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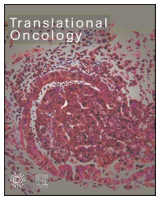

Original Research

Identification and validation of serum MUC17 as a non-invasive early warning biomarker for screening of gastric intraepithelial neoplasia

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ABSTRACT

Background: The early diagnosis and treatment of Gastric Intraepithelial Neoplasia (GIN) are pivotal for improving the survival rates of patients with gastric cancer (GC). Regrettably, reliable noninvasive biomarkers for GIN screening are currently lacking.

Methods: mRNA data from the GEO database, pan-cancer data from the TCGA database, and a gene list of exocrine proteins were subjected to integrated analysis to identify a noninvasive biomarker for GIN. The scRNA-seq data analysis, IHC and Elisa were employed to validate the expression of the biomarker in the serum and tissues of clinical patients across different pathological stages.

Results: MUC17 has been identified as a non-invasive diagnostic marker for GIN. It is upregulated in GIN prior to the onset of gastric carcinogenesis and downregulated in other tumors, with high GC specificity. The area under the curve values of serum MUC17 for differentiating chronic gastritis (CG) from low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN), and early gastric cancer (EGC) were 0.8788, 0.8544, and 0.9513, respectively. Additionally, low plasma MUC17 levels were found to be significantly lower in gastric ulcer (GU), gastric neuroendocrine tumor (GNET), and gastrointestinal stromal tumor (GIST) compared to GIN. The AUC for differentiating between GIN and GU, GNET, or GIST was 0.7803, 0.9244 and 0.9796, respectively.

Conclusions: These findings suggest that plasma MUC17 levels hold substantial promise as a screening biomarker for individuals with GIN and EGC, effectively identifying high-risk groups that necessitate further gastroscopy.

Introduction

Gastric cancer (GC), which accounts for approximately 800,000 deaths annually, ranks as the third leading cause of cancer-related mortality worldwide and the fifth most common cancer overall [1]. The intestinal subtype of GC is characterized by a complex, multi-step progression beginning with inflammation and advancing through stages of intestinal metaplasia, atrophy, and intraepithelial neoplasia (GIN), which includes both low-grade intraepithelial neoplasia (LGIN) and high-grade intraepithelial neoplasia (HGIN), ultimately culminating in early gastric cancer (EGC) or advanced gastric cancer (AGC) [2]. Despite substantial advancements in our understanding of gastric cancer, the factors and mechanisms underlying its occurrence and progression remain incompletely elucidated. Complex molecular pathways are deemed crucial in the pathogenesis of gastric cancer. Various molecular pathways, along with their respective upstream and downstream mediators, play significant roles in its development. Notable examples include the SOX family transcription factors and the STAT3 signaling pathway, among others [3–5]. This potential signaling pathways and their mediators also offer an expanding array of therapeutic targets for the treatment of gastric cancer. For instance, clinical trials focusing on STAT signaling molecules are progressively being undertaken and have yielded promising outcomes [6]. Nonetheless, the five-year survival rate for patients with advanced gastric cancer remains low. Conversely, the five-year survival rate can increase to 95 % if intervention and treatment are administered during the pre-or early stages of gastric cancer [7]. The majority of individuals with GIN and EGC exhibit no conventional symptoms, a critical factor contributing to delayed diagnosis and

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potentially fatal outcomes [8]. Endoscopic screening for patients with GIN and EGC, along with the adoption of continuous monitoring or endoscopic submucosal dissection (ESD) treatment, are essential measures for improving the survival outcomes of patients with GC [9]. The practice of endoscopic screening for GIN and EGC has been successfully implemented in large asymptomatic populations in South Korea and Japan [10,11]. However, in other countries, the timely establishment of a national gastroscopy screening program is hindered by disparities in access to healthcare services and the substantial economic burden on health systems. Despite the substantial burden on medical resources imposed by annual gastroscopy, the detection rate of stomach cancer and precancerous lesions remains low [12]. The absence of effective initial screening methods contributes to gastric cancer detection rates remaining below 20 % [13]. A gastroscopy screening program for gastric precancerous lesions in rural Shandong Province, China, demonstrated detection rates of 1.83 % for LGIN and 0.22 % for HGIN [14]. Despite the advantages of endoscopy in diagnosing gastrointestinal disorders, a significant number of asymptomatic individuals are reluctant to undergo routine gastroscopy due to concerns related to scheduling, cost, and the invasive nature of the procedure. Consequently, there is a critical need to develop non-invasive and cost-effective serum biomarkers for preliminary screening prior to gastroscopy. Implementing such serological biomarker screening could effectively identify key target populations that require more detailed gastroscopic examination.

Currently, the majority of serological biomarkers for GC are primarily concentrated on gastric carcinogenesis rather than on the precarcinogenic stages. These biomarkers, such as carcinoembryonic antigen (CEA), carbohydrate antigen 19–9 (CA199), and cancer antigen 125 (CA125), predominantly exhibit elevated expression levels in the advanced stages of the disease [15]. These tumor biomarkers exhibit limited organ specificity, frequently leading to their elevation across a range of tumor types, thereby diminishing their specificity for the detection of GC. Current serological screening markers for patients with GIN and EGC encompass pepsinogens I and II (PGI and PGII), gastrin-17, and anti-Helicobacter pylori (H. pylori) antibodies [16,17]. Most of these markers are correlated with the condition of the stomach, particularly indicating an atrophic state of the gastric mucosa [18,19]. Nevertheless, no research has definitively demonstrated whether or not they accurately identify patients with GIN and EGC. Serological biomarkers for GIN are scarce. Consequently, our research concentrated on the stage of GIN that precedes gastric carcinogenesis, with the aim of identifying a non-invasive diagnostic biomarker suitable for initial screening prior to gastroscopy. This biomarker must exhibit significantly elevated levels in the serum of patients not only with GIN and EGC but also with AGC. The identification of such a biomarker could enhance the breadth and efficacy of screening programs.

In this study, we identified a serum diagnostic biomarker through a comprehensive analysis of encoded exocrine protein gene lists and transcriptome sequencing data from multiple clinical samples available in public databases. To ensure high specificity for GC, we excluded genes that are also upregulated in other gastrointestinal (GI) tumors. Consequently, we identified a serum biomarker that is both an early diagnostic indicator at the onset of GIN and highly specific for GC. Serum samples were collected from clinical patients at various stages of GC occurrence and progression to assess the diagnostic efficacy of this biomarker. Additionally, we examined the expression levels of this biomarker in other stomach disorders and evaluated its potential as a differential diagnostic marker.

Materials and method

Data acquisition and processing

Gene expression profiles were obtained from the GEO database, and the corresponding accession numbers for the datasets listed as follows: GSE55696, including 19 samples of CG tissue, 19 samples of LGIN tissue, 20 samples of HGIN tissue, and 19 samples of EGC tissue; GSE130823, comprising 46 samples of CG tissue, 17 samples of LGIN tissue, 14 samples of HGIN tissue, and 16 samples of EGC tissue; GSE224056, consisting of 5 patient-matched pairs of AGC and adjacent normal gastric tissues; GSE66229, containing 300 AGC tissues and 100 normal control tissues. Gene expression profiles from two supplementary datasets were utilized to validate candidate genes for their differential diagnostic value. The first dataset, GSE73336, includes 4 mouse gastric ulcer samples along with their corresponding normal tissues. The second dataset, GSE26942, comprises 3 gastrointestinal stromal tumor tissue samples, 202 GC tissue samples, and 12 adjacent normal tissues. The R software (version 4.2.0) utilizing the "limma" package was employed for the processing and analysis of count data. RNA sequencing data for pan-cancer analyses were obtained from The Cancer Genome Atlas (TCGA) cancer cohorts, accessible via the UCSC Xena browser. For pan-cancer types other than GC, genes exhibiting low expression and down-regulation were individually identified in comparison to normal tissue using the "tinyarray" package within the R software environment. The Tumor Immunity Estimation Resource 2.0 was employed to visualize the expression profiles of individual genes across comprehensive pan-cancer datasets. Single-cell RNA sequencing data for this study were sourced from GSE183904 and subsequently processed using the "Seurat" package within the R software environment. Immunohistochemistry images depicting MUC17 expression in various cancerous and normal tissues were acquired from the Human Protein Atlas.

The list of exocrine protein genes can be obtained through the following sources (Table S1): 1. The Human Protein Atlas, which provides data on protein subcellular localization (https://www.proteinatlas.org/). This database includes predictions of the human secretome using three different methods (SignalP4 [20], Phobius [21] and SPOCTOPUS [22]) for signal peptides, as well as the MDSEC [23] and final predictions resulting from manual annotation. 2. Cellular Components (CC) terms in the Gene Ontology Database are also utilized to identify exocrine protein genes. Relevant terms include GO:0005576 (extracellular region), GO:0005615 (extracellular space), GO:0031012 (extracellular matrix) and GO:0005614 (interstitial matrix).

Patients and samples

Serum samples were collected from a cohort comprising 100 patients diagnosed with AGC, 40 patients with EGC, 40 patients with HGIN, 40 patients with LGIN, 40 patients with CG, and 40 healthy donors. Additionally, for the purpose of differential diagnosis with other diseases, serum samples were obtained from 120 patients, including 40 patients with gastric ulcer (GU), 40 patients with gastric neuroendocrine tumor (GNET), and 40 patients with gastrointestinal stromal tumor (GIST). Baseline statistics for the clinical data of the aforementioned patients are presented in Table 1. The diagnoses for all patients were confirmed by clinicians and verified through pathological examination following gastroscopy or surgical intervention. Serum samples were collected from all patients prior to any examination or treatment. All serum samples were stored in cryovials at −80 °C until analysis.

A total of 100 paraffin-embedded gastric tissue samples were procured from the pathology department and sectioned into 5 μm slices for immunohistochemistry (IHC) analysis. These samples were derived from patients who had undergone either endoscopic biopsy or surgical resection, including 20 cases of CG, 20 cases of LGIN, 20 cases of HGIN, 20 cases of EGC, 20 cases of AGC, and 20 samples of normal gastric tissue. The study adhered to the principles outlined in the Declaration of Helsinki and received approval from the Ethics Committee of The Second Hospital of Hebei Medical University (Ethical Approval Code: 2023-R495).

Sandwich enzyme-linked immunosorbent assay (ELISA)

The concentration of MUC17 in the serum of all patients was

Table 1
Clinical characteristics of LGIN, HGIN, EGC, AGC, CG, GU, GIST and GNET patients and healthy donors.

	Control $n = 40$	LGIN $n = 40$	HGIN $n = 40$	EGC $n = 40$	AGC $n = 100$
Age (years)	52.48 ±8.79	53.90 ±11.46	51.50 ±7.51	54.13 ±8.32	55.82 ±13.28
Gender					
Male (%)	20(50.0)	28(70.0)	23(57.5)	22(55.0)	60(60.0)
Female (%)	20(50.0)	12(30.0)	17(42.2)	18(45.0)	40(40.0)
Hp					
Position (%)	10(25.0)	12(30.0)	12(30.0)	18(45.0)	36(36.0)
Negative (%)	30(75.0)	28(70.0)	28(70.0)	22(55.0)	64(64.0)
Location					
Cardia		9	7	8	14
Fundus		3	3	5	13
Gastric body		6	12	6	29
Gastric antrum		16	8	13	24
Pylorus		6	10	8	20

	CG $n = 40$	GU $n = 40$	GIST $n = 40$	GNET $n = 40$	*P* value
Age (years)	21.63 ±9.080	53.23 ±8.43	50.50 ±11.18	52.23 ±5.609	0.130
Gender					0.510
Male (%)	18(45.5)	23(57.5)	21(52.5)	20(50.0)	
Female (%)	22(55.0)	17(42.5)	19(47.5)	20(50.0)	
Hp					0.278
Position (%)	11(27.5)	13(32.5)	9(22.5)	10(25.0)	
Negative (%)	29(72.5)	23(67.5)	31(77.5)	30(74.5)	
Location					0.106
Cardia		2	3	5	
Fundus		7	12	11	
Gastric body		10	11	11	
Gastric antrum		2	7	7	
Pylorus		9	7	6	

quantified using the MUC17 ELISA Kit (ENOVA Bio Co Ltd, Nanjing, China). A 50-μL volume of either the standard (provided by the manufacturer, with varying concentration gradients), the sample (serum from all patients in our study), or the sample diluent (blank) was added to a 96-well plate pre-coated with MUC17 antibody. Subsequently, 100 μL of HRP-conjugate reagent was introduced to each well, and the plate was incubated at 37 °C for 1 hour. Each well was treated with 500 μL of a 20X wash solution, as supplied by the manufacturer, and allowed to stand for 30 s before the liquid was discarded. This washing procedure was repeated five times. Subsequently, the contents of each well were aspirated and replaced with 50 μL each of chromogen solution A and chromogen solution B, both provided by the manufacturer. The plate was then incubated at 37 °C in the dark for 15 min. The reaction was terminated by adding 50 μL of the stop solution, also supplied by the manufacturer. Absorbance was measured at 450 nm using a microplate reader.

Immunohistochemical staining

The tissue sections were generally subjected to antigen retrieval by boiling in 0.1 M sodium citrate, followed by deparaffinization using a clearing reagent, and rehydration through a graded ethanol series. Endogenous peroxidase activity was inhibited by treatment with 3 % hydrogen peroxide. Subsequently, the slides were incubated overnight at 4 °C with a rabbit anti-MUC17 antibody (1:50, Affinity). The following day, a biotin-labeled anti-rabbit secondary antibody was applied for 20 min at room temperature. The slides were subsequently blocked with 5 % goat serum for 1 hour. Thereafter, the sections were counterstained with hematoxylin, stained with diaminobenzidine (DAB), and finally mounted. Imaging of the sections was performed using an Olympus microscope (Japan). The integrated optical density (IOD), reflecting the intensity and area of staining, was quantified using Image-Pro Plus 6.0 software to obtain the IHC results.

Statistical analysis

The mean ± standard deviation is used to represent data. The statistical significance between two groups was calculated using Student's *t*-test or the Mann–Whitney U test. For the analysis of continuous variables among three or more groups, one-way analysis of variance (ANOVA) or the Kruskal–Walli's test was employed. The statistical relationships were examined using Spearman's correlation test. The diagnostic performance was assessed by using the AUC and 95 % confidence interval (CI). A statistical significance threshold of $P < 0.05$ was used to all analyses, which were carried out using either GraphPad Prism 9.0 or R software 4.1.2.

Results

Identification of serum biomarkers in patients with GIN and GC

The aim of this study was to identify a biomarker suitable for initial screening and for the identification of high-risk individuals who require a comprehensive examination via gastroscopy. This biomarker must exhibit significantly elevated levels in the blood of individuals with GIN, EGC, and AGC. To achieve this objective, we conducted an analysis of RNA expression profiling data obtained from public databases, following the methodology depicted in Fig. 1. The mRNA expression profiles were sourced from the GSE55696 and GSE130823 databases to identify genes upregulated during GIN. Analysis revealed that 1662 genes were upregulated in LGIN tissues, 1287 genes in HGIN tissues, and 1882 genes in EGC tissues, compared to CG tissues ($LogFC > 1$ and $pvalue < 0.05$). These findings are depicted in a volcano plot (Fig. 2A). Gene lists from the three groups were intersected to identify genes consistently upregulated across LGIN, HGIN, and EGC tissues. This methodology was similarly applied to the GSE130823 database. By intersecting 1088 upregulated genes in the LGIN group, 826 upregulated genes in the HGIN group, and 833 upregulated genes in the EGC group, a total of 267 genes were identified that demonstrated consistent upregulation from LGIN to EGC. Volcano plots were utilized to illustrate the upregulated genes within each specific group (Fig. 2B). The results derived from intersecting data from the two databases were deemed more accurate. Consequently, 73 genes were identified as upregulated from LGIN to EGC based on these two public datasets (Fig. 2C). To further investigate the continuous upregulation of genes in advanced GC, mRNA expression profiles of GC tissues and normal tissues were obtained from TCGA database. A total of 2619 mRNAs were identified as upregulated in advanced GC ($LogFC > 1$ and $pvalue < 0.05$) and were presented using a heatmap (Fig. 2D).

The identification of secretory protein biomarkers in plasma, as a noninvasive diagnostic technique, is clinically significant due to its reproducibility and cost-effectiveness. To identify potential biomarkers for GIN and GC detectable in blood, the list of up-regulated genes was intersected with the list of genes encoding extracellular proteins. This gene list comprised 6656 genes encoding proteins that can be secreted into the extracellular space and enter the bloodstream. Ultimately, 20 genes were identified that exhibited continuous upregulation during the progression from LGIN to AGC (Fig. 2E). The 20 hub genes are: CLDN7, EPHB2, F12, HPSE, KLK6, KLK8, LEFTY1, LIF, LRP8, RARRES1, TNFRSF11B, TNFRSF12A, CEACAM7, DMBT1, TNFSF9, ULBP2, TUBB3, ANXA13, MUC17, CLRN3. These genes have the potential to serve as serological screening markers prior to gastroscopy, facilitating the identification of individuals with gastric mucosal lesions, including LGIN, HGIN, EGC, and AGC, who may subsequently require further gastroscopic evaluation or endoscopic treatment.

To identify a biomarker with high GC specificity that is upregulated in a minimal number of carcinomas, we downloaded pan-cancers mRNA

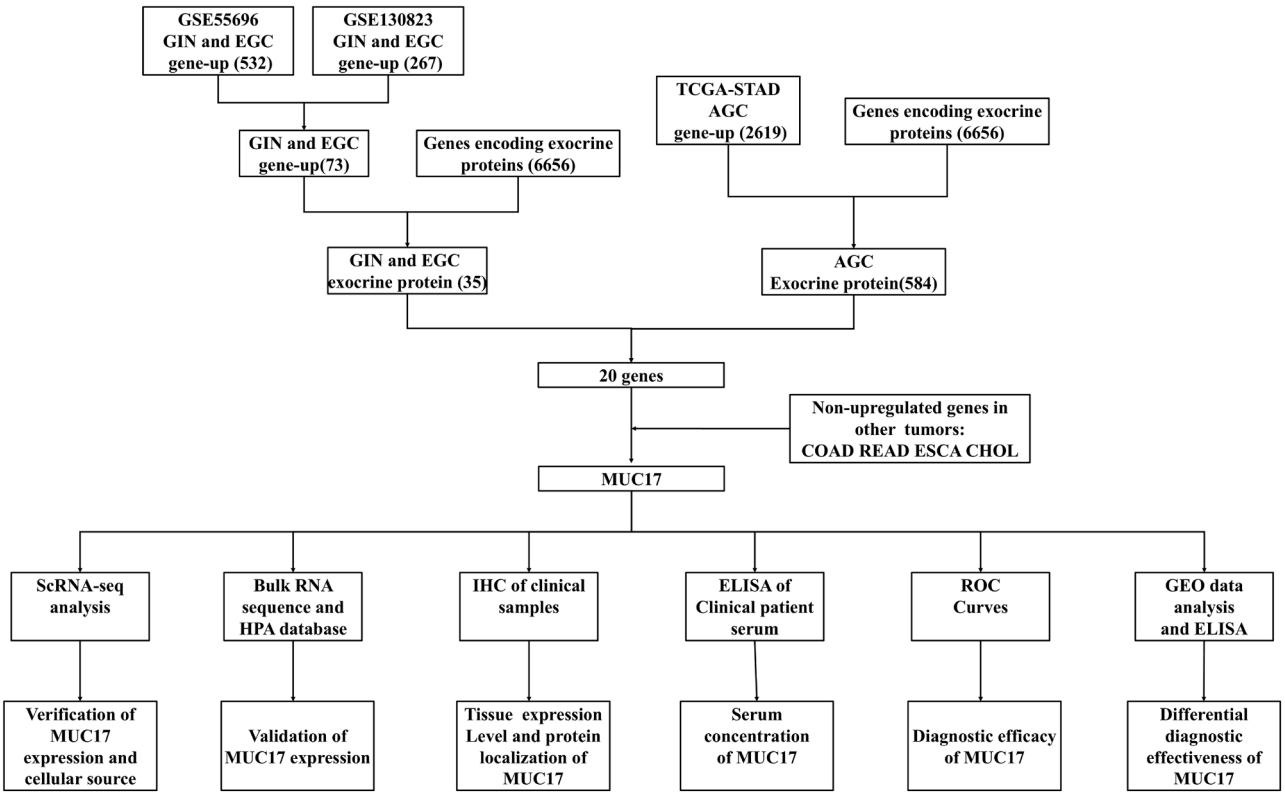

Fig. 1. Workflow of this study.

expression data from the Xena database. Genes with no significant difference in expression between tumor and normal groups and down-regulations in tumors were selected for each tumor type respectively (Table S2). Initially, we intersected genes that are not up-regulated in expression in gastrointestinal tumors, including colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), esophageal carcinoma (ESCA), and cholangiocarcinoma (CHOL), with the 20 genes mentioned above. Unexpectedly, only a single gene, MUC17, was characterized. We employed the TIMER2.0 platform to illustrate the expression levels of MUC17 across various cancer types, as presented in Fig. 3A. The data reveal a pronounced overexpression of MUC17 in GC compared to the majority of other tumors. Although the expression of MUC17 is elevated in the tumor tissues of Pancreatic Adenocarcinoma (PAAD) and Esophageal Carcinoma (ESCA), statistical analysis indicates that these differences are not significant. In the context of COAD and READ, MUC17 demonstrated a pattern of elevated expression in normal tissues relative to tumor tissues, however, statistical analysis revealed no significant differences in expression levels. Consequently, MUC17 exhibited higher specificity in GC, suggesting its potential as an organ-specific serum diagnostic marker. Subsequently, we examined the positive rate of MUC17 across various tumor types, categorizing samples with expression levels exceeding the average of the normal group as positive. As illustrated in Fig. 3B, MUC17 expression in GC presented a higher positive rate compared to other tumors.

Validated expression of MUC17 from epithelial cell in GC tissue using single-cell RNA-seq

To elucidate the expression pattern and protein localization of MUC17 in GC tissues, we analyzed data from the single-cell dataset GSE183904. Each cluster was assigned a distinct color based on the single-cell transcriptome analysis (Fig. 4A), which demonstrated that MUC17 is predominantly expressed in the epithelial cells of GC tissue (Fig. 4B and C). Furthermore, reclustering of all epithelial cells within the GC tissue identified 15 distinct clusters (Fig. 4D). MUC17 exhibited elevated expression levels in the 12 subgroup and moderate expression in the 1, 2, and 3 subgroups (Fig. 4E and F). The distinct expression pattern of MUC17 demonstrated significant heterogeneity across clusters within tumor epithelial cells. Consequently, our findings highlight that MUC17 expression is specifically upregulated by certain clusters of epithelial cells in GC tissues, with its deposition in the extracellular matrix. This suggests its potential utility as a valuable serum marker for the diagnosis of GC.

Validation of MUC17 expression using public data

To assess the gene expression levels of MUC17 across various pathological stages of GC, we analyzed the GSE55696 dataset. The findings indicated that, compared to CG group, MUC17 expression was significantly elevated in the LGIN, HGIN, and EGC groups ($p < 0.05$) (Fig. 5A). Consistent results were observed in the GSE130823 dataset, where MUC17 expression in the LGIN, HGIN, and EGC groups was also significantly higher than in the CG group ($p < 0.05$) (Fig. 5B). Additionally, MUC17 expression was upregulated in the AGC stages in both the GSE224056 and GSE66229 datasets ($p < 0.05$) (Fig. 5C and D). We subsequently investigated the differential expression of MUC17 between cancerous and normal tissues using immunohistochemistry data from the Human Protein Atlas (HPA) datasets to validate the protein expression levels of MUC17 across various tumor tissues. MUC17 expression was no significantly different in COAD, READ, LIHC, LUAD, PRAD and BRCA tissues compared to normal tissues, and only a small amount of MUC17 expression was found in normal colorectal tissue, as shown by the arrows in Fig. 5E.

Validation of MUC17 expression and localization using immunohistochemistry

To elucidate the expression and protein localization of MUC17 across

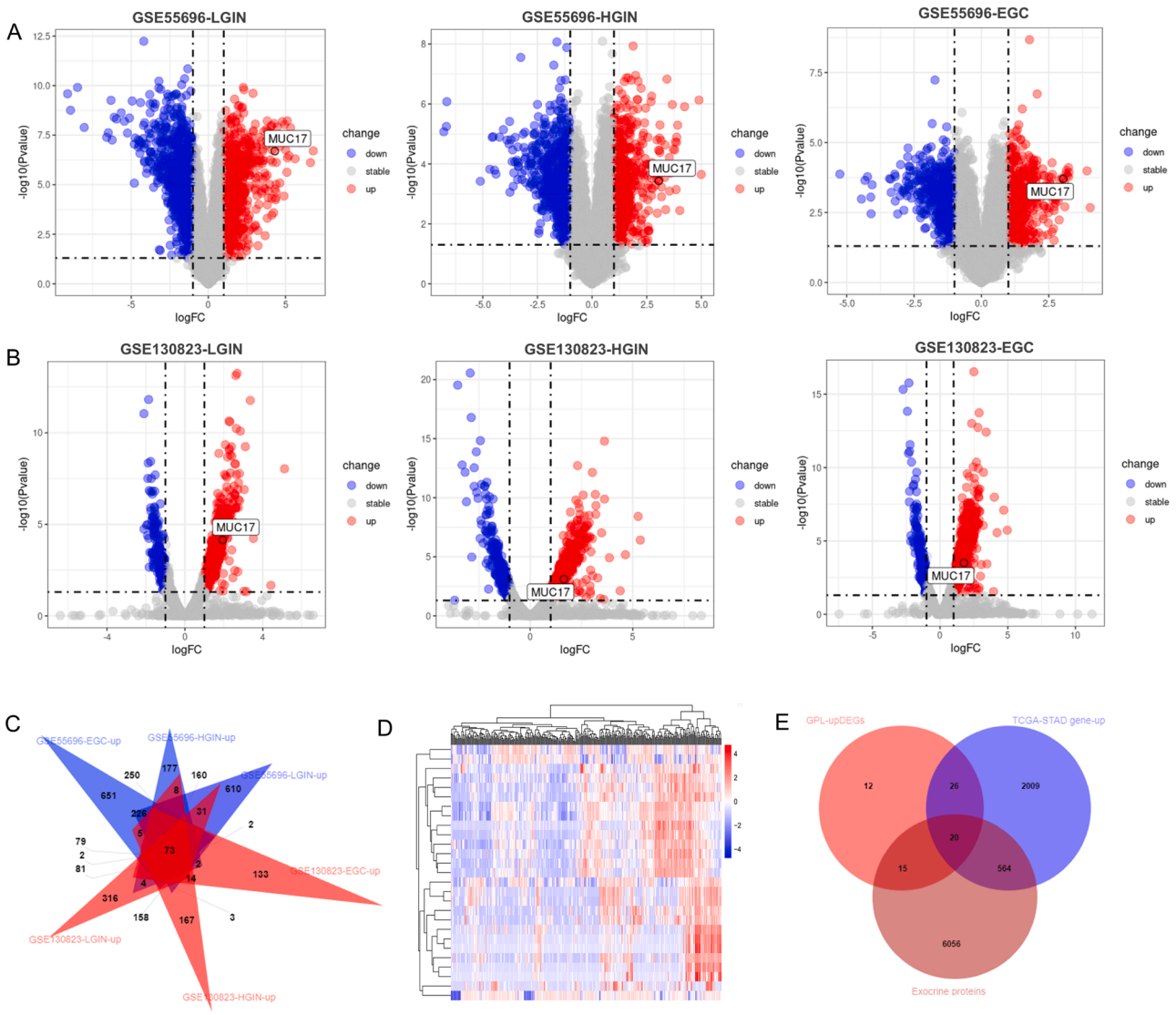

Fig. 2. Schematic representation of the screening process for serological biomarkers from LGIN to AGC. (A) Up-regulated genes were identified in the LGIN, HGIN, and EGC groups within the GSE55696 dataset. (B) Similarly, up-regulated genes were identified in the LGIN, HGIN, and EGC groups within the GSE130823 dataset. (C) By intersecting the results from both datasets, 73 genes were identified that exhibited continuous up-regulation from LGIN to EGC. (D) Up-regulated genes in AGC tissues were identified using the TCGA dataset. (E) 20 hub genes were identified exhibited continuous upregulation in the progression from LGIN to AGC and could be detected in serum.

different pathological stages of GC, we quantified MUC17 levels in human stomach tissues using IHC. Our study included 20 samples each from CG, LGIN, HGIN, EGC, and AGC. Representative images of the IHC are presented in Fig. 6A, illustrating the localization of MUC17 within both the epithelial cells and the extracellular matrix of gastric tissues across various pathological stages. The IHC results further indicated a significant upregulation of MUC17 expression in LGIN, HGIN, EGC, and AGC compared to CG group ($P < 0.05$, Fig. 6B). Additionally, correlation analyses were performed to investigate the relationship between MUC17 expression and disease progression in GC. The results indicate a positive correlation between MUC17 expression levels and the progression of GC patients ($r = 0.8696$, $p < 0.0001$, Fig. 6C).

Validation of MUC17 expression using ELISA in serum samples

To evaluate the potential utility of MUC17 as a noninvasive biomarker for gastric lesion in the early stage, we performed a quantitative ELISA to measure serum MUC17 levels across various cohorts. These cohorts comprised 40 healthy individuals as the normal control group, 40 patients with CG, 40 patients with LGIN, 40 patients with HGIN, 40 patients with EGC, and 100 patients with AGC. The mean plasma concentration of MUC17 was 1.266 ± 0.3703 ng/ml in healthy individuals, 1.163 ± 0.3088 ng/ml in CG patients 1.669 ± 0.3182 ng/ml in LGIN patients, 1.691 ± 0.4257 ng/ml in HGIN patients, 1.841 ± 0.3248 ng/ml in EGC patients and 2.076 ± 0.4668 ng/ml in AGC patients (Fig. 7A). Fig. 7B demonstrates that serum MUC17 levels are positively correlated with the progression of gastric lesions from CG to AGC ($r = 0.5537$, $p < 0.0001$). The correlation between MUC17 expression in tissue and serum was assessed by analyzing IHC data alongside ELISA results obtained from the same patient cohort. As illustrated in Fig. 7C, there is a significant positive correlation between serum MUC17 levels and MUC17 expression in gastric lesion tissues ($r = 0.5380$, $p < 0.001$). The predominant source of elevated MUC17 expression in serum is likely the secretion from gastric lesion tissue, indicating its potential utility as a serum biomarker.

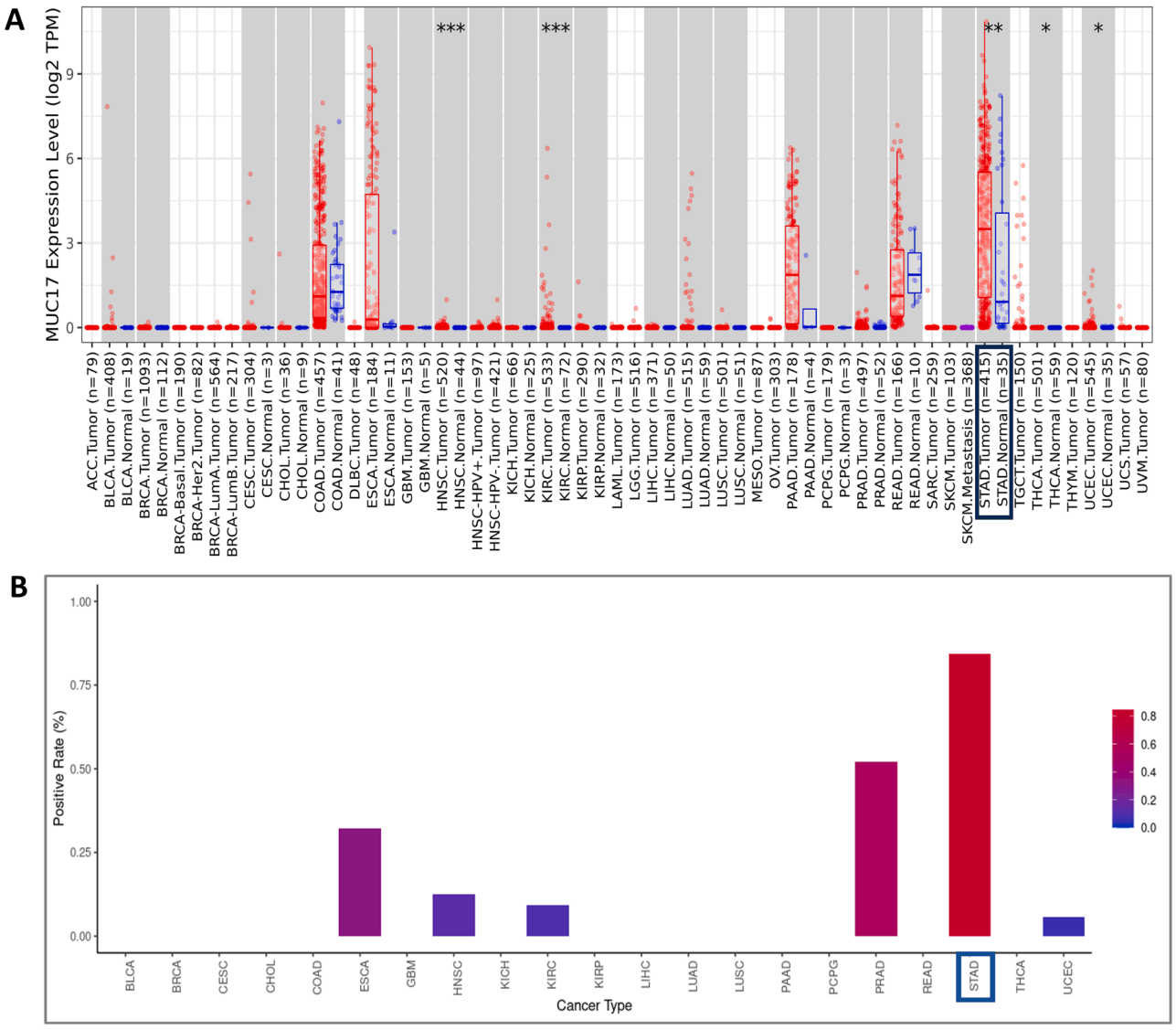

Fig. 3. Pan-cancer analysis of MUC17. (A) mRNA expression levels of MUC17 were analyzed in different cancer types from TCGA data in TIMER2.0. *ns* $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (B) The positive rate of MUC17 expression in various tumors.

Validation of the diagnostic efficacy of serum biomarker MUC17

ROC curve analysis was performed to evaluate the diagnostic efficacy of MUC17 based on its mRNA expression levels. The analysis revealed that MUC17 achieved an AUC of 0.7562 for distinguishing LGIN tissue from CG tissue, an AUC of 0.7579 for differentiating HGIN tissue from CG tissue, and an AUC of 0.7258 for distinguishing EGC tissue from CG tissue in the GSE55696 dataset (Fig. 8A). Furthermore, MUC17 demonstrated an AUC of 0.8800 for discriminating GC tumor tissue from normal tissue in the GSE224056 dataset (Fig. 8B). Similar results are obtained in the GSE130823 and GSE66229 dataset, the AUC values of the ROC curves corresponding to LGIN, HGIN, EGC and GC were 0.8711, 0.7705, 0.7487 and 0.7154 respectively (Fig. 8C and D).

To evaluate the sensitivity and specificity of MUC17 in non-invasive diagnostics, we constructed ROC curves to elucidate the diagnostic accuracy of serum MUC17 concentrations in identifying LGIN, HGIN, EGC, and AGC. The area under the curve (AUC) values were 0.8788 for discriminating LGIN from CG, 0.8544 for discriminating HGIN from CG, 0.9513 for discriminating EGC from CG and 0.9780 for discriminating AGC from CG, respectively (Fig. 8E). Details concerning the diagnostic values of the remaining groups are delineated in Table 2. The findings indicate that serum levels of MUC17 are elevated during the early stages of GC progression, underscoring its significant diagnostic value for patients with GIN. The capacity to accurately diagnose GIN underscores the potential of MUC17 as a serological marker for preliminary screening, preceding comprehensive endoscopic examinations. Early identification of patients with intraepithelial neoplasia is feasible. Annual endoscopy is recommended for these patients with GIN.

Identification of MUC17 as a biomarker for the differential diagnosis of other gastric diseases

Based on the aforementioned findings, MUC17 demonstrates reduced expression in the tissues of CG patients compared to those with GIN, indicating its potential utility in the differential diagnosis between CG and GIN. Clinically, GIN often manifests as a mass during endoscopic evaluation; nevertheless, community hospitals frequently encounter difficulties in attaining precise diagnoses. This raises the question of whether serum MUC17 could be employed in the differential diagnosis of other stomach disorders such as GU, GNET and GIST.

To validate our aforementioned hypothesis, this study identified that the expression of MUC17 in GIST tissues within the GSE26942 dataset

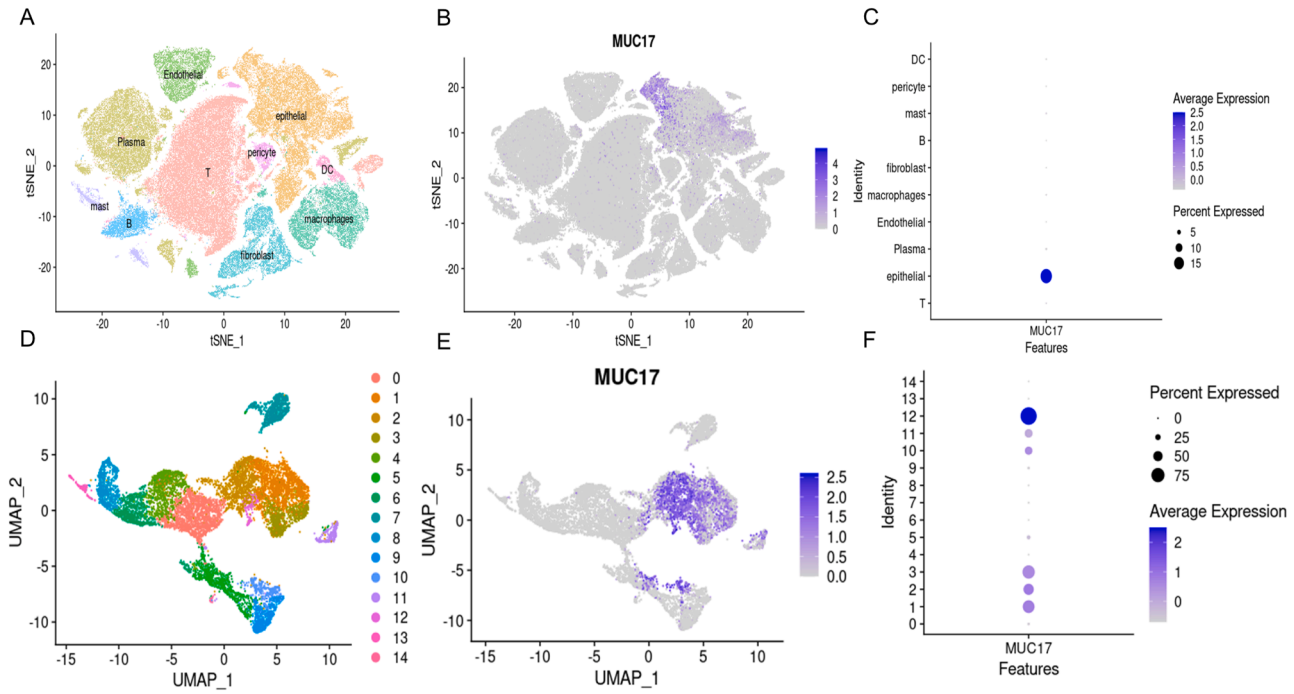

Fig. 4. The expression and localization of MUC17 were verified by single-cell RNA sequencing data of GC. (A) The t-SNE plot of each single cell under analysis. One cluster is represented by each color. The graphic has annotations for each sort of cell. (B, C) The t-SNE plot and dot plot are color-coded on a gradient from gray to blue to indicate the expression levels of MUC17 across various cell types within GC tissues. (D) The UMAP plot illustrating the distribution of epithelial cells within GC tissue is presented. Each color represents one cluster. (E, F) The UMAP plot and dot plot are color-coded (from gray to blue) to represent the expression levels of MUC17 in epithelial cells.

was significantly lower than in GC tissues and comparable to that in normal tissues (Fig. 9A). Additionally, MUC17 expression was undetectable in mouse ulcer tissues within the GSE73336 dataset, indirectly suggesting that MUC17 also exhibits low expression in GU tissues relative to normal tissues. These findings from publicly available datasets imply that MUC17 may serve as a potential differential diagnostic marker for GC.

To evaluate the diagnostic utility of MUC17 in differentiating it from other gastric pathologies, serum samples were obtained from 40 patients with GU, 40 patients with GNET, and 40 patients with GIST. The ELISA results demonstrated significant variations in MUC17 serum levels among these patient groups compared to the GIN group ($p < 0.001$) (Fig. 9B). For the different groups, the mean plasma MUC17 concentrations were 1.312 ± 0.3108 ng/ml for GU patients, 0.9991 ± 0.2985 ng/ml for GNET patients and 0.8023 ± 0.4414 for GIST patients. Detailed results of the multiple comparisons are shown in Table S3. ROC curve analysis was performed to further illustrate the diagnostic value of plasma MUC17 for GIN patients. MUC17 demonstrated AUC value of 0.7803, 0.9244, and 0.9331 for distinguishing GIN patients from those with GU, GNET, and GIST, respectively (Fig. 9C). Ultimately, the results showed that plasma MUC17 was able to discriminate effectively between patients with GIN and three other gastric diseases, thus indicating its potential as a diagnostic biomarker.

MUC17 regulates the progression of LGIN to EGC

To further investigate the molecular mechanisms by which MUC17 promotes the progression of precancerous lesions and gastric cancer, we stratified LGIN patients in the GSE55696 dataset into high-expression and low-expression groups based on the median expression level of MUC17. Differential gene expression analysis was conducted between groups exhibiting high and low MUC17 expression (Fig. 10A; Table S4) to elucidate the molecules involved by which MUC17 may exert its effects. Subsequently, the up-regulated genes in the differentially expressed genes underwent KEGG enrichment analysis (Fig. 10B) to identify associated molecular pathways. The identified pathways were then compared with those enriched in all upregulated pathways within the LGIN patient group (Fig. 10C) to identify potential pathways in which MUC17 may be involved. The items highlighted in the red box in the figure represent the intersections between MUC17-involved molecular pathways and the overall upregulated pathways. This analytical approach was similarly applied to patients with HGIN (Fig. 10D–F) and EGC (Fig. 10G–I) within the GSE55696 dataset. Additionally, the same methodology was employed for patients with LGIN (Fig.S1 A–C; Table S5), HGIN (Fig. S1 D–F), and EGC (Fig. S1 G–I) within the GSE130823 dataset. As illustrated in the figure, the upregulated differential genes in the MUC17 high expression group in different pathological stages of gastric cancer progression were enriched in various metabolic pathways, such as vitamin digestion and absorption, thiamine metabolism, steroid hormone biosynthesis, retinol metabolism, nucleotide metabolism, nitrogen metabolism, mineral absorption, glycolysis/gluconeogenesis, fat digestion and absorption, and amino acid metabolism, among others. These findings imply that MUC17 may play a role in modifying tumor metabolic patterns during the early stages of cancer progression. Additionally, MUC17 is potentially involved in multiple carcinogenic signaling pathways, including the PI3K-AKT, JAK-STAT, HIF-1, TNF, and PPAR pathways, among others.

Discussion

Gastric cancer ranks among the most prevalent malignancies in humans; however, the prognosis for affected patients remains dismal, with a mere 20 % five-year survival rate for individuals diagnosed at advanced stages of the disease [24]. Due to the poor prognosis associated with GC, which is often diagnosed at advanced stages or with metastases, the overall survival rate for individuals afflicted with this disease remains significantly low [25]. The prompt identification and accurate diagnosis of GC are essential for optimizing therapeutic

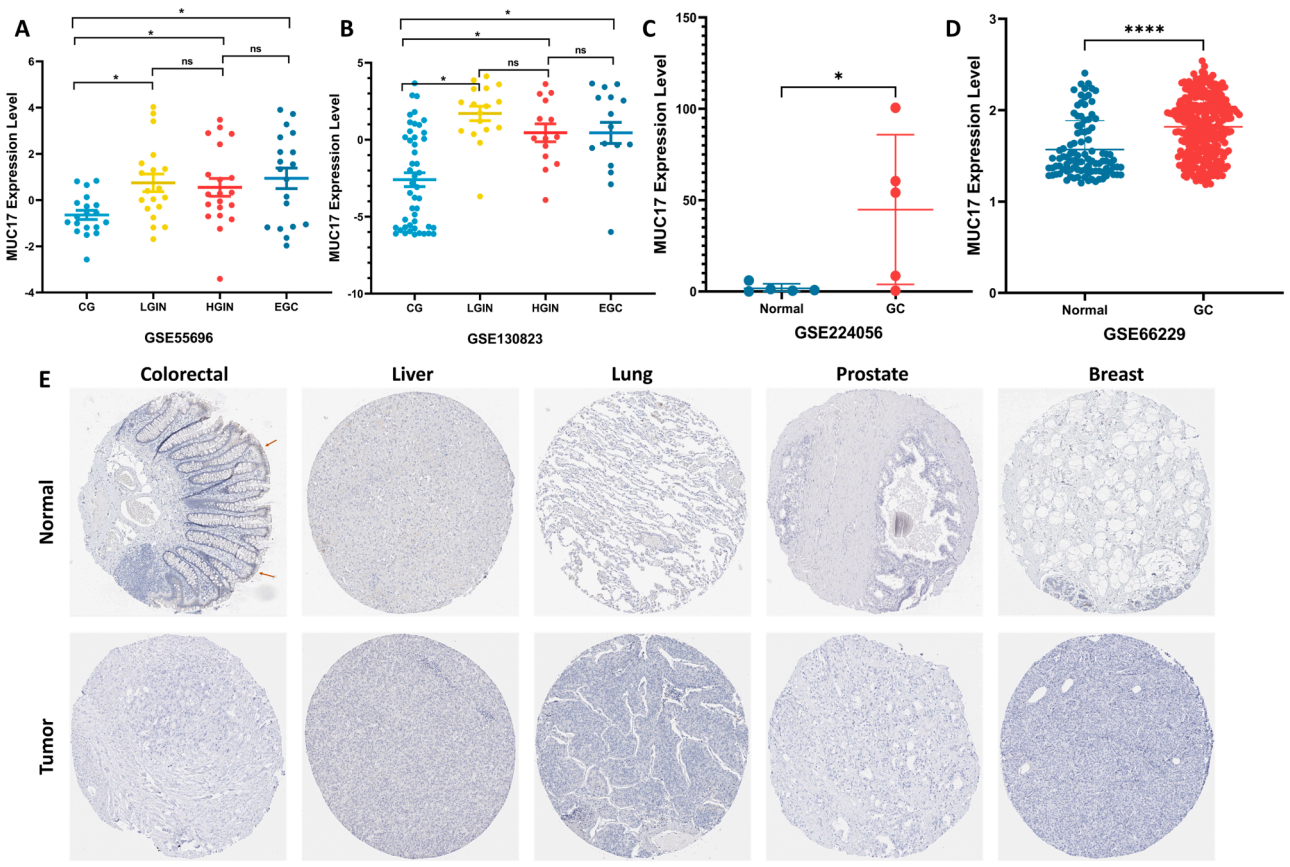

Fig. 5. Validation of MUC17 expression and localization. (A, B, C, D) Gene expression levels of MUC17 in different pathological stages of GC from GSE55696, GSE130823, GSE224056 and GSE66229. Data are shown as the mean±SEM. *ns* $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (E) Protein expression of MUC17 in COAD, READ, LIHC, LUAD, PRAD and BRCA tissues and normal tissues from the HPA database.

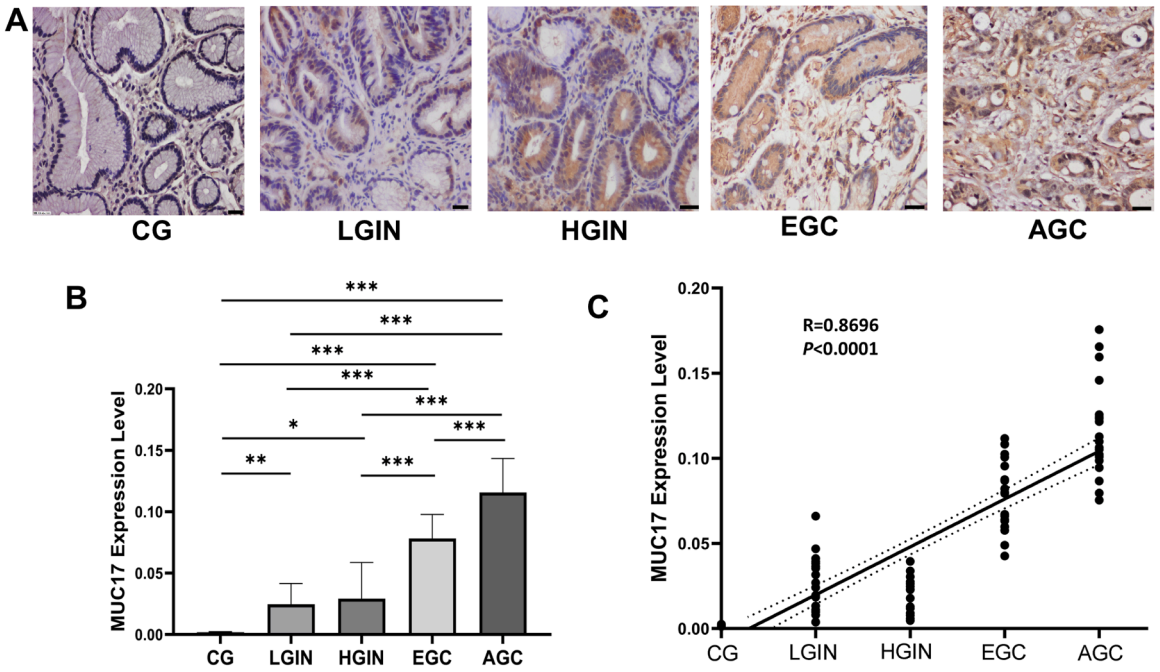

Fig. 6. Validation of MUC17 expression and localization. (A) Representative images of IHC for MUC17 in the different pathological stages of GC. (B) The quantitative statistical results of MUC17 expression by IHC. Data are shown as the mean ± SEM. *ns* $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (C) The correlation between the different pathological stages and MUC17 expression.

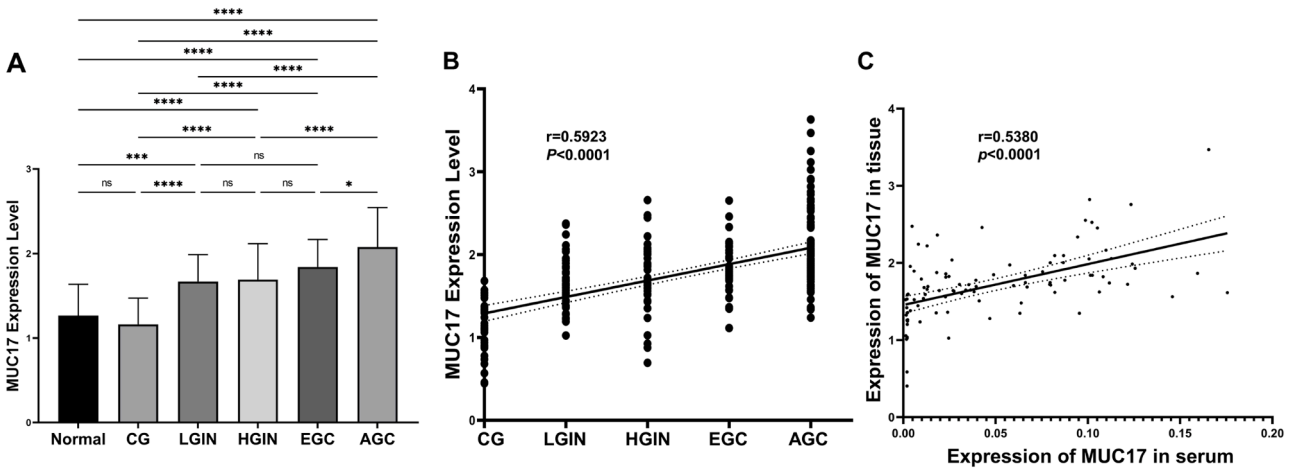

Fig. 7. Expression level of MUC17 in serum. (A) Serum MUC17 expression levels from patients with Normal, CG, LGIN, HGIN, EGC, and AGC. Data are shown as the mean ± SEM. *ns* $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (B) The relationship between the various clinical phases and MUC17 expression. (C) The correlation of MUC17 expression in tissue and serum from IHC results and Elisa results in the same patient.

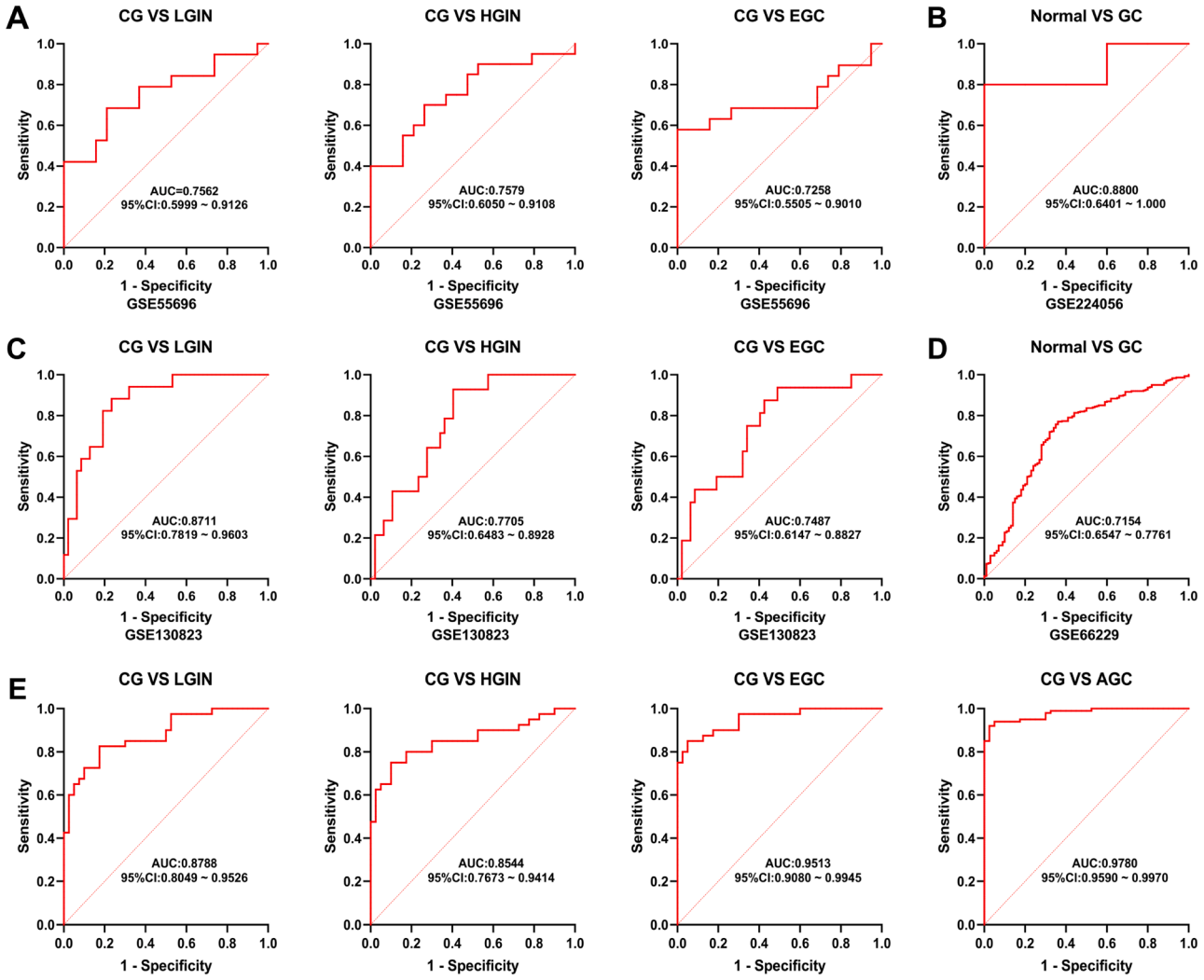

Fig. 8. Assessment of diagnostic efficacy of MUC17. (A, B, C, D) The diagnostic efficacy of MUC17 base on mRNA expression level across various groups in different datasets. (E) The diagnostic efficacy of serum MUC17 concentration across different clinical stages of GC.

Table 2
The diagnostic value of MUC17 for LGIN, HGIN, EGC and AGC.

	Cut-off	Sensitivity (%)	Specificity (%)	Youden index (%)	AUC (95 %CI)	*p*
LGIN	1.380	82.5	82.5	65	0.8788 (0.8049–0.9526)	<0.0001
HGIN	1.511	75	90	65	0.8544 (0.7673–0.9414)	<0.0001
EGC	1.552	85	95	80	0.9513 (0.9080–0.9945)	<0.0001
AGC	1.582	92	97.5	89.5	0.9780 (0.9590–0.9970)	<0.0001

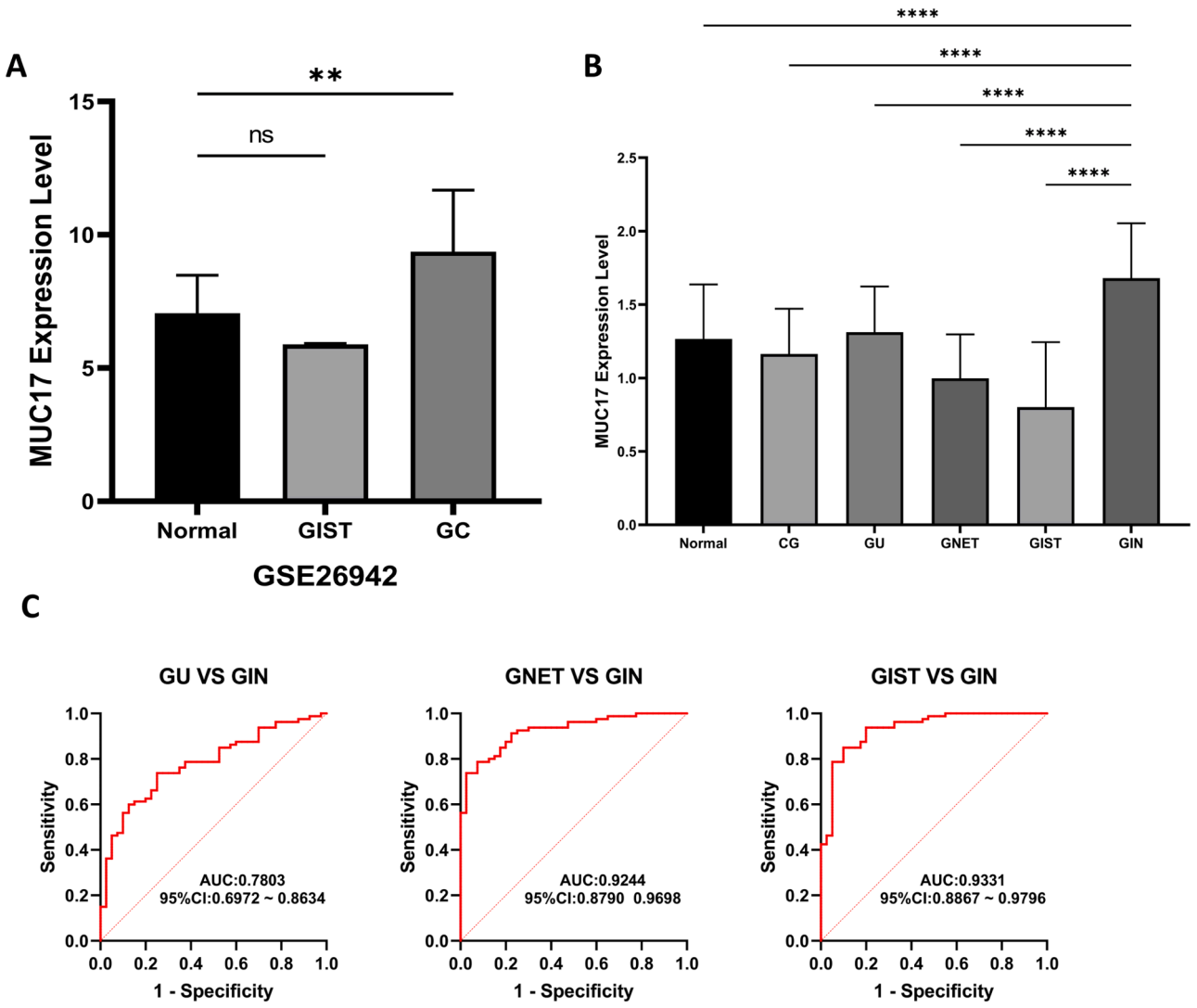

Fig. 9. To assess the differential diagnostic efficacy of MUC17 in GIN and other gastric diseases. (A) MUC17 expression levels in the normal, GIST, and GC groups in the GSE26942 dataset. (B) Serum MUC17 expression levels from normal control group and patients with CG, GU, GIST, GNET and GIN. Data are shown as the mean ± SEM. *ns* $p \geq 0.05$, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. (C) Evaluating the differential diagnostic efficacy of MUC17 expression across different gastric diseases.

outcomes and enhancing patient survival rates [26]. Currently, upper gastrointestinal endoscopy is extensively employed in the screening of gastric cancer. The advent of sophisticated techniques, including magnifying endoscopy, autofluorescence imaging, narrow-band imaging, and laser confocal endoscopy, has significantly enhanced the detection rates of precancerous lesions [27–29]. However, these methodologies continue to be relatively expensive, both in terms of financial resources and the requirement for highly skilled medical personnel. Furthermore, accurate diagnosis remains heavily dependent on the expertise of experienced physicians, with a persistent risk of missed diagnoses [30]. Despite the benefits of endoscopy, it remains an invasive procedure that carries risks associated with intubation and sedation, potential infections, and the possibility of false negative or positive results, as well as overdiagnosis. These factors deter certain individuals from undergoing gastroscopy, potentially resulting in the exclusion of a segment of the population with GIN or EGC. Consequently, there is a critical need for noninvasive and cost-effective serum biomarkers for the primary screening of GIN and EGC prior to gastroscopy. Next-generation sequencing (NGS) and single-cell RNA sequencing offer novel, comprehensive methods for examining the genome and transcriptome [31]. Recently, Multiple omics-based studies have been employed to analyze clinical samples from cancer patients in the pursuit of diagnostic biomarkers and druggable targets [32–34]. Serum is integral to clinical diagnostics due to its accessibility, minimally invasive nature, and broad

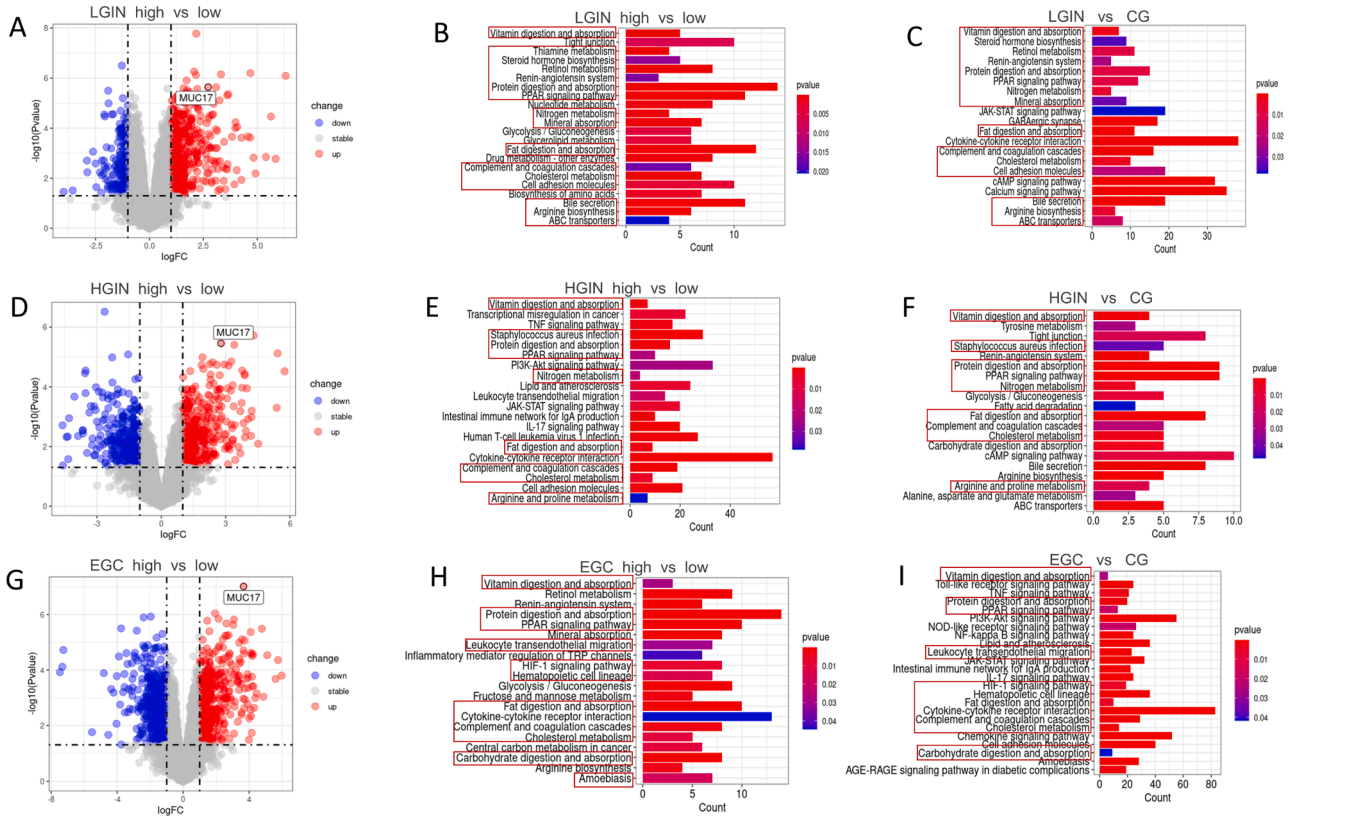

Fig. 10. The molecular mechanisms of MUC17 in the development of gastric cancer, as analyzed within the GSE55696 dataset. (A, D, G) In the GSE55696 dataset, up-regulated genes were found in patients with high expression of MUC17 compared to patients with low expression in the LGIN, HGIN, and EGC groups, respectively. (B, E, H) KEGG pathway enrichment analysis of up-regulated genes in patients with high expression of MUC17 in the LGIN, HGIN, and EGC groups, respectively. (C, F, I) KEGG pathway enrichment was analyzed in LGIN, HGIN and EGC groups compared with CG group.

patient acceptance. Emerging biomarkers such as microRNA, long non-coding RNA (lncRNA), microRNA (miRNA), circular RNA (circRNA), serum proteins, and cell-derived vesicles exemplify blood-based liquid biopsies that hold promise for future diagnostic advancements [35–37]. Studies have demonstrated that promoter methylation of specific tumor-associated genes can function as a non-invasive and effective biomarker for cancer detection in body fluid samples [38]. However, the diagnostic utility of these biomarkers for GIN has not yet been quantitatively assessed. There is a pressing need for additional research into serological biomarkers that can be utilized before endoscopic surveillance programs to identify high-risk groups necessitating gastroscopy [39].

Numerous prior studies have focused on the disparities in biomarkers between patients with GC and healthy individuals [40,41]. However, there is a paucity of studies specifically examining tumor biomarkers at the intraepithelial neoplasia stage. We analyzed mRNA transcriptome sequencing data from GIN and GC in public databases and a curated list of exocrine protein genes to find early-stage GIN serologic biomarkers. We identified 20 key genes with increased expression in both GIN and GC. Previous studies indicate that most GC diagnostic markers lack organ specificity. Therefore, we used TCGA pan-cancer data to identify GC-specific serological diagnostic markers. Ultimately, our findings indicate that MUC17 demonstrates organ-specific expression for GC in comparison to other cancer types. This serum biomarker holds potential for facilitating preliminary screening processes prior to gastroscopy in large-scale population programs, thereby reducing healthcare costs and aiding in the identification of individuals at risk for gastric precancerous lesions.

The MUC17 gene, one of 21 mucin genes (MUC1-MUC21) found in the q22.1 region of chromosome 7 between MUC12 and SERPINE1, belongs to the mucin family of high molecular weight glycosylated proteins [42]. The 14.2 kb mRNA that is produced from the full-length MUC17 coding sequence has 13 exons. Two variations that encode membrane anchoring and secretory forms are produced via alternative splicing [43]. As a result, the presence of an exocrine variant of MUC17 was identified as an extracellular biomarker of GIN, suggesting its putative use as a noninvasive testing method. Then, through single-cell transcriptome data analysis, we observed that MUC17 was predominantly expressed in epithelial cells in GC tissues. Notably, there was a substantial degree of heterogeneity among epithelial cells in GC tissues. Analysis of mRNA bulk-seq data from public databases also revealed that MUC17 was significantly overexpressed in GIN and GC. We verified the expression of MUC17 in some cancers and corresponding normal tissues in the HPA database and found that there was only a small amount of MUC17 expression in normal colorectal cancer, which was consistent with the TCGA results. In addition, we validated the expression levels of MUC17 in normal gastric tissues, LGIN, HGIN, EGC and AGC tissues through immunohistochemistry. Our results showed a notable rise in MUC17 expression from LGIN to AGC patients, supporting its use as a gastroscopy screening marker for GIN and GC. Subsequently, serum samples were collected from healthy individuals and patients diagnosed with CG, LGIN, HGIN, EGC, and AGC to validate the serum concentration of MUC17. The results obtained were consistent with the previously mentioned bioinformatics data. The levels of MUC17 are elevated in patients with LGIN and exhibit a progressive increase as the condition advances to AGC. Early elevation of this biomarker in initial GIN stages allows for noninvasive serum screening to identify individuals with precancerous lesions before gastroscopy, enabling thorough gastroscopy and annual monitoring. To assess MUC17′s diagnostic efficacy, ROC curves and AUC were analyzed, revealing MUC17′s strong potential for identifying GIN, EGC, and AGC. Unfortunately, although the expression level of MUC17 increased with the progression of the GC process and

showed a positive correlation, there was no significant difference in serum MUC17 levels between LGIN and HGIN in this study. However, this does not impede the ability of MUC17 as a primary serological screening marker before undergoing gastroscopy. This approach allows gastroscopists to conduct a comprehensive examination of the key population. At the end of the study, we assessed serum MUC17 levels in CG, GU, GNET, and GIST patients to ascertain whether MUC17 could effectively differentiate between EGC and other stomach disorders. The results showed notable differences in MUC17 expression levels in the serum of CG, GU, GNET, and GIST patients compared to EGC patients. The ROC curve demonstrated high sensitivity and specificity. This suggests that MUC17 can be utilized as a serological marker in the differential diagnosis of EGC, underscoring its robust disease specificity.

To elucidate the molecular mechanisms by which MUC17 regulates precancerous lesions and gastric cancer, we analyzed differences between high and low MUC17 expression in LGIN, HGIN, and EGC patient groups using datasets GSE55696 and GSE130823. We longitudinally compared the continuously up-regulated pathways during the progressive pathological stages from LGIN to EGC between the high and low MUC17 expression groups. Additionally, we conducted a horizontal comparison of the intersection between these up-regulated pathways in the high MUC17 expression group and the overall up-regulated pathways at each pathological stage. These pathways may be regulated by MUC17. Figs. 10B, E, H and S1 B, E, H demonstrate a continuous up-regulation of several pathways within the high MUC17 expression group during the pathological progression from LGIN to EGC, including vitamin digestion and absorption, protein digestion and absorption, PPAR signaling pathway, nitrogen metabolism, and fat digestion and absorption. Recent research has demonstrated that gastric cancer facilitates its rapid proliferation and survival through metabolic reprogramming [44,45]. This process potentially enhances the suitability of malignant cells for growth by altering glycolysis, fatty acid metabolism, amino acid metabolism, and other metabolic pathways during the initial stages of oncogenic transformation [46–48]. We therefore hypothesize that MUC17 may influence disease progression by modulating cellular metabolic pathways. Nonetheless, further rigorous experimental validation is required to substantiate this hypothesis. Notably, MUC17 consistently up-regulates the PPAR signaling pathway. This aligns with the findings of Yang et al. [49], who reported a significant reduction in PPAR γ (peroxisome proliferator-activated receptor γ) expression in MUC17 knockdown cells. Furthermore, Zou et al. [50] showed that PPAR δ and interferon gamma promote the transformation and tumorigenesis of gastric progenitor cells in mice, while Liu et al. [51] demonstrated that PPAR δ dysregulation of the Ccl20/Ccr6 axis facilitates gastric adenocarcinoma carcinogenesis by remodeling the tumor microenvironment. PPAR plays a critical role in regulating multiple genes associated with glucose and lipid metabolism, lipogenesis, and inflammation during tumor progression [52]. This is consistent with the observed upregulation of several metabolic pathways, including disorders in glucose and lipid metabolism, in the high MUC17 expression group. The findings presented in this study indicate that MUC17 may play a role in the pathogenesis of gastric cancer by modulating the mechanisms of metabolic reprogramming and certain cancer-related signaling pathways. However, the precise mechanisms by which MUC17 regulates these signaling pathways and tumor metabolism remain unclear. Consequently, further rigorous experimental investigations are required to substantiate its efficacy.

In this study, we undertook a comprehensive analysis of MUC17 expression across various stages of gastric lesions. We evaluated its potential as a serum diagnostic marker using both our own cohort and publicly available datasets. Furthermore, we characterized the expression levels of MUC17 in other gastric diseases to assess its utility as a differential diagnostic indicator. Although our findings provide significant insights, it is essential to recognize the study's limitations. To further substantiate the efficacy of this diagnostic marker, it is imperative to increase the serum sample size and undertake multi-center studies. In this study, we concentrated on the precancerous stage of GC and identified MUC17 as a potential biomarker for the non-invasive diagnosis of patients with GIN and EGC, thereby facilitating the early detection of GC. Despite advancements in gastroscopy technology, the widespread implementation of gastroscopy is hindered by its invasiveness and the substantial medical burden it imposes. Consequently, some individuals are reluctant to undergo the procedure. MUC17 offers a viable alternative for preliminary screening, enabling the identification of patients prior to gastroscopy. If a patient shows elevated MUC17 in noninvasive screening, we recommend comprehensive and regular gastroscopy. Although MUC17 is less effective for distinguishing between LGIN and HGIN, it does not affect its effectiveness in screening patients with GIN and EGC. The process of identifying and validating new biomarkers for clinical application is both lengthy and complex. Additional clinical validation is necessary to evaluate the reliability of our current findings and to ascertain the potential clinical utility of MUC17.

Conclusions

In summary, serum MUC17 demonstrates robust predictive efficacy for both GIN and EGC. It holds potential as an effective preliminary serum screening biomarker prior to gastroscopy, facilitating the identification of high-risk populations for GC. This contributes valuable diagnostic information for clinical practitioners.

Ethical approval statement

The study was censored and approved by the Ethics Committee of the Second Hospital of Hebei Medical University (Approval No 2023-R495) and all the participants signed an informed consent form. All procedures performed in this study involving human participants were guided by the Helsinki Declaration.

Availability of data and materials

The dataset does not require specific permission for access and is publicly available for use. The data that support the findings of the study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Bingxue Yang: Writing – original draft, Software, Methodology, Funding acquisition, Conceptualization. **Xiaoli Xie:** Writing – review & editing, Methodology, Funding acquisition, Data curation. **Xiaoxu Jin:** Writing – review & editing, Methodology, Funding acquisition, Data curation. **Xiuhong Huang:** Software, Methodology. **Yujian He:** Software, Methodology. **Kaige Yin:** Supervision. **Chenguang Ji:** Supervision. **Li Liu:** Supervision. **Zhijie Feng:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2024.102207.

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