China. Utilizing the WHO Classification of Digestive System Tumors (5th edition) guidelines [9], specimens were classified into AH (48), LGD (30), and HGD groups (45). Two deputy chief pathologists, specializing in digestive pathology, independently evaluated the specimens in a double-blinded manner. Disagreements were resolved through joint review to achieve consensus. Histomorphologic features such as background mucosal inflammation, intestinal metaplasia, lesion boundaries, mature differentiation, nuclear characteristics, nucleus to cytoplasm ratio, nucleoli, polymorphism, and cell polarity were documented. IHC expression of MUC5AC, MUC6, MUC2, CD10, P53, and Ki67 in all cases was also assessed.

Hematoxylin and eosin (H&E) and immunohistochemical (IHC) staining

Specimens were fixed in 10% buffered formalin, paraffin embedded, and sectioned into 3-4-μm-thick slices for hematoxylin and eosin (HE) and immunohistochemistry (IHC) staining. Primary antibodies against MUC2 (mAb M53), MUC5AC (mAb 45M1), P53 (mAb MX008), Ki67 (mAb MX006) (ready to use, MaixinBio, Fuzhou, China), MUC6 (mAb MRQ-20), and CD10 (mAb UMAB235) (ready to use, ZSGB-BIO, Beijing, China) were used. IHC was performed using the Ultra View DAB Detection Kit on a Ventana Benchmark Ultra automated IHC staining system (Roche Diagnostics). Appropriate negative and positive controls were used to validate staining quality. EDTA (pH 9.0) was employed for antigen retrieval.

Pathologic evaluation of IHC

MUC5AC, MUC6, and MUC2 staining was observed in cell cytoplasm, while CD10 exhibited glandular secretory margin staining. A positive result for MUC5AC, MUC6, MUC2, and CD10 was defined as ≥5% cell expression in the lesion [1]. P53 and Ki67 exhibited nuclear staining. P53 expression was categorized into 'wild-type pattern' (10-50% of lesion cells exhibiting scattered positive staining of varying intensities) and 'mutation-type pattern' (≥60% of lesion cells exhibiting diffuse strong positivity [missense mutation] or complete loss of expression [nonsense mutation]) [8]. Ki67 staining was classified as 'pit neck pattern' (expression confined to the bottom of gastric pits and the neck of gastric glands), 'polarity pattern' (expression predominantly confined to the middle and upper third of the tumor glands), or 'diffuse pattern' (diffuse distribution pattern) [10, 11].

Statistical analysis

Data analysis was conducted using SPSS (version 23.0 for Windows; IBM, Armonk, NY, USA). Categorical data are presented as n (%), and inter-group comparisons use the Fisher exact probability method. The significance level was set at $\alpha=0.05$, adjusted for pairwise comparison to $\alpha=\alpha/[k\ (k-1)/2]=0.0167$, where k represents the number of compared groups.

Table 1. Morphologic characteristics in different gastric mucosal lesions

Factor	Group			*P* value
	AH (n=48)	LGD (n=30)	HGD (n=45)	
Inflammation				0.029
Mild	19 (39.58%)	21 (70.00%)	25 (55.56%)	
Severe	29 (60.42%)	9 (30.00%)	20 (44.44%)	
Intestinal metaplasia				<0.001
-	17 (35.42%)	12 (40.00%)	32 (71.11%)	
+	31 (64.58%)	18 (60.00%)	13 (28.89%)	
Boundary				<0.001
+	8 (16.67%)	30 (100%)	40 (88.89%)	
-	40 (83.33%)	0 (0%)	5 (11.11%)	
Differentiation				<0.001
+	47 (97.92%)	4 (13.33%)	0 (0%)	
-	1 (2.08%)	26 (86.67%)	45 (100%)	
Morphology				<0.001
Rod-shape	1 (2.08%)	29 (96.67%)	1 (2.22%)	
Circular	47 (97.92%)	1 (3.33%)	44 (97.78%)	
Nucleus-to-cytoplasm Ratio				<0.001
≥50%	31 (64.58%)	1 (3.33%)	42 (93.33%)	
<50%	17 (35.42%)	29 (96.67%)	3 (6.67%)	
Nucleolus				0.001
+	23 (47.92%)	28 (6.67%)	16 (35.56%)	
-	25 (52.08%)	2 (93.33%)	29 (64.44%)	
Polymorphism				0.005
+	0 (0%)	0 (0%)	5 (11.11%)	
-	48 (100%)	30 (100%)	40 (88.89%)	
Polarities				<0.001
+	43 (89.58%)	30 (100%)	10 (22.22%)	
-	5 (10.42%)	0 (0%)	35 (77.78%)	

+, present; -, absent; AH, atypical hyperplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia.

Results

Histomorphologic characteristics in various gastric mucosal lesions

The histomorphological characteristics across the AH, LGD, and HGD groups demonstrated notable variations (summarized in **Table 1**). The AH group exhibited a more pronounced inflammatory background compared to the relatively milder inflammation observed in the LGD and HGD groups; however, this difference was not significant (P=0.029). Significant differences were observed among the three groups in terms of histological structure, including lesion boundaries, surface differentiation (**Figure 1A-C**), and cellular morphology (including cell shape, nucleus-to-cytoplasm ratio, nucleoli, polymorphism, and cell polarity) (P<0.01).

Histomorphological characteristics with pairwise comparisons

Pairwise comparison between the three groups revealed structural and cellular differences, but no significant differences were found in cell morphology (P=1.000) or polymorphism (P=0.168) between AH and HGD.

Immunohistochemical characteristics in various gastric mucosal lesions

Immunohistochemical findings, summarized in **Table 2**, showed polarized MUC5AC and MUC6 expression patterns in the AH group. MUC5AC was predominantly expressed on the surface epithelium and neck glands, with minimal expression in the deep glands (**Figure 1D**). MUC6 displayed strong expression in the deep

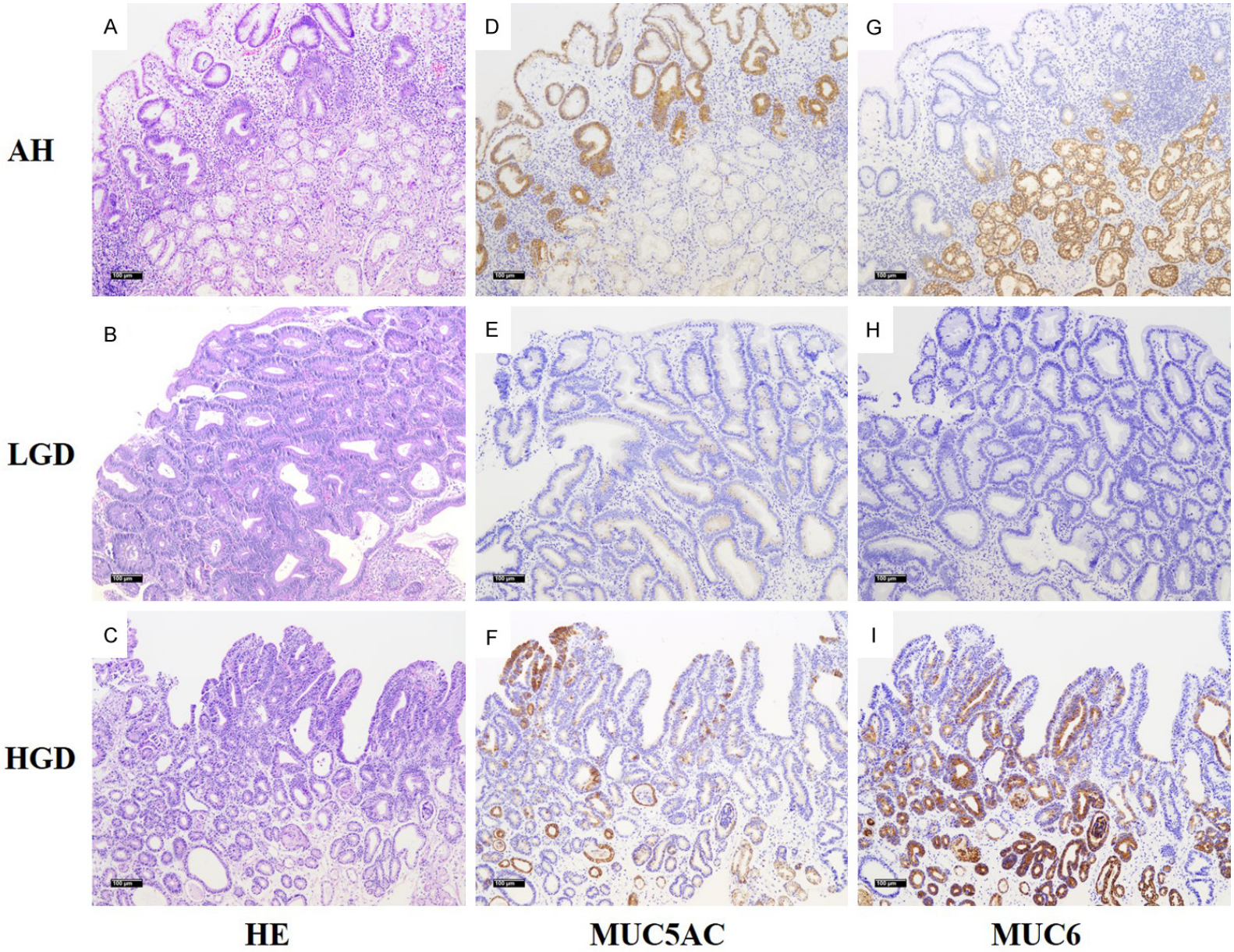

Figure 1. Histologic features and immunopositivity of MUC5AC and MUC6 in various gastric mucosal lesions. H&E staining (A-C) demonstrates a mature surface differentiation in AH (A) and an immature surface differentiation in LGD (B) and HGD (C). The expression of MUC5AC and MUC6 exhibit a polarized pattern in AH (D, G) while showing a lack of polarity in LGD and HGD (E, H and F, I) (H&E, IHC×100).

Table 2. Immunohistochemical characteristics in different gastric mucosal lesions

Factor	Group			*P* value
	AH (n=48)	LGD (n=30)	HGD (n=45)	
MUC5AC				<0.001
+	48 (100%)	5 (16.67%)	26 (57.78%)	
-	0 (0%)	25 (83.33%)	19 (42.22%)	
MUC6				<0.001
+	48 (100%)	4 (13.33%)	24 (53.33%)	
-	0 (0%)	26 (86.67%)	21 (46.67%)	
MUC2				0.593
+	31 (64.58%)	18 (60.00%)	32 (71.11%)	
-	17 (35.42%)	12 (40.00%)	13 (28.89%)	
CD10				<0.001
+	8 (16.67%)	15 (50.00%)	7 (15.56%)	
-	40 (83.33%)	15 (50.00%)	38 (84.44%)	
P53				<0.001
Wild-type pattern	48 (100%)	28 (93.33%)	15 (33.33%)	
Mutation-type pattern	0 (0%)	2 (6.67%)	30 (66.67%)	
Ki67				<0.001
Pit neck pattern	46 (95.83%)	0 (0%)	8 (17.78%)	
Polarity pattern	0 (0%)	23 (76.67%)	5 (11.11%)	
Diffuse pattern	2 (4.17%)	7 (23.33%)	32 (71.11%)	

+, present; -, absent; AH, atypical hyperplasia; LGD, low-grade dysplasia; HGD, high-grade dysplasia.

glands, variable in the neck glands, and was absent in surface epithelium (**Figure 1G**). The polarity expression rate was decreased in both the LGD (**Figure 1E**, **1H**) and HGD (**Figure 1F**, **1I**) groups, with focal expression lacking polarity. MUC2 expression rates were consistently high across all three groups, showing no significant difference. CD10 expression was lower in the AH and HGD groups and higher in the LGD group.

A 'mutation-type pattern' of P53 was predominantly observed in the HGD group (**Figure 2C**), contrasting with the 'wild-type pattern' observed in the AH (**Figure 2A**) and LGD groups (**Figure 2B**). Ki67 exhibited a predominant 'pit neck pattern' in the AH group (**Figure 2D**), a 'polarity pattern' in the LGD group (**Figure 2E**), and a 'diffuse pattern' in the HGD group (**Figure 2F**).

Immunohistochemistry characteristics with pairwise comparisons

Pairwise immunohistochemical comparisons among the three groups for MUC5AC, MUC6, P53, and KI67 revealed significant differences, except for P53 between the AH and LGD groups and MUC5AC and MUC6 between LGD and HGD.

Discussion

This retrospective study analyzed morphologic and immunohistochemical characteristics of 123 gastric mucosal biopsy specimens collected over three years at our hospital, including 48 cases of atypical hyperplasia (AH), 30 cases of LGD, and 45 cases of HGD.

AH, commonly arising from gastritis, intestinal metaplasia, and epithelial injury of gastric mucosa [12], is characterized by ill-defined boundaries and a pronounced inflammatory background. The cells in AH typically exhibit a circular morphology, prominent nucleoli, and a high nucleus-to-cytoplasm ratio. Despite these features, cellular polarity is often preserved, and surface maturation is evident, indicating a benign nature [13]. This aligns with our findings, underscoring the importance of the "gradient of maturation" in AH, where structural and cellular alterations diminish from the deeper to the superficial mucosal layers.

LGD is characterized by minimal architectural disruption and mild to moderate cytological atypia. Histologically, it presents with elongated/oval, polarized, and basally located nuclei, and mild to moderate mitotic activity [9]. Additionally, glands typically appear dilated beneath the adenomatous layer [10, 14]. Our study observed neatly arranged palisaded nuclei, predominantly confined to the lower halves of the cells, with immature surface differentiation and rare nucleoli. A low nucleus to cytoplasm ratio and maintained cellular polarity were also noted. In contrast, HGD exhibits pronounced architectural disarray, such as complex glandular branching or fusion. Neoplastic cells tend to

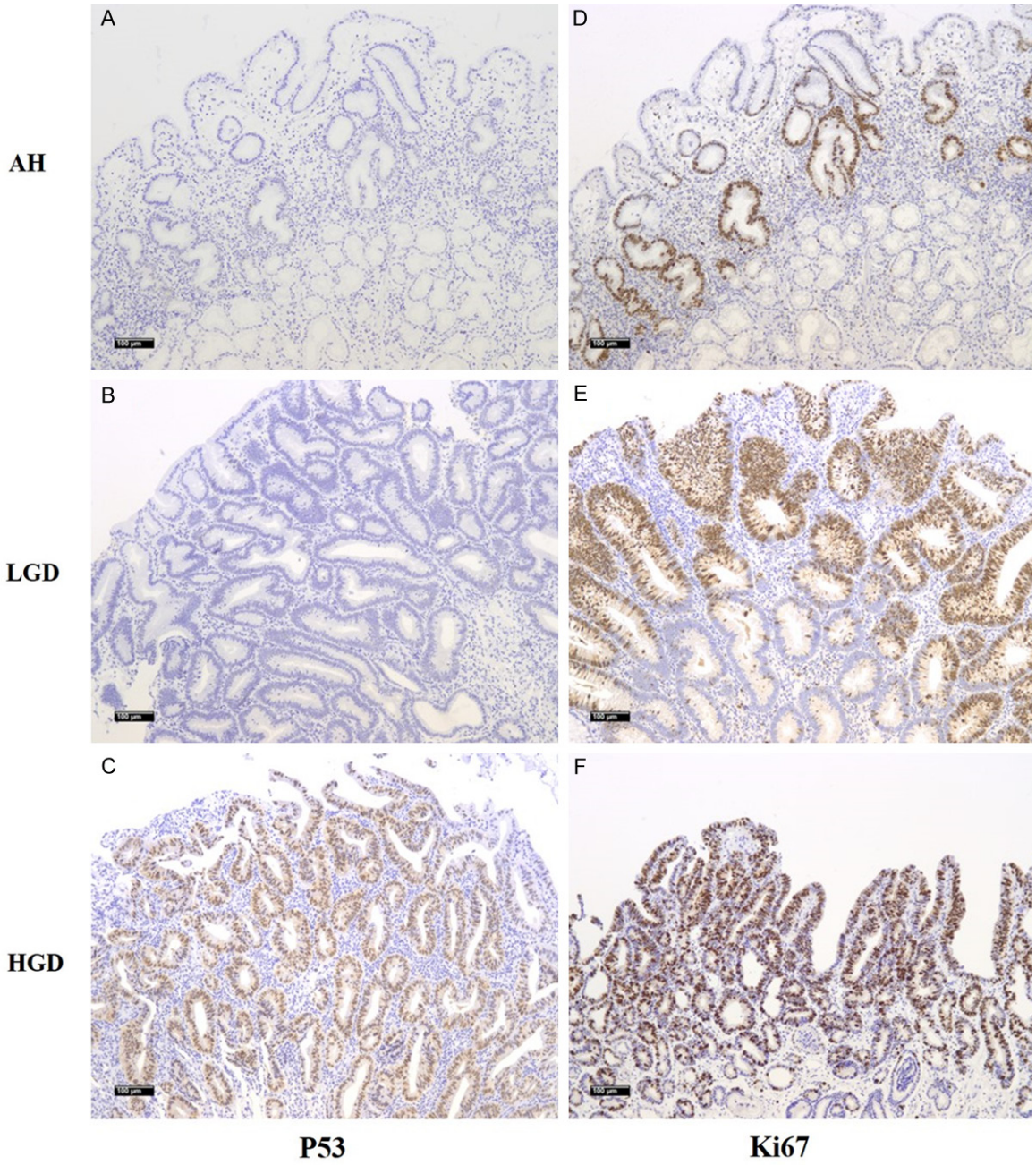

Figure 2. Immunopositivity of P53 and Ki67 in various gastric mucosal lesions. Expression of P53 demonstrates different patterns: 'wild-type pattern' in AH (A) and LGD (B), and a 'mutation pattern' in HGD (C). Ki67 also varies: 'pit neck pattern' in AH (D), a 'polarity pattern' in LGD (E), a 'diffuse pattern' in HGD (F) (H&E, IHC×100).

be cuboidal, rather than columnar, with a high nucleus-to-cytoplasm ratio, numerous mitoses, and nuclei located within the luminal zone of the epithelium, indicating a loss of polarity [9]. The diagnosis of HGD in this study was supported by the presence of irregularly shaped, branching, and folding complex tubular structures [15]. Notably, the absence of nuclear polarity and mature surface differentiation was observed, along with voluminous and irregularly shaped nuclei, prominent nucleoli, and an increased nucleus to cytoplasm ratio.

Mucins, high-molecular-weight glycoproteins rich in O-linked oligosaccharides and N-glycan chains, are encoded by 21 mucin (MUC) genes

in the human genome. These genes encode two categories of mucins: secreted and membrane-bound. In the gastric mucosa, the predominant mucins include membrane-bound MUC1 and secreted MUC5AC and MUC6 [16]. MUC5AC is typically expressed in the surface mucous epithelium of normal gastric mucosa, while MUC6 expression is elevated in the mucous neck cells of fundic and pyloric glands of the gastric mucosa. CD10, marking the microvilli on the luminal surface of the small intestine, and MUC2, observed in the perinuclear areas of goblet cells in the adult intestine, are generally not expressed in the normal gastric mucosa [17]. MUC5AC and MUC6 are regarded as gastric-type markers, while MUC2 and CD10 are intestinal-type markers. Gastric cancer mucin phenotypes, categorized into intestinal, gastric, combined, and unclassified types based on the expression of these markers, have varied implications for malignancy [18]. Notably, gastric-type mucin phenotypes are critical to identify due to their increased malignant potential in early invasion and metastasis stages [19]. Abnormal expression or absence of mucin distribution can signal abnormal differentiation and maturation of gastric mucosal glands, serving as a molecular marker for malignant malignancy [20, 21]. Our study found that AH typically exhibited distinct surface differentiation and maturation, with only one case lacking this maturation trend. Immunohistochemically, MUC5AC was expressed on the mucosal surface, and MUC6 in the mucous neck cells of fundic and pyloric glands, indicating a polarity expression pattern suggestive of surface differentiation and maturation. In contrast, all cases of HGD and 86.67% of LGD lacked this surface epithelial maturation and differentiation trend. However, 13.33% of LGD cases showed localized surface differentiation, implying that the transition from intestinal metaplasia to LGD was a gradual process. Therefore, the combined detection of MUC5AC and MUC6 can aid in assessing surface mature differentiation and distinguishing between neoplastic and non-neoplastic conditions.

P53, a tumor suppressor protein, plays a pivotal role in cell cycle regulation, DNA replication, and preventing uncontrolled cell division during tumor growth [22]. Mutations or aggregation of the P53 can lead to a loss of its normal tumor-suppressing function, thereby promoting tumor progression [23]. While wild-type P53 plays a crucial role in preventing carcinogenesis through apoptosis induction and genetic repair, mutant P53 is associated with an increased risk of carcinogenesis. The intensity of TP53 expression correlates with the degree of dysplasia, with rates in AH cases ranging from 1% to 5%, increasing to 65% in LGDs, and up to 75% in HGDs. Additionally, 50%-90% of adenocarcinomas exhibit TP53 mutations [24]. The overexpression of the p53 protein was observed to increase in correlation with the assigned histological classification [10]. Our study found a progressive increase in 'mutation-type pattern' expression rates across AH, LGD, and HGD groups [0% (0/48), 6.66% (2/30), and 66.66% (30/45), respectively], aligning with the literature, albeit at lower rates.

Ki67, a nuclear protein, is indicative of cellular division and proliferation, as it is present during the S, G1, G2, and M phases of the cell cycle, but absent in the G0 phase. In normal gastric mucosa, Ki67-positive cells are typically located at the base of gastric pits and the neck of gastric glands, denoting the proliferative zone of the mucosa. Notably, Ki67-positive cells are sporadically distributed [25]. In cases of intestinal metaplasia and hyperplasia, the proliferative zone of Ki67 shifts downward [10], a finding echoed in our research. In the present study, we observed distinct Ki67 expression patterns: 'pit neck pattern' in AH, 'polarity pattern' in LGD, and 'diffuse pattern' in HGD. These patterns aid in differentiating AH, LGD, and HGD, beyond mere reliance on expression indices.

Tumor grade is known to correlate with p53 overexpression and Ki-67-positivity, both indicative of tumor aggressiveness [26-28]. However these studies predominantly focused on the expression index of P53 and Ki67, with less emphasis on expression patterns. Our study highlights the importance of MUC5AC, MUC6, P53, and Ki67 expression patterns over positive rates in distinguishing gastric atypical hyperplasia and dysplasia. While morphologic features and immunohistochemical profiles can establish a diagnosis in most cases, distinguishing between AH and HGD can be challenging, especially when P53 exhibits wild-type expression and surface epithelium erosion or

loss. Distinguishing between LGD and AH, particularly in cases of intestinal metaplasia, is difficult due to their similar cellular morphology. In such cases, P53 immunohistochemistry, primarily in the wild-type, offers limited diagnostic utility. Therefore, further research into more sensitive and specific diagnostic indicators is necessary.

In conclusion, our study provides a comprehensive analysis of the morphologic characteristics and IHC expression in gastric mucosal biopsies. In differentiating atypical hyperplasia and dysplasia, we recommend a diagnostic approach that incorporates an evaluation of lesion boundaries, maturation gradients, glandular structure, cellular morphology, nucleus-to-cytoplasm ratio, nucleolus characteristics, and cellular polarity. Integrating these assessments with immunohistochemistry is recommended for achieving a precise pathological diagnosis. This combined methodological approach establishes a robust framework for clinical diagnosis and treatment planning. Significantly, our findings highlight that the expression patterns of MUC5AC, MUC6, P53, and Ki67 are more informative than mere reliance on expression indices during the diagnostic process.

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Disclosure of conflict of interest

None.

Address correspondence to: Ruixue Lei, Department of Pathology, Anyang Tumor Hospital/The Affiliated Anyang Tumor Hospital of Henan University of Science and Technology, No. 1 Huanbin North Road, Anyang 455000, Henan, China. Tel: +86-18317328633; E-mail: 55931944@qq.com

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