Label-free quantitative proteomics of gastric high-grade intraepithelial neoplasia

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Abstract. Early detection and diagnosis are key to improving the survival rate and reducing the fatality rate linked to gastric cancer. The precancerous lesion of gastric cancer is referred to as gastric high-grade intraepithelial neoplasia (HGIN). Both the sensitivity and specificity of current biomarkers that aid in the diagnosis of gastric HGIN are still relatively low. Furthermore, proteomic data on gastric HGIN are still scarce. The present study aimed to explore candidate protein biomarkers for gastric HGIN screening with proteomics and bioinformatics technology. A total of 10 serum samples were collected and categorized into two groups, i.e., the gastric HGIN and the healthy control groups. Label-free quantification in conjunction with liquid chromatography with tandem mass spectrometry was employed to identify the probable biomarkers for gastric HGIN. Furthermore, differentially expressed proteins (DEPs) were quantified by proteomics analysis. In total, 1,192 distinct serum proteins were discovered between the gastric HGIN group and the healthy control group. DEPs were identified in the further analyses, utilizing a threshold of a 1.5-fold difference in expression level ($P<0.05$) in comparison with the control group. There were 18 upregulated and 12 downregulated proteins in the gastric HGIN group in comparison with the control group. Bioinformatics analyses were performed using Gene Ontology and KEGG pathway enrichment analyses. The GO analysis revealed that the DEPs were enriched in biological processes such as 'cellular', 'biological regulation', 'multicellular organismal', 'developmental' and 'reaction to stimulus processes', localized to 'cell', 'intracellular' and 'protein-containing complex', and involved in molecular functions such as 'molecular function modulator', 'binding' and 'catalytic activity'. The KEGG pathway enrichment analysis manifested that the DEPs were predominantly enriched in 'antigen processing and presentation', 'diabetic cardiomyopathy', 'Epstein-Barr virus infection', 'herpes simplex virus 1 infection', 'human immunodeficiency virus 1 infection' and 'human cytomegalovirus infection'. In conclusion, the present data provide more biological information for the formation of gastric HGIN and clues for further research on the pathogenesis of early gastric cancer.

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Introduction

Gastric cancer is the predominant gastrointestinal malignancy at present. In 2020, there were >1 million new cases of gastric cancer globally and ~769,000 deaths. As a result, it ranks 5th in incidence and 4th in mortalities related to malignant tumors (1). Due to the invasiveness of endoscopic screening and the lack of ideal biomarkers, the early diagnosis rate of gastric cancer is <10% and the proportion of patients at the intermediate and late stage is higher (2). The 5-year survival rate is ~90% for early gastric cancer, while that of intermediate and advanced gastric cancer is only ~30% and the overall therapeutic effect is not ideal (3). Therefore, early detection and diagnosis are key to improving the survival rate and reducing the fatality rate of gastric cancer. However, early gastric cancer and precancerous lesions are mostly asymptomatic or cause nonspecific symptoms (4,5). Gastric cancer is often mistaken for benign diseases, such as gastritis and dyspepsia, in the early stage of the disease, and it fails to get the attention of patients so they easily miss the best treatment period. Most patients have developed advanced gastric cancer when diagnosed, which requires chemotherapy, radiotherapy, surgery and other treatments, increasing the physical and psychological burden of patients and endangering the safety of patients in serious cases (6,7). Currently, gastric cancer diagnosis primarily relies on gastroscopy and pathological diagnosis (8). However, gastroscopy and pathological biopsy are invasive examinations, which are difficult for patients to accept and have a high cost, and they also have certain requirements regarding the operation ability and experience of doctors, so the opportunity for early diagnosis is usually missed (9).

Gastric high-grade intraepithelial neoplasia (HGIN) is a precancerous lesion. Over 80% of gastric HGIN progresses to adenocarcinoma (10). If the lesions can be detected and treated in time at the gastric HGIN stage, the therapeutic effect on patients is significantly improved. Appropriate biomarkers can improve the rates of early diagnosis of gastric cancer and help achieve its prompt treatment (11). Although studies on

biomarkers for gastric cancer have been gradually carried out at home and abroad, only a small number of biomarkers can be used for auxiliary diagnosis of clinical gastric cancer so far (12,13), such as carcinoembryonic antigen, carbohydrate antigen 19-9 (CA19-9), CA724 and α-fetoprotein, but the detection rate and accuracy are low, which are not sufficient to replace conventional pathological diagnosis. The gastric HGIN-related diagnostic sensitivity of of 77.8% and specificity of 81.8%, are still relatively low (14,15).

Blood is an ideal sample for detecting tumor biomarkers because some tumor-related specific proteins are released into the blood, and blood sampling is simple, rapid, and minimally invasive, so samples are relatively easy to obtain. Abnormal protein expression plays a principal function in the occurrence as well as the development of gastric cancer (16,17). Proteomic studies have been reported on gastric cancer, and certain differentially expressed proteins (DEPs), such as fibrinogen α-chain, apolipoprotein A-II, vitronectin and clusterin isoform 1, have been screened (18,19). Another study from Singapore, comparing plasma proteomics of normal individuals and patients with early and advanced gastric cancer found that the level of component 9 (C9) in patients with gastric cancer was significantly higher than that in normal subjects, and the subsequent blind test study on 119 plasma samples showed that the sensitivity of C9 could be as high as 90% at a specificity of 74% (20). However, due to different etiologies and different stages of the disease, the results of various research groups were different (21). In addition, proteomic data on gastric HGIN are still scarce.

Label-free quantification (LFQ) is one of the most popular high-throughput screening techniques in quantitative proteomics. The approach may detect >1,000 proteins in a serum sample with a single needle, after which it employs tandem mass spectrometric analysis to compare the relative protein content in up to 10 distinct samples concurrently (22). In addition, this technology has the advantages of high throughput, high sensitivity, low-abundance protein detection, accurate quantification, good repeatability, reliable identification results and detection of a wide range of proteins, and is widely used in the analysis and exploration of DEPs (23). In the present study, LFQ together with liquid chromatography with tandem mass spectrometry (LC-MS/MS) was employed to compare the peripheral blood's protein expression of patients with gastric HGIN and healthy controls, and the protein expression profile in serum was analyzed biologically, providing a basis for finding biomarkers for gastric HGIN.

Patients and methods

Patients and plasma samples. The present study was approved by the Ethics Committee of the Affiliated Hospital of Putian University (Putian, China; approval no. 202006) (24). All patients enrolled in this study were from the Affiliated Hospital of Putian University (Putian, China), with the enrollment period from January 2022 to March 2022. Each patient who participated signed a written informed consent form before the study began. All patients were divided into a gastric HGIN group and healthy control group, meeting the following inclusion and exclusion criteria: The gastric HGIN group was pathologically confirmed as HGIN, and endoscopic ultrasonography and computed tomography did not confirm metastasis, and there was no previous history of gastric malignancy. The healthy control group underwent gastroscopy to exclude gastric HGIN. The following conditions were excluded in both groups: Associations with other malignant tumors, serious systemic diseases and serious primary diseases. A total of 10 serum samples were collected and all personnel was from the Affiliated Hospital of Putian University. Male individuals with a higher incidence of gastric cancer were selected as the case group, and age-matched healthy individuals were chosen from the same period as the control group. There were 5 cases in the gastric HGIN group and 5 cases in the healthy control group. Among them, 5 patients in the gastric HGIN group underwent endoscopic submucosal dissection (ESD) at the Affiliated Hospital of Putian University, and were diagnosed with HGIN by postoperative pathology. The protocol was performed according to standard procedures. Centrifugation of blood samples was conducted at 1,300 x g for 10 min at 4˚C and immediately stored at -80˚C.

Protein extraction and trypsin digestion. The centrifugation process was crucial in the isolation of cellular debris contained in the serum sample (12,000 x g at 4˚C for 10 min). After centrifugation, the supernatant was transferred into another centrifuge tube. The Pierce™ Top 14 Abundant Protein Depletion Spin Columns Kit (Thermo Fisher Scientific, Inc.) was utilized to separate the top 14 high-abundance proteins. Afterward, the concentration of protein was ascertained utilizing a bicinchoninic acid kit (cat. no. P0011; Beyotime Institute of Biotechnology) as per the manufacturer's specifications.

The same amount of each sample protein was taken for enzymatic hydrolysis. An appropriate amount of standard protein (cat. no. 26620; Thermo Fisher Scientific, Inc.) was added and the volume was adjusted to be consistent with the lysate. A total of 5 mM dithiothreitol was utilized for the reduction of the protein solution to facilitate its digestion for 30 min at 56˚C. Subsequently, it underwent alkylation with 11 mM iodoacetamide for 15 min at room temperature, which was performed in the dark. The alkylated samples were transferred to ultrafiltration tubes, centrifuged at 12,000 x g for 20 min at room temperature, replaced with 8 M urea for 3 times and then replaced with displacement buffer for 3 times. Finally, trypsin (Promega Corporation) was introduced at 1:50 trypsin-to-protein mass ratios for digestion overnight at 37˚C. The peptides were subjected to centrifugation at 12,000 x g for 10 min at room temperature and then recovered once with ultrapure water, and the two peptide solutions were combined.

LC-MS/MS analysis. The tryptic peptides were dissolved in solvent A (0.1% formic acid, 2% acetonitrile/in water) and directly loaded onto a home-made reversed-phase analytical column (25 cm length, 100 μm inner diameter) (cat. no. ZTC18M096; MilliporeSigma). The liquid phase gradient was set as follows: 0-68 min, 4-20% solvent B (0.1% formic acid in 90% acetonitrile); 68-82 min, 20-32% B; 82-86 min, 32-80% B; 86-90 min, 80% B, all at a constant flow rate of 500 nl/min on an EASY-nLC 1200 ultra-performance LC system (Thermo Fisher Scientific, Inc.).

The separated peptides were analyzed in Exploris 480™ (Thermo Fisher Scientific, Inc.) with a nano-electrospray ion source. The electrospray voltage applied was 2.3 kV and the compensation voltages were -45 and -70 V. The full MS scan resolution was set to 60,000 for a scan range of 400-1,200 m/z. Up to 25 of the most abundant precursors were then selected for further MS/MS analyses with 30 sec dynamic exclusion. The higher-energy collisional dissociation fragmentation was performed at a normalized collision energy of 27%. The fragments were detected in the Orbitrap at a resolution of 30,000. The fixed first mass was set at 110 m/z. The automatic gain control target was set at 75%, with an intensity threshold of 1E4 ions/sec and the MS2 maximum injection time was set at 100 msec. The MS proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with database identifier PXD036991 (https://www.ebi.ac.uk/pride/profile/reviewer_pxd036991).

Database search. The resulting MS/MS data were processed using Proteome Discoverer search engine (v2.4.0.305) (https://www.thermofisher.cn/cn/zh/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-software/multi-omics-data-analysis/proteome-discoverer-software.html?erpType=Global_E1). Tandem mass spectra were searched against the *Homo sapiens* database (78,120 entries) concatenated with a reverse decoy database. Trypsin (Full) was specified as the cleavage enzyme, allowing up to 2 missing cleavages. The mass tolerance for precursor ions was set at 10 ppm in the first search and the mass tolerance for fragment ions was set at 0.02 Da. Carbamidomethyl on Cys was specified as a fixed modification, and oxidation (methionine; M), acetylation on protein N-term, Met-loss (M) and Met-loss+acetyl (M) were specified as variable modifications. The quantitative method was set as LFQ. The false discovery rate was adjusted to <1% and the minimum score for modified peptides was set to >40. The minimum peptide length was set at 6. All of the other parameters in Proteome Discoverer were set to default values.

Bioinformatics analysis. Gene Ontology (GO) annotations were acquired from the eggNOG database (v.5.0) (http://eggnog5.embl.de/#/app/home). The eggNOG-mapper software (v.2.0) (http://eggnog-mapper.embl.de/) analyzed these GO categories: Cellular component, molecular function and biological process. The Kyoto Encyclopedia of Genes and Genomes (KEGG) online service tool KAAS (v.2.1) (https://www.genome.jp/tools/kaas/) annotated the protein descriptions and then mapped using the KEGG mapper (v.5.0) (https://www.kegg.jp/kegg/mapper/) to their respective pathways. WoLFPSort (v.0.2) (https://www.genscript.com/wolf-psort.html) was used to investigate the subcellular localization. P<0.05 was deemed statistically significant for both GO terms and KEGG pathways. Gene count thresholds for the GO terms and KEGG pathways were established at ≥20 and ≥4, respectively.

Statistical analysis. Clinical parameter data were expressed as the mean ± standard deviation. Continuous variables were compared using Student's t-test and the Mann-Whitney U-test depending on whether the data were normally distributed. The protein data along with the intensity values were log-transformed with base 2 and median normalization was carried out to eliminate random errors and sample bias. DEPs were identified by a two-tailed Student's t-test. The pathway enrichment analysis was performed by a two-tailed Fisher's exact test. P<0.05 (two-tailed) was considered to indicate a statistically significant difference. Data were analyzed and visualized with SPSS 21.0 (IBM, Corp.) or GraphPad Prism 7.0 (GraphPad Software, Inc.).

Table I. Demographics and clinicopathological characteristics of patients and healthy controls.

Characteristics	Patients	Healthy controls	P-value
Sex, n (%)			1.000
Male	5 (100)	5 (100)	
Female	0 (0)	0 (0)	
Mean age ± SD, years	62.6±3.8	59.8±0.8	0.168
Pathology, n (%)			0.004
Gastric HGIN	5 (100)	0	
Normal	0	5 (100)	

HGIN, high-grade intraepithelial neoplasia.

Results

Characteristics of patients with gastric HGIN and healthy controls. A total of 10 serum samples were collected in this study. In the gastric HGIN group, 5 cases were male, with an average age of 62.6±3.8 years. All cases were diagnosed with HGIN after ESD. The healthy control group consisted of 5 cases, all male, with an average age of 59.8±0.8 years. There was no rmarked difference in terms of age between the patients with gastric HGIN and the healthy controls (P>0.05, two-tailed Student's t-test) (Table I).

Identification of DEPs. LFQ in conjunction with LC-MS/MS enabled the discovery of 1,192 serum proteins. Upregulated and downregulated DEPs were defined as proteins with expression ratio thresholds of >1.5-fold increase or <0.67-fold decrease (P<0.05, two-tailed Student's t-test), respectively. In comparison with the control group, 18 proteins were upregulated and 12 proteins were downregulated in the gastric HGIN group (Table II; Fig. 1).

GO analysis. GO analysis evaluated the biological process, cellular component and molecular function terms enriched by the DEPs. Concerning the biological process analysis, the leading five modulated processes were 'cellular', 'biological regulation', 'multicellular organismal', 'developmental' and 'reaction to stimulus processes'. Regarding the cellular component analysis, the most prevalent proteins were primarily linked to the 'cell', 'intracellular' and 'protein-containing complex'. On the other hand, the molecular function analysis indicated that the prevalent molecular functions were 'molecular function modulator', 'binding' and 'catalytic activity' (Fig. 2A).

Table II. Differentially expressed proteins between the gastric HGIN group and the control group.

A, Upregulated proteins

Protein accession no.	Protein description	Gene name	HGIN/CON ratio	HGIN/CON P-value
F6SYF8	Dickkopf-related protein 3	DKK3	4.698	0.030
P55259	Pancreatic secretory granulemembrane major glycoprotein GP2	GP2	2.938	0.022
A0A0G2JMX5	Leukocyte immunoglobulin-like receptor subfamily B member 5	LILRB5	2.802	0.005
O76076	CCN family member 5	CCN5	2.667	0.036
A0A0B4J2D9	Immunoglobulin κ variable 1D-13	IGKV1D-13	2.639	0.029
Q9NQ38	Serine protease inhibitor Kazal-type 5	SPINK5	2.585	0.029
E7ENL6	Collagen α-3(VI) chain	COL6A3	2.383	0.032
H0YCU9	Transgelin (Fragment)	TAGLN	2.381	0.031
Q92484	Acid sphingomyelinase-like phosphodiesterase 3a	SMPDL3A	2.009	0.026
Q12864	Cadherin-17	CDH17	1.995	0.050
A0A1W2PRS4	Junctional adhesion molecule-like	JAML	1.964	0.023
Q9BRV8	Suppressor of IKBKE 1	SIKE1	1.836	0.035
A0A0A0MSN4	Angiotensin-converting enzyme	ACE	1.748	0.031
P17931	Galectin-3	LGALS3	1.738	0.042
Q15828	Cystatin-M	CST6	1.611	0.007
P16070	CD44 antigen	CD44	1.538	0.035
P61769	β-2-microglobulin	B2M	1.53	0.037
Q9NPY3	Complement component C1q receptor	CD93	1.516	0.003

B, Downregulated proteins

Protein accession no.	Protein description	Gene name	HGIN/CON ratio	HGIN/CON P-value
P30101	Protein disulfide-isomerase A3	PDIA3	0.659	0.034
P28070	Proteasome subunit β type-4	PSMB4	0.618	0.043
P26447	Protein S100-A4	S100A4	0.591	0.018
A0A0J9YVY3	Immunoglobulin heavy variable 7-4-1	IGHV7-4-1	0.551	0.049
Q12882	Dihydropyrimidine dehydrogenase [NADP(+)]	DPYD	0.539	0.024
B5MDQ0	DNA excision repair protein ERCC-6-like	ERCC6L	0.531	0.044
O95897	Noelin-2	OLFM2	0.484	0.050
O14793	Growth/differentiation factor 8	MSTN	0.456	0.007
P04406	Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	0.421	0.033
Q9HBB8	Cadherin-related family member 5	CDHR5	0.415	0.040
Q8N1N4	Keratin, type II cytoskeletal 78	KRT78	0.337	0.038
P01705	Immunoglobulin λ variable 2-23	IGLV2-23	0.279	0.033

HGIN, high-grade neoplasia; CON, control.

The major subcategories (>40%) for the gastric HGIN proteins were extracellular (50%), cytoplasm (13.33%), cytoplasm/nucleus (10%) and plasma membrane (10%) (Fig. 2B) as per the subcellular structural localization evaluations.

To determine whether the DEPs were remarkably enriched in certain functional terms, GO enrichment analysis was employed to analyze the DEPs. GO enrichment analysis identifies significant enrichment of DEPs in specific functional terms

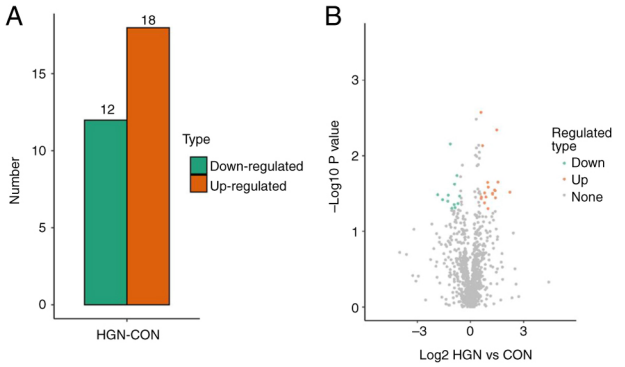

Figure 1. Quantification of DEPs. (A) The quantity of DEPs utilizing a threshold of 1.5-fold. (B) A volcanic scatter plot showing a threshold of 1.5. DEP, differentially expressed protein; HIGN, high-grade neoplasia; CON, control.

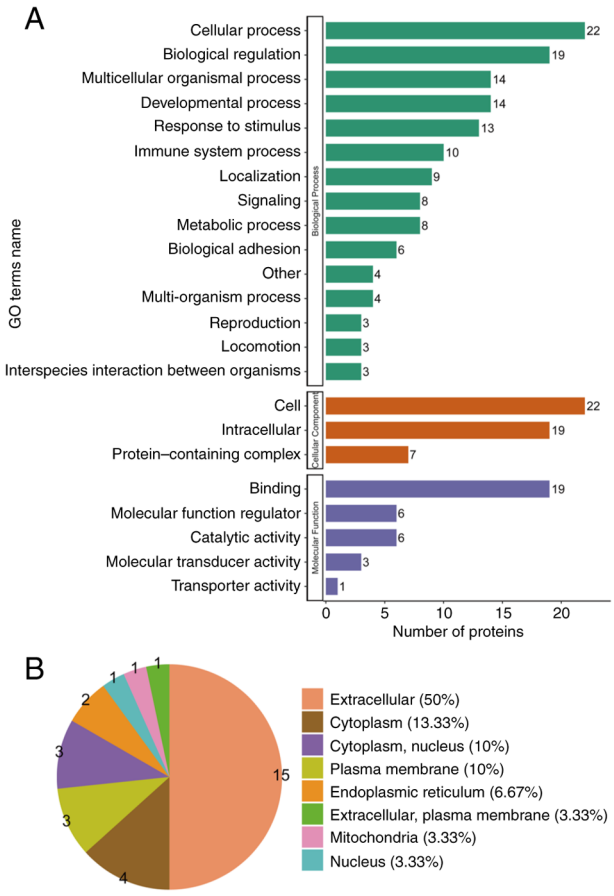

Figure 2. GO annotation analysis revealing the possible biomarkers principally engaged in the gastric HGIN group. (A) GO terms' names, namely cellular component, molecular function and biological process in HGIN-linked proteins. (B) Subcellular structural localization evaluation in HGIN-linked proteins. GO, gene ontology; HGIN, high-grade intraepithelial neoplasia.

through statistical methods (P<0.05), whereas GO categorization describes the distribution profile of DEPs across GO hierarchy without emphasizing statistical significance. The core distinction resides in the application of statistical testing to assess functional specificity enrichment. In the category biological process, the most significantly enriched items were 'mononuclear cell migration', 'positive modulation of cell killing' and 'hemopoiesis modulation' (Fig. 3A). In cellular component, the most significantly enriched items were 'brush border membrane', 'recycling endosome membrane' and 'microvillus' (Fig. 3B). In the category molecular function, the most significantly enriched items were 'transforming growth factor beta receptor binding' and 'phosphoric diester hydrolase activity' (Fig. 3C).

KEGG pathway enrichment analysis. The proteins were integrated into the KEGG database and Fisher's exact test P-values were important to determine the proteins' enrichment levels in order to identify the pathways influenced by the DEPs. The KEGG pathway enrichment analysis manifested that the DEPs were predominantly enriched in 'antigen processing and presentation', 'diabetic cardiomyopathy', 'Epstein-Barr virus infection', 'herpes simplex virus 1 infection', 'human immunodeficiency virus 1 infection' and 'human cytomegalovirus infection' (Fig. 4).

Discussion

In the present study, 30 DEPs were identified in the gastric HGIN group in comparison with the healthy control group, including 18 and 12 upregulated and downregulated proteins, correspondingly. Bioinformatics analysis showed that the functions of the DEPs mainly included 'cellular process', 'biological regulation', 'multicellular organismal process', 'binding, molecular function regulator' and 'catalytic activity'. The major signaling pathways included 'antigen processing and presentation', 'diabetic cardiomyopathy', 'Epstein-Barr virus infection', 'herpes simplex virus 1 infection', 'human immunodeficiency virus 1 infection' and 'human cytomegalovirus infection'. DEP functions, as well as the signaling pathways engaged, can avail an experimental foundation for determining the mechanism of occurrence and advancement of gastric HGIN. The proteomic characteristics and data of gastric HGIN analyzed in the study were different from those of previous gastric cancer proteomics and were heterogeneous (25,26). The discrepancy may be linked to the different stages of gastric cancer in each study.

Collagen alpha-3 (VI) chain (COL6A3) is among the collagen type VI, which is the predominant structural extracellular matrix protein (27). It is primarily expressed in stromal cancer-associated fibroblasts (CAFs) (28). CAFs are a central determinant in the progression of malignancies, as affirmed by a previous study (29). COL6A3 expression was remarkably varied in the normal mucosa in contrast with cancerous tissues, with the greatest and lowest expression levels in primary colorectal cancer and adenoma, respectively. COL6A3 expression was positively directly proportional to the stage of prostate, breast and colorectal cancer (30). Certain studies have reported that the level of expression of COL6A3 in gastric cancer tissue is higher than in normal tissue (31,32). Therefore, COL6A3 could be used as a tumor diagnostic marker. COL6A3 functioned in tumorigenesis as well as in the progression of cholangiocarcinoma via the E2F1/LMCD1-AS1/miR-345-5p/COL6A3 axis (33). In a study on colon cancer, stromal COL6A3 could enhance tumor growth by regulating Hippo and Wnt signaling (34).

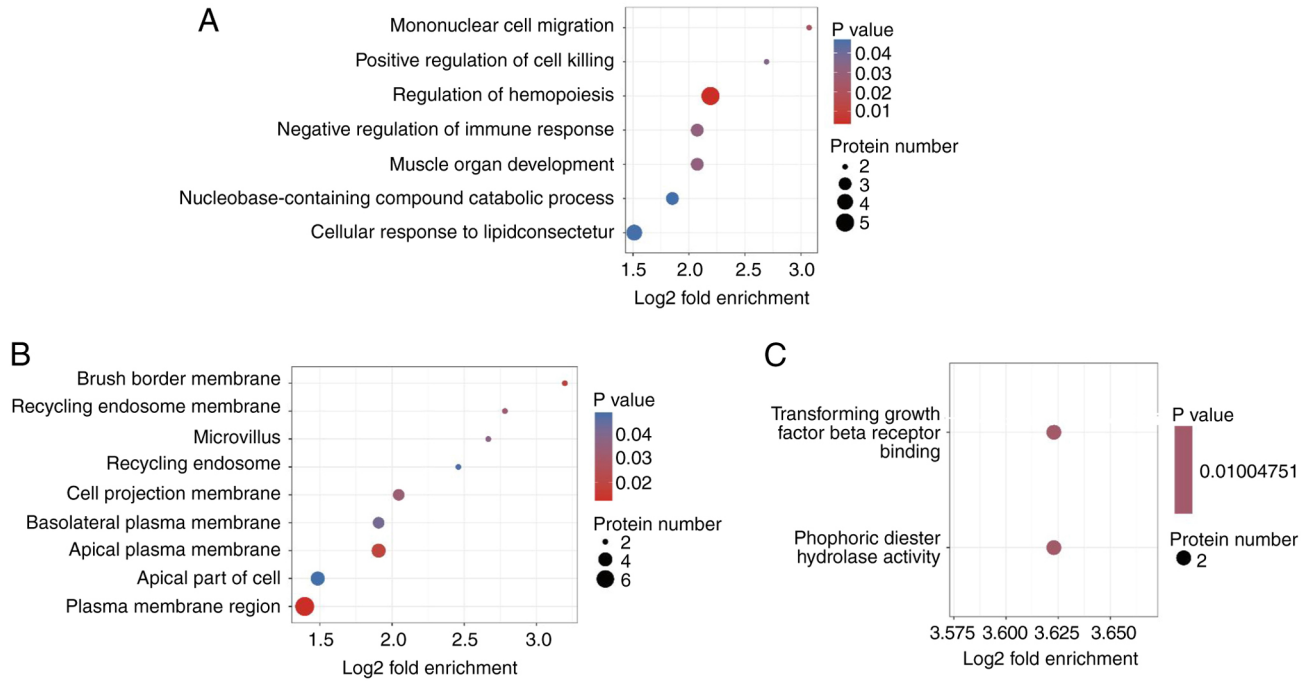

Figure 3. GO enrichment analyses. (A) GO enrichment analyses for biological process. (B) GO enrichment analyses for cellular component. (C) GO enrichment analyses for molecular function. GO, gene ontology.

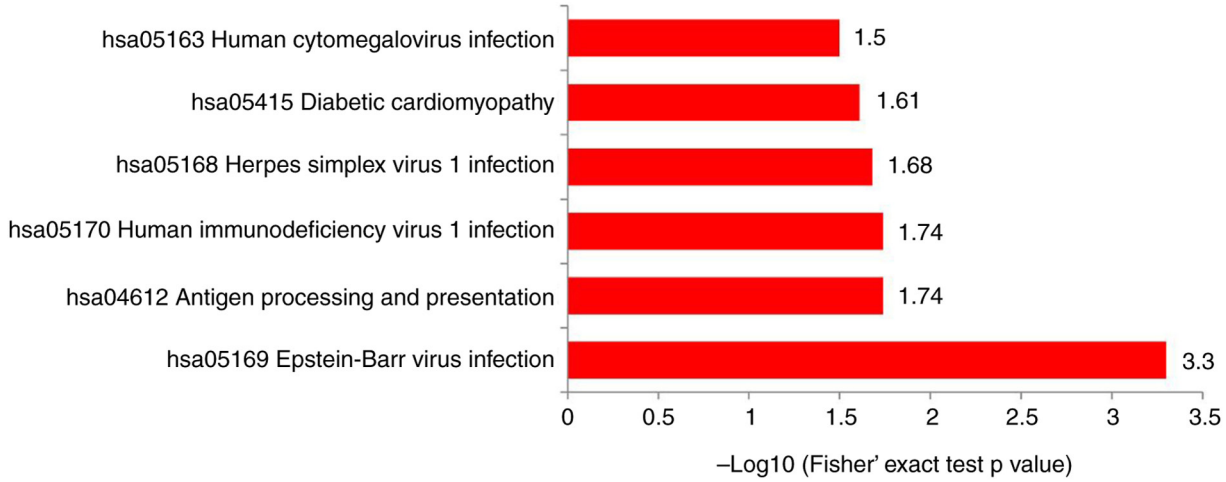

Figure 4. Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis comparing differentially expressed proteins.

COL6A3 knockout lowered cell proliferation and invasion, and enhanced cell apoptosis in cancer cell lines (28), further confirming the relationship between COL6A3 and tumor progression. In this study, COL6A3 expression was upregulated, indicating that it may be engaged in the development of gastric HGIN, but the specific mechanism of action remains to be further studied.

Cadherin-17 (CDH17), also referred to as human peptide transporter-1 or liver-intestine cadherin, encompasses seven homologous repeated domains. As one of the cadherin superfamily members, it functions in intercellular conjunction (35). CDH17 can regulate the Ca^{2+}-dependent homophilic cell-cell adhesion, suggesting that it may be related to the occurrence of tumors (36). In general, CDH17 is expressed not only on the enterocytes' basolateral surfaces but also on goblet cells in the small and large intestines selectively and is rarely discovered in the stomach or liver of a healthy adult (37,38). Previous documentation affirms that the elevated expression level of CDH17, contrary to the lowered levels of various classical cadherins, is linked to cholangiocarcinoma as well as gastric, liver, colorectal and pancreatic cancers, highlighting that CDH17 may have a central function in tumor progression (39). In many of these malignancies, CDH17 expression is linked to tumor stage or unfavorable survival of patients (40). Therefore, CDH17 can be utilized as a sensitive tumor marker for the identification of metastasis of unclear primary origin as well as their subsequent assignation to the gastrointestinal tract (41). Multiple studies on gastric cancer have affirmed that overexpression of CDH17 was positively linked to the histological stage and tumor invasion of gastric cancer, demonstrating

that CDH17 expression can be utilized as a valuable indicator for anticipating the progression of gastric cancer (42). High expression of CDH17 can promote cell adhesion and proliferation and participates in the formation and development of gastric cancer mainly via three signaling pathways in the cells, i.e. the Wnt/β-catenin pathway, the NF-κB signaling pathway and the Ras/Raf/MEK/ERK MAPK signaling pathway (43). In the present study, the expression of CDH17 was upregulated in the gastric HGIN group, suggesting that CDH17 influences the occurrence and development of the disease. However, since we are studying precancerous lesions of gastric cancer, which is at a different disease stage from previous studies, whether it has the same mechanism of action needs to be proved by further studies.

The crucial element of the extracellular matrix (ECM), hyaluronic acid (HA) is essentially linked to a complex transmembrane adhesion glycoprotein known as cluster of differentiation 44 (CD44) (44). Numerous studies have affirmed that CD44 is not only important in the various physiological processes, for instance, diverse immune functions, organ development and hematopoiesis (45,46), but also in pathological processes, particularly tumors (47). Increasing evidence infers that CD44 is remarkably overexpressed in various cancer types such as lymphoma, breast, colon, endometrial, prostate, ovarian, gallbladder and oral squamous cell carcinoma, which is linked to aggressive biological behavior (48,49). CD44 expression was also upregulated in previous studies on gastric precancerous lesions and gastric cancer (50). CD44 interacts with different ECM components, cytokines and proteins that exist in the tumor microenvironment. The interaction between ligands, including osteopontin and HA, in addition to matrix metalloproteinases, with the CD44 receptor, can trigger numerous cellular signaling pathways, thus enhancing tumor advancement and aggressiveness (51). In the present study, CD44 expression was upregulated in the gastric HGIN group. Although the role of CD44 in gastric HGIN remains elusive, it may be involved in the formation of HGIN.

The protein disulfide isomerase A3 (PDIA3) was identified as the most remarkable downregulated protein based on the various ratios in protein expression and the related P-values. PDIA3, also referred to as endoplasmic reticulum resident protein 57, is among the principal members of the PDI gene family. It has been discovered as one of the primary enzymatic chaperones that are essential in the reconstruction of misfolded proteins inside the endoplasmic reticulum (52). It has been ascertained that in some malignancies, the expression of PDIA3 is negatively linked to the degree of differentiation. In uterocervical cancer, PDIA3 expression was downregulated in contrast with normal tissue, as well as cervical intraepithelial neoplasia (53). Another study showed that low expression of PDIA3 was significantly linked to poor cause-specific survival in patients with papillary thyroid carcinoma (54). In cancer immunity, PDIA3 creates a complex with major histocompatibility complexes (MHC) class I to enhance antigen processing, thus triggering an immune response against cancerous cells (55). On the contrary, when MHC class I expression is inadequate, cancerous cells can evade the cytotoxic impacts of immune cells that can be seen in gastric cancer (56). In the present study, downregulation of PDIA3 was detected, which indicated the possibility of inactivation of gastric HGIN as a tumor suppressor during the development of HGIN, but its potential impact requires further study.

The present study has certain limitations. First of all, the sample size included in this study is small and the screened DEPs have not been further verified. In the future, it is necessary to further expand the sample and use enzyme-linked immunosorbent assay and other experimental methods to verify the DEPs. Secondly, due to the small sample size, in order to take into account the reliability of the experimental data, only males with a relatively higher incidence of gastric cancer were selected as experimental subjects in this study (57), and it is required to expand the sample to further include females in the study.

To conclude, utilizing LFQ coupled with LC-MS/MS, it was found that certain proteins, such as COL6A3, CDH17, CD44 and PDIA3, could be candidate protein biomarkers for gastric HGIN. However, additional studies are indispensable to ascertain the possible functions of these candidate proteins in the development of gastric HGIN. The present data provide additional biological information regarding the formation of gastric HGIN and provide clues for further research on the pathogenesis of early gastric cancer.

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Availability of data and materials

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with database identifier PXD036991 (https://www.ebi.ac.uk/pride/profile/reviewer_pxd036991).

Authors' contributions

NL, CH and JG designed the study. LL and XH collected data and patients. NL, LL and XH performed the research. NL and CH analyzed the data. NL, CH and JG wrote the manuscript. All authors contributed to editorial changes to the manuscript. NL and LL confirm the authenticity of the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Affiliated Hospital of Putian University (approval no. 202006). Informed consent was obtained from all subjects involved in the study. Patients were fully aware of the purpose, methods, potential therapeutic benefits and possible adverse reactions of the study, and willingly agreed to participate and fully cooperate with the doctors.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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