GBMN GEORGIAN BIOMEDICAL & MEDICAL NEXUS SINCE 2022

VOLUME 4 · ISSUE 4
OCTOBER-DECEMBER 2026

ORIGINAL ARTICLE

Proliferative and TP53 Alterations Across Gastric Precancerous Lesions and Adenocarcinoma

Beka Dangadze[1, ID*], George Didava[1, ID], George Burkadze[1, ID], Shota Kepuladze[1, ID]
[1] Department of Molecular Pathology, Tbilisi State Medical University, Tbilisi, Georgia

Received: 2026-08-19 · Accepted: 2026-10-14 · Published: 2026-11-18

ABSTRACT

Background. Gastric adenocarcinoma develops through a multistep process involving progressive histopathological and molecular alterations. Assessment of biomarkers associated with cellular proliferation and altered p53 expression may improve the characterization of gastric precursor lesions across increasing histologic severity.

Objectives. To investigate the immunohistochemical expression of Ki67 and p53 across gastric histopathologic categories and to evaluate their association with increasing histologic severity.

Methods. This retrospective study included 136 formalin-fixed, paraffin-embedded gastric tissue specimens representing normal gastric mucosa, chronic atrophic gastritis, complete intestinal metaplasia, incomplete intestinal metaplasia, low-grade dysplasia, high-grade dysplasia, intestinal-type gastric adenocarcinoma, and diffuse-type gastric adenocarcinoma. Immunohistochemical staining for Ki67 and p53 was performed using Leica antibodies. Ki67 expression was evaluated as the labeling index, while p53 staining was classified as wild-type or aberrant (overexpression/null pattern). Differences in Ki67 among diagnostic groups were analyzed using the Kruskal–Wallis test followed by Dunn–Bonferroni post hoc comparisons, with $p<0.05$ considered statistically significant.

Results. Ki67 expression differed significantly across the diagnostic categories, demonstrating the lowest proliferative activity in normal gastric mucosa and the highest levels in invasive adenocarcinomas ($p<0.001$). High-grade dysplasia exhibited significantly greater proliferative activity than low-grade dysplasia. p53 immunophenotypes also differed among the diagnostic groups, with aberrant staining particularly frequent in high-grade dysplasia and diffuse-type adenocarcinoma. Incomplete intestinal metaplasia demonstrated higher proliferative activity than complete intestinal metaplasia.

Conclusions: Ki67 labeling indices differed markedly across gastric histopathologic categories and were highest in dysplasia and adenocarcinoma. p53 immunohistochemical patterns also varied among diagnostic groups, with aberrant staining particularly frequent in high-grade dysplasia and diffuse-type adenocarcinoma. The p53 distribution was not uniformly monotonic across the examined categories. These findings support further investigation of Ki67 and p53 immunophenotypes in longitudinal and molecularly characterized cohorts.

Keywords. Gastric adenocarcinoma; Gastric carcinogenesis; Gastric dysplasia; Immunohistochemistry; Intestinal metaplasia; Ki67; P53; TP53; Cell proliferation.

DOI: https://doi.org/10.52340/GBMN.2026.01.01.197

BACKGROUND

Gastric cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide, accounting for a substantial proportion of cancer-associated deaths despite continuous advances in diagnostic methods and therapeutic strategies.[1] The prognosis of gastric cancer largely depends on the stage at diagnosis, emphasizing the importance of identifying precursor lesions with an increased risk of malignant transformation.[2] Consequently, understanding the biological mechanisms underlying gastric carcinogenesis has become an important focus of both diagnostic pathology and translational cancer research.

The development of intestinal-type gastric adenocarcinoma is generally described by the Correa cascade, a multistep process characterized by the sequential progression from chronic gastritis and mucosal atrophy to intestinal metaplasia, epithelial dysplasia, and ultimately invasive carcinoma.[3] Among these precursor lesions, intestinal metaplasia and dysplasia represent critical stages in neoplastic progression. Intestinal metaplasia is further classified

Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.

into complete and incomplete subtypes, with incomplete intestinal metaplasia considered to possess a greater risk of progression toward dysplasia and invasive carcinoma. Similarly, high-grade dysplasia is widely recognized as an immediate precursor of gastric adenocarcinoma and therefore requires careful pathological evaluation.[4]

Cellular proliferation and alterations in tumor-suppressor pathways are important features of tumor development. Ki67 is a well-established marker of proliferative activity and has been widely used to evaluate epithelial turnover and tumor aggressiveness in gastric lesions. Increased Ki67 expression has been associated with progressive neoplastic transformation and unfavorable clinicopathological features. The tumor suppressor protein p53 plays a crucial role in maintaining genomic integrity by regulating cell-cycle progression, DNA repair, and apoptosis. Aberrant p53 immunohistochemical expression, including overexpression and complete loss of staining, may serve as a surrogate for underlying TP53 abnormalities, although immunohistochemistry does not directly establish TP53 mutation status.[5]

Although numerous studies have investigated Ki67 or p53 individually, fewer studies have evaluated their expression across a broad range of gastric histopathologic categories, from non-neoplastic mucosa to invasive adenocarcinoma. Simultaneous assessment of these biomarkers may provide additional information regarding proliferative activity and p53 immunophenotypic heterogeneity across these lesions.

The present study aimed to evaluate the immunohistochemical expression of Ki67 and p53 in gastric precancerous lesions and gastric adenocarcinoma and to determine their association with increasing histologic severity. Particular emphasis was placed on comparing complete and incomplete intestinal metaplasia, low-grade and high-grade dysplasia, and the two major histological subtypes of gastric adenocarcinoma.

METHODS

We conducted a retrospective observational study using archival formalin-fixed, paraffin-embedded (FFPE) gastric tissue specimens obtained from the Department of Pathology. A total of 136 cases representing the examined gastric histopathologic categories were included. The study cohort comprised normal gastric mucosa (n=10), chronic atrophic gastritis (n=10), complete intestinal metaplasia (n=30), incomplete intestinal metaplasia (n=30), low-grade dysplasia (n=16), high-grade dysplasia (n=16), intestinal-type gastric adenocarcinoma (n=12), and diffuse-type gastric adenocarcinoma (n=12). Histopathological diagnoses were established according to the 5th edition of the World Health Organization (WHO) Classification of Digestive System Tumors and confirmed independently by two experienced pathologists. We excluded cases with inadequate tissue preservation, insufficient material for immunohistochemical analysis, extensive autolysis, or equivocal histopathological findings.

We retrieved available clinicopathological information from pathology reports and institutional medical records. Patient age and sex were available for the overall cohort. Other clinicopathological variables were not sufficiently complete for consistent subgroup analysis and were therefore not used as study outcomes.

All hematoxylin and eosin (H&E)-stained slides were reviewed prior to immunohistochemical evaluation. Intestinal metaplasia was subclassified as complete or incomplete according to established histomorphological criteria. Dysplastic lesions were categorized as low-grade or high-grade based on the degree of architectural complexity and cytological atypia. Gastric adenocarcinomas were classified according to the Lauren classification into intestinal-type and diffuse-type carcinomas.

Immunohistochemical staining was performed on 4-µm-thick sections prepared from FFPE tissue blocks. Sections were deparaffinized, rehydrated, and subjected to heat-induced antigen retrieval according to the manufacturer's recommendations. Endogenous peroxidase activity was blocked before primary antibody incubation. Immunostaining was performed using a Leica automated immunostaining platform with a polymer-based detection system (Novolink™, Leica Biosystems, Newcastle upon Tyne, UK). Diaminobenzidine (DAB) was used as the chromogen, followed by hematoxylin counterstaining. Appropriate positive and negative control tissues were included in each staining run.

The primary antibodies included anti-Ki67 (clone MM1, Leica Biosystems) and anti-p53 (clone DO-7, Leica Biosystems). Both antibodies were used according to the manufacturer's validated protocols.

Two pathologists independently evaluated immunohistochemical slides, blinded to the available clinicopathological information.

Ki67 expression was evaluated as the percentage of positively stained nuclei among at least 500 representative epithelial or tumor cells in areas

Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.

demonstrating the highest proliferative activity (hot spots). The Ki67 labeling index was expressed as the percentage of positively stained nuclei.

p53 immunoreactivity was interpreted using a pattern-based approach and classified as wild-type, overexpression, or null expression. Diffuse strong nuclear staining involving the great majority of lesioned cells was interpreted as an overexpression pattern. Complete absence of nuclear staining in lesioned cells was classified as a null pattern only when non-neoplastic cells on the same slide showed retained nuclear staining as an internal positive control. Heterogeneous nuclear staining that did not meet either aberrant pattern was classified as wild-type.

Statistical analyses were performed using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Ki67 values were summarized primarily as median with interquartile range (IQR) because group comparisons were performed using nonparametric methods.

Comparisons among the eight diagnostic groups were performed using the Kruskal–Wallis test for Ki67, followed by Dunn–Bonferroni post hoc analysis for pairwise comparisons. Categorical p53 patterns were summarized descriptively across diagnostic groups. Because of the small group sizes and sparse cells in several p53 categories, asymptotic χ^2 results were not used to support claims of ordered progression. Statistical significance for the Ki67 analyses was defined as a two-sided p-value <0.05.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Institutional Ethics Committee of Tbilisi State Medical University (TSMU.18.02.2019.N3). All tissue specimens were obtained from archived diagnostic material, and patient confidentiality was maintained throughout the study by anonymizing all clinical information prior to analysis.

RESULTS

A total of 136 gastric tissue specimens were included in the analysis. The study cohort comprised normal gastric mucosa (n=10), chronic atrophic gastritis (n=10), complete intestinal metaplasia (n=30), incomplete intestinal metaplasia (n=30), low-grade dysplasia (n=16), high-grade dysplasia (n=16), intestinal-type gastric adenocarcinoma (n=12), and diffuse-type gastric adenocarcinoma (n=12). The mean patient age was 60.6 ± 10.5 years, with a range of 30–79 years. Seventy-two patients were male (52.9%), and 64 were female (47.1%).

Ki67 proliferative activity differed significantly among the eight diagnostic groups (Kruskal–Wallis H=124.92, p<0.001). Normal gastric mucosa demonstrated the lowest proliferative activity, with a median Ki67 index of 1.0% (interquartile range [IQR], 0.0–2.0%). A modest increase was observed in chronic atrophic gastritis, with a median Ki67 index of 5.0% (IQR, 3.0–6.0%). Complete intestinal metaplasia showed a further increase to a median of 13.0% (IQR, 11.0–18.0%), while incomplete intestinal metaplasia exhibited a median Ki67 index of 25.0% (IQR, 18.0–28.8%).

Proliferative activity increased substantially in dysplastic lesions. The median Ki67 index was 42.5% (IQR, 39.0–44.3%) in low-grade dysplasia and 58.5% (IQR, 56.5–61.8%) in high-grade dysplasia. The highest values were identified in invasive carcinomas, with median indices of 71.5% (IQR, 64.0–84.0%) in intestinal-type adenocarcinoma and 73.5% (IQR, 71.0–76.0%) in diffuse-type adenocarcinoma.

Adjusted pairwise comparisons demonstrated significant differences in Ki67 expression across several adjacent categories within the intestinal pathway. Ki67 expression was higher in chronic atrophic gastritis than in normal gastric mucosa (adjusted p=0.005), in complete intestinal metaplasia than in chronic atrophic gastritis (adjusted p<0.001), and in incomplete intestinal metaplasia than in complete intestinal metaplasia (adjusted p<0.001) (**Fig.1** and **Fig.2**).

FIGURE 1. Ki67 immunostaining demonstrating increased proliferative activity in low-grade dysplasia arising in a background of intestinal metaplasia, 200x

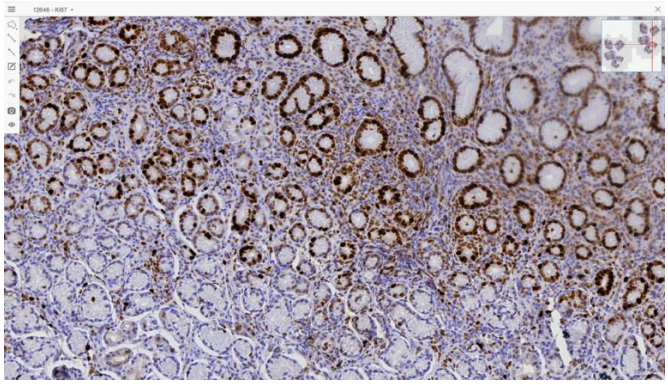
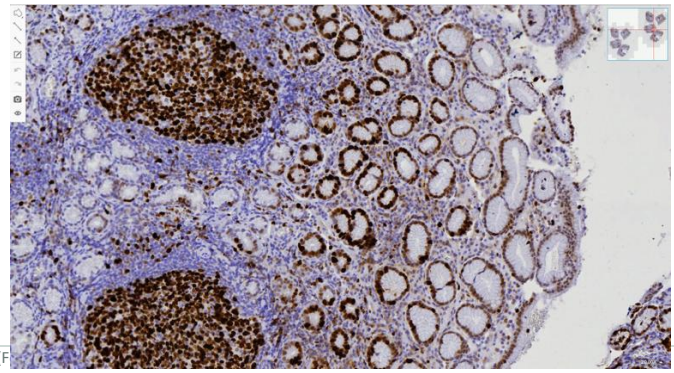


FIGURE 2. Ki67 immunostaining showing markedly increased proliferative activity in high-grade dysplasia, 200×



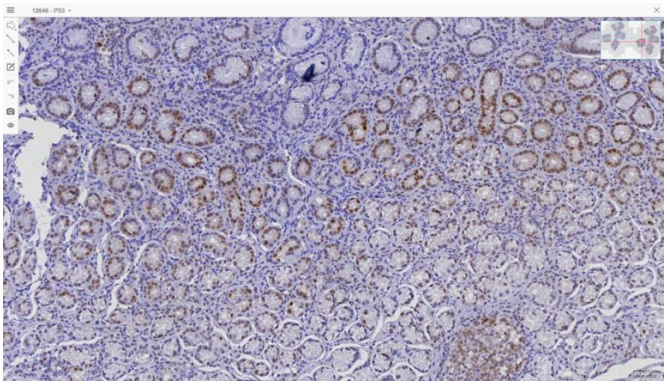
ISSN (Online): 2720-8796 · ISSN (Print): 2720-7994.
Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.

Significant increases were also observed from incomplete intestinal metaplasia to low-grade dysplasia (adjusted p<0.001), from low-grade to high-grade dysplasia (adjusted p<0.001), and from high-grade dysplasia to intestinal-type adenocarcinoma (adjusted p=0.003). Although diffuse-type adenocarcinomas demonstrated a slightly higher median Ki67 index than intestinal-type tumors, the difference between the two carcinoma subtypes was not statistically significant (adjusted p=0.582).

Ki67 labeling indices showed a strong ordered increase across the categories comprising the intestinal pathway from normal mucosa through intestinal-type adenocarcinoma. Diffuse-type adenocarcinoma was not interpreted as a terminal stage of this pathway and was considered separately as a histologic comparator.

p53 immunohistochemical patterns varied across the diagnostic groups. Wild-type p53 expression was present in all normal gastric mucosa cases. In chronic atrophic gastritis, 6 of 10 cases (60.0%) retained a wild-type pattern, whereas overexpression and null patterns were each observed in 2 cases (20.0%). Among complete intestinal metaplasia cases, wild-type expression was identified in 24 of 30 cases (80.0%), while 2 cases (6.7%) showed overexpression and 4 cases (13.3%) demonstrated a null pattern. In incomplete intestinal metaplasia, 26 of 30 cases (86.7%) showed wild-type expression, and 4 cases (13.3%) demonstrated overexpression; no null-pattern cases were identified (**Fig.3**).

FIGURE 3. p53 immunohistochemical staining demonstrating a wild-type expression pattern in intestinal metaplasia with low-grade dysplasia, 200×



Low-grade dysplasia retained a wild-type p53 pattern in 12 of 16 cases (75.0%). Aberrant expression was observed in the remaining 4 cases, equally divided between overexpression and null patterns. A marked change was identified in high-grade dysplasia, in which only 4 of 16 cases (25.0%) retained wild-type expression. Overexpression was present in 6 cases (37.5%), and a null pattern was also identified in 6 cases (37.5%). Thus, aberrant p53 expression increased from 25.0% in low-grade dysplasia to 75.0% in high-grade dysplasia.

Among intestinal-type adenocarcinomas, 8 of 12 cases (66.7%) demonstrated wild-type p53 expression, and 4 cases (33.3%) showed overexpression. In contrast, diffuse-type adenocarcinomas showed the highest frequency of aberrant p53 expression: only 2 of 12 cases (16.7%) retained a wild-type pattern, while 8 cases (66.7%) showed a null pattern and 2 cases (16.7%) showed overexpression.

When overexpression and null staining were combined as an aberrant p53 category, the frequencies were 0% in normal gastric mucosa, 40.0% in chronic atrophic gastritis, 20.0% in complete intestinal metaplasia, 13.3% in incomplete intestinal metaplasia, 25.0% in low-grade dysplasia, 75.0% in high-grade dysplasia, 33.3% in intestinal-type adenocarcinoma, and 83.3% in diffuse-type adenocarcinoma. The distribution was not monotonic across the diagnostic categories; aberrant staining was particularly frequent in high-grade dysplasia and diffuse-type adenocarcinoma.

Comparison of precursor lesions showed that incomplete intestinal metaplasia had significantly higher Ki67 expression than complete intestinal metaplasia, supporting increased proliferative activity in the incomplete subtype. However, aberrant p53 expression was slightly more frequent in complete than in incomplete intestinal metaplasia. This finding suggests that the enhanced proliferative activity of incomplete intestinal metaplasia was not uniformly accompanied by an increased frequency of abnormal p53 staining in this cohort (**Tabl.1**).

TABLE 1. Ki67 and p53 expression in gastric precancerous lesions and adenocarcinoma

Histopathological Group	n	Median Ki67 (%)	Wild-type p53, n (%)	Aberrant p53*, n (%)
Normal gastric mucosa	10	1.0 (0.0–2.0)	10 (100.0)	0 (0.0)
Chronic atrophic gastritis	10	5.0 (3.0–6.0)	6 (60.0)	4 (40.0)
Complete intestinal metaplasia	30	13.0 (11.0–18.0)	24 (80.0)	6 (20.0)
Incomplete intestinal metaplasia	30	25.0 (18.0–28.8)	26 (86.7)	4 (13.3)
Low-grade dysplasia	16	42.5 (39.0–44.3)	12 (75.0)	4 (25.0)
High-grade dysplasia	16	58.5 (56.5–61.8)	4 (25.0)	12 (75.0)
Intestinal-type adenocarcinoma	12	71.5 (64.0–84.0)	8 (66.7)	4 (33.3)
Diffuse-type adenocarcinoma	12	73.5 (71.0–76.0)	2 (16.7)	10 (83.3)
Overall	136	—	—	—

**Aberrant p53 includes both overexpression and null-pattern immunostaining*

Georgian Biomedical and Medical Nexus (Former Georgian Biomedical News).
ISSN (Online): 2720-8796 · ISSN (Print): 2720-7994.
Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.

High-grade dysplasia demonstrated a median Ki67 index of 58.5% and aberrant p53 expression in 75.0% of cases. Compared with low-grade dysplasia, these findings indicate higher proliferative activity and more frequent aberrant p53 staining in high-grade dysplasia.

The carcinoma groups displayed similarly high proliferative activity but differed in p53 phenotype. Intestinal-type and diffuse-type adenocarcinomas had comparable Ki67 indices, while diffuse-type adenocarcinoma showed a higher frequency of aberrant p53 expression, predominantly the null pattern. This immunophenotypic difference should be regarded as exploratory and warrants evaluation in larger, molecularly characterized cohorts.

Overall, Ki67 expression differed markedly across the examined gastric histopathologic categories, with higher values in dysplasia and carcinoma. p53 patterns were heterogeneous and did not show a uniform stepwise increase; aberrant staining was particularly frequent in high-grade dysplasia and diffuse-type adenocarcinoma.

DISCUSSION

Gastric carcinogenesis is a multistep process characterized by morphological and molecular alterations. Identifying biomarkers associated with different histopathologic states may improve the biological characterization of gastric precursor lesions. In the present study, Ki67 proliferative activity and p53 expression patterns showed distinct distributions across the examined gastric diagnostic categories.

The most consistent finding was the marked increase in Ki67 expression across categories of increasing histologic severity within the intestinal pathway. Low proliferative activity was observed in normal mucosa and chronic atrophic gastritis, followed by higher values in intestinal metaplasia, dysplasia, and intestinal-type adenocarcinoma. These cross-sectional findings agree with previous reports indicating increased cellular proliferation in increasingly advanced gastric lesions. The markedly elevated Ki67 labeling index observed in high-grade dysplasia further supports its characterization as an advanced precursor lesion.[6]

p53 immunohistochemical patterns also differed across the examined categories. Wild-type staining predominated in normal gastric mucosa and most metaplastic and low-grade dysplastic lesions, whereas aberrant patterns were frequent in high-grade dysplasia and diffuse-type adenocarcinoma.[5] Importantly, the distribution was not uniformly monotonic: aberrant p53 staining was more frequent in chronic atrophic gastritis than in either intestinal-metaplasia subgroup. It was less frequent in intestinal-type adenocarcinoma than in high-grade dysplasia. Accordingly, the present data support histologic-group differences in p53 immunophenotype rather than a continuous accumulation of p53 abnormalities.

Comparison of complete and incomplete intestinal metaplasia revealed higher proliferative activity in the incomplete subtype, consistent with previous evidence that incomplete intestinal metaplasia is associated with greater gastric cancer risk. Differences in p53 expression between the two metaplasia subtypes were less pronounced, indicating that the higher Ki67 labeling index in incomplete intestinal metaplasia was not accompanied by a higher frequency of aberrant p53 staining in this cohort.

Among invasive tumors, both intestinal-type and diffuse-type adenocarcinomas demonstrated high Ki67 expression. Diffuse-type adenocarcinomas exhibited a higher frequency of aberrant p53 staining than intestinal-type tumors. Because diffuse-type carcinoma is not a terminal stage of the Correa intestinal pathway, it should be interpreted as a biologically distinct comparator rather than as a more advanced ordinal category. The observed difference in p53 immunophenotype is exploratory and requires molecular validation.

The present study has several limitations. Its retrospective, cross-sectional design and reliance on archival tissue may introduce selection bias and do not permit demonstration of temporal progression or prediction of malignant transformation within individual patients. The relatively small numbers in several diagnostic groups limit precision, particularly for categorical p53 patterns. Sparse p53 categories also limit the reliability of conventional asymptotic contingency testing. The absence of molecular genetic analysis prevents direct inference of TP53 mutation status from p53 immunohistochemistry. In addition, detailed clinicopathological covariates and longitudinal follow-up were not sufficiently complete for robust subgroup or outcome analyses. These limitations should be considered when interpreting the findings.

Overall, the results indicate that Ki67 and p53 provide complementary descriptive information across gastric histopathologic categories. The findings support further evaluation of these markers in larger longitudinal cohorts with molecular characterization and clinically relevant outcome data.

Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.

CONCLUSIONS

The present study demonstrated marked differences in Ki67 proliferative activity across gastric histopathologic categories, with the highest values observed in dysplasia and adenocarcinoma. p53 immunohistochemical patterns also varied among diagnostic groups, with aberrant staining particularly frequent in high-grade dysplasia and diffuse-type adenocarcinoma. However, p53 abnormalities did not show a uniform stepwise increase across the examined categories.

These findings characterize Ki67 and p53 immunophenotypes across a broad spectrum of gastric lesions but do not establish prediction of malignant progression, molecular TP53 status, or clinical surveillance utility. Further longitudinal studies incorporating molecular analysis and larger cohorts are required to determine the potential diagnostic or prognostic value of these markers.

ACKNOWLEDGEMENTS

The authors express their sincere gratitude to the staff of the Department of Molecular Pathology, Tbilisi State Medical University, for their assistance in specimen processing and technical support throughout this study.

REFERENCES

1. Zhou L, Han B, Yuan Y, Dong Z, Shi Y, Zheng R. The global burden of stomach cancer and its risk factors from 1990 to 2021: findings from the Global Burden of Disease Study 2021. *BMC Public Health 2025 25:1*. 2025;25(1):2678-. doi:10.1186/S12889-025-23901-Y.
2. Yada T, Yokoi C, Uemura N. The Current State of Diagnosis and Treatment for Early Gastric Cancer. *Diagn Ther Endosc*. 2013;2013:241320. doi:10.1155/2013/241320.
3. Li W, Zhang T. Precancerous pathways to gastric cancer: a review of experimental animal models recapitulating the correa cascade. *Front Cell Dev Biol*. 2025;13:1620756. doi:10.3389/FCELL.2025.1620756/FULL.
4. Du S, Yang Y, Fang S, et al. Gastric Cancer Risk of Intestinal Metaplasia Subtypes: A Systematic Review and Meta-Analysis of Cohort Studies. *Clin Transl Gastroenterol*. 2021;12(10):e00402. doi:10.14309/CTG.0000000000000402.
5. Janic A, Abad E, Amelio I. Decoding p53 tumor suppression: a crosstalk between genomic stability and epigenetic control? *Cell Death Differ*. 2024;32(1):1. doi:10.1038/S41418-024-01259-9.
6. Yang J, Wang S, Li X, et al. Signaling pathways and targeted interventions for precancers. *Signal Transduction and Targeted Therapy 2025 11:1*. 2026;11(1):9-. doi:10.1038/s41392-025-02342-4.
7. Cristian M, Aşchie M, Deacu M, et al. Comparison of Ki67 Proliferation Index in Gastrointestinal Non-Hodgkin Large B-Cell Lymphomas: The Conventional Method of Evaluation or AI Evaluation? *Diagnostics*. 2023;13(17):2775. doi:10.3390/DIAGNOSTICS13172775.
8. Li W, Zhang T. Precancerous pathways to gastric cancer: a review of experimental animal models recapitulating the correa cascade. *Front Cell Dev Biol*. 2025;13:1620756. doi:10.3389/FCELL.2025.1620756/FULL.

Downloaded from gbmn.org. For personal use only. No other uses without permission. Copyright © 2022. All rights reserved.