Differentially expressed genes *ASPN, COL1A1, FN1, VCAN* and *MUC5AC* are potential prognostic biomarkers for gastric cancer

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Abstract. Gastric cancer (GC) is one of the most common malignancies worldwide. To the best of our knowledge, no biomarkers have been widely accepted for the early diagnosis and prognostic prediction of GC. This study aimed to identify potential novel prognostic biomarkers for GC. The dataset GSE29272, which originates from the public database Gene Expression Omnibus, was employed in the present study. The online tool GEO2R was used to calculate the differentially expressed genes (DEGs) in GSE29272 between tumour tissues and adjacent tissues. CytoHubba and MCODE plugins of Cytoscape software were used to obtain hub genes and modules of DEGs. The online tools Database for Annotation, Visualisation and Integrated Discovery and Search Tool for the Retrieval of Interacting Genes were employed to conduct Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway analysis, and to construct protein-protein interaction networks. A total of 117 DEGs were extracted from GSE29272. In addition, 15 hub genes and seven modules were identified in the 117 DEGs. The enrichment analysis revealed that they were mainly enriched in GO biological process and cellular component domains, and the 'ECM-receptor interaction', 'focal adhesion', 'metabolism of xenobiotics by cytochrome P450' and 'drug metabolism' pathways. The hub genes asporin (*ASPN),* collagen type I α1 chain (*COL1A1),* fibronectin 1 (*FN1),* versican *(VCAN)* and mucin 5AC *(MUC5AC)* were demonstrated to have prognostic value for patients with GC. The *ASPN* and *VCAN* genes were significantly associated with overall survival and disease-free survival (log-rank P=0.025, 0.038, 0.0014 and 0.015, respectively). *COL1A1* and *FN1* were significantly associated with overall survival (log-rank P=0.013 and 0.05, respectively), and *MUC5AC* was significantly associated with disease-free survival (log-rank P=0.027). Results from the present study suggested that *ASPN, COL1A1, FN1, VCAN* and *MUC5AC* may represent novel prognostic biomarkers for GC.

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Introduction

Gastric cancer (GC) is one of the most common causes of tumour-associated mortality worldwide (1). GC in East Asia represents ~50% of all GC cases (2). The high incidence of GC is partly due to the popular application of endoscopy (3). Despite advances in the diagnosis and treatment options of GC, the prognosis remains poor, and the 5-year survival rate of patients with GC is <20% (4). The commonly used biomarkers, carcinoembryonic antigen and cancer antigen 19-9, possess limited sensitivity and specificity in clinical application, which results in unsatisfactory levels of early diagnosis of GC (5).

Some molecules have been recently documented for their prognostic value in the screening and diagnosis of patients with GC. For instance, carbohydrate antigen 72-4 (CA72-4), an independent prognostic marker, has a good prognostic value for overall and relapse-free survival for patients with GC (6). As a prognostic marker, CA72-4 is widely used in various types of tumour, including pancreatic cancer, lung cancer and ovarian carcinoma (7-9). The sensitivity of CA72-4 in ovarian carcinoma is ~47% at the time of primary diagnosis (7). In addition, after analysis of the receiver operating characteristic curve of CA72-4, the area under the curve is ~88.4% in lung cancer (8). The sensitivity of CA72-4 is 25.5% in pancreatic cancer, which is higher than that in benign pancreatic disease (9). Although several proteins have recently been reported to be associated with prognosis for patients with GC, specific biomarkers for early diagnosis and prognostic assessment of GC are still lacking (10-12). Therefore, establishing novel tumour markers with sufficient sensitivity and specificity is therefore crucial in order to improve the value of early diagnosis and prognostic prediction for patients with GC.

High-throughput sequencing (HTS) is an increasingly widely used tool that has significant roles in numerous life science fields, including early diagnosis, tumour grading and prognostic assessment (13). Databases containing HTS datasets are well acknowledged and have an increasingly significant role in the early diagnosis and prognostic prediction of various types of malignancy. The Gene Expression Omnibus (GEO)

database (https://www.ncbi.nlm.nih.gov/geo/) is a public functional genomics data source supporting Minimum Information About a Microarray Experiment-compliant data submissions. The GEO database contains array- and sequence-based data, providing users with experimental and curated gene expression information. In the present study, the GEO database was employed to identify novel prognostic biomarkers for patients with GC; these novel insights may aid in the development of individual treatments.

Materials and methods

Patient data collection. The GC expression profile dataset GSE29272 [GPL96, (HG-U133A), Affymetrix Human Genome U133A Array; Affymetrix; Thermo Fisher Scientific, Inc., Waltham, MA, USA] in the GEO database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29272, accessed January 10, 2018) was used in the present study. This dataset includes 134 normal gastric tissue samples and 134 GC tissue samples (14,15).

Data processing. The online tool, GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/, accessed January 10, 2018) was applied to determine the differentially expressed genes (DEGs) in normal gastric tissues and GC tissues (16). Adjusted P-values were used to reduce the false positive rate using the Benjamini and Hochberg false discovery rate method by default. Adjusted P≤0.05 and |log fold change (FC)|≥1.5 were set as cut-off values. A total of 117 DEGs were then identified, including 43 upregulated and 74 downregulated genes. Eventually, the top 15 genes were determined as hub genes ranked by the Degree method in cytoHubba, a plugin in Cytoscape 3.6.0 software (17,18).

Enrichment analysis of tissue expression, gene ontology (GO) terms and kyoto encyclopedia of genes and genomes (KEGG) pathways of DEGs. GO analysis, including biological process (BP), cellular component (CC) and molecular function (MF) domains, is a tool widely used for annotating specific genes and gene products, and for assembling biological features for high-throughput genome and transcriptome data (19). KEGG is a database resource used to understand high-level functions and utilities of a biological system from molecular-level information by genome sequencing and other high-throughput experimental technologies (20). The Database for Annotation, Visualisation and Integrated Discovery (DAVID) version 6.7 (https://david-d.ncifcrf.gov/, accessed January 16, 2018) was used to identify detailed tissue expression, GO terms, including BP, CC, and MF, and KEGG pathways associated with the 117 DEGs (21,22).

Protein-protein interaction (PPI) network and modules analysis. The online resource Search Tool for the Retrieval of Interacting Genes (STRING' https://string-db.org/cgi/input.pl) was used to construct relationships for hub proteins. Subsequently, the Molecular Complex Detection (MCODE) plugin in Cytoscape 3.6.0 software was used to screen modules of the PPI network with the following default settings: Degree cut-off, 2; node score cut-off, 0.2; K-core, 2; maximum depth, 100. Eventually, enrichment analysis of GO terms and KEGG pathways was performed on DAVID using the 117 DEGs and the genes in the different modules.

Table I. Top 15 hub genes of differentially expressed genes, with high degrees.

Gene	Degree	Adjusted P-value	Log FC
FN1	39	$7.92x10^{43}$	1.752388
TIMP1	36	$1.87x10^{63}$	2.109944
SPP1	35	$1.77x10^{41}$	2.640095
MMP7	33	$2.05x10^{17}$	1.616738
CA2	33	$1.22x10^{29}$	-2.8229
COL1A2	31	$4.10x10^{39}$	1.578489
SPARC	30	$7.63x10^{44}$	1.887773
MUC5AC	30	$2.12x10^{40}$	-3.4951
VCAN	30	$7.31x10^{26}$	1.559823
APOE	29	$7.79x10^{35}$	1.561487
COL3A1	29	$7.42x10^{42}$	1.625067
COL1A1	29	$8.31x10^{33}$	1.51057
THBS2	27	$8.67x10^{50}$	2.163794
ASPN	27	$4.46x10^{37}$	2.303273
BGN	27	$2.91x10^{50}$	1.728095

FC, fold change.

Expression levels, correlation and survival analysis of hub genes. The online resource Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/index.html, accessed January 18, 2018), which originated from The Cancer Genome Atlas database, was used to determine the overall survival (OS) and disease-free survival (DFS) outcomes of hub genes (23). Furthermore, the genes associated with OS and/or DFS were applied for further analysis, including Pearson correlation analysis and analysis of expression levels in tumour and normal tissues.

Results

Identification of DEGs and hub genes. There were 134 GC tissues and 134 normal gastric tissue samples analysed in this study. Firstly, the GEO2R tool was employed to identify DEGs using the following cut-off values: Adjusted P≤0.05 and |logFC|≥1.5. As a result, a total of 117 DEGs were identified, including 43 upregulated and 74 downregulated genes. Subsequently, a PPI network of 117 DEGs was constructed using STRING (Fig. 1). Furthermore, 15 hub genes out of the 117 DEGs were determined by the Degree method using the following criteria: P≤0.05 and |log fold change (FC)|≥1.5. The 15 hub genes were as follows: Fibronectin 1 (*FN1*), TIMP metallopeptidase inhibitor 1, secreted phosphoprotein 1, matrix metalloproteinase 7, carbonic anhydrase 2, collagen type I α2 chain, secreted protein acidic and cysteine rich, mucin 5AC (*MUC5AC*), versican (*VCAN*), apolipoprotein E, collagen type III α1 chain, collagen type I α1 chain (*COL1A1*), thrombospondin 2, asporin (*ASPN*) and biglycan (Table I).

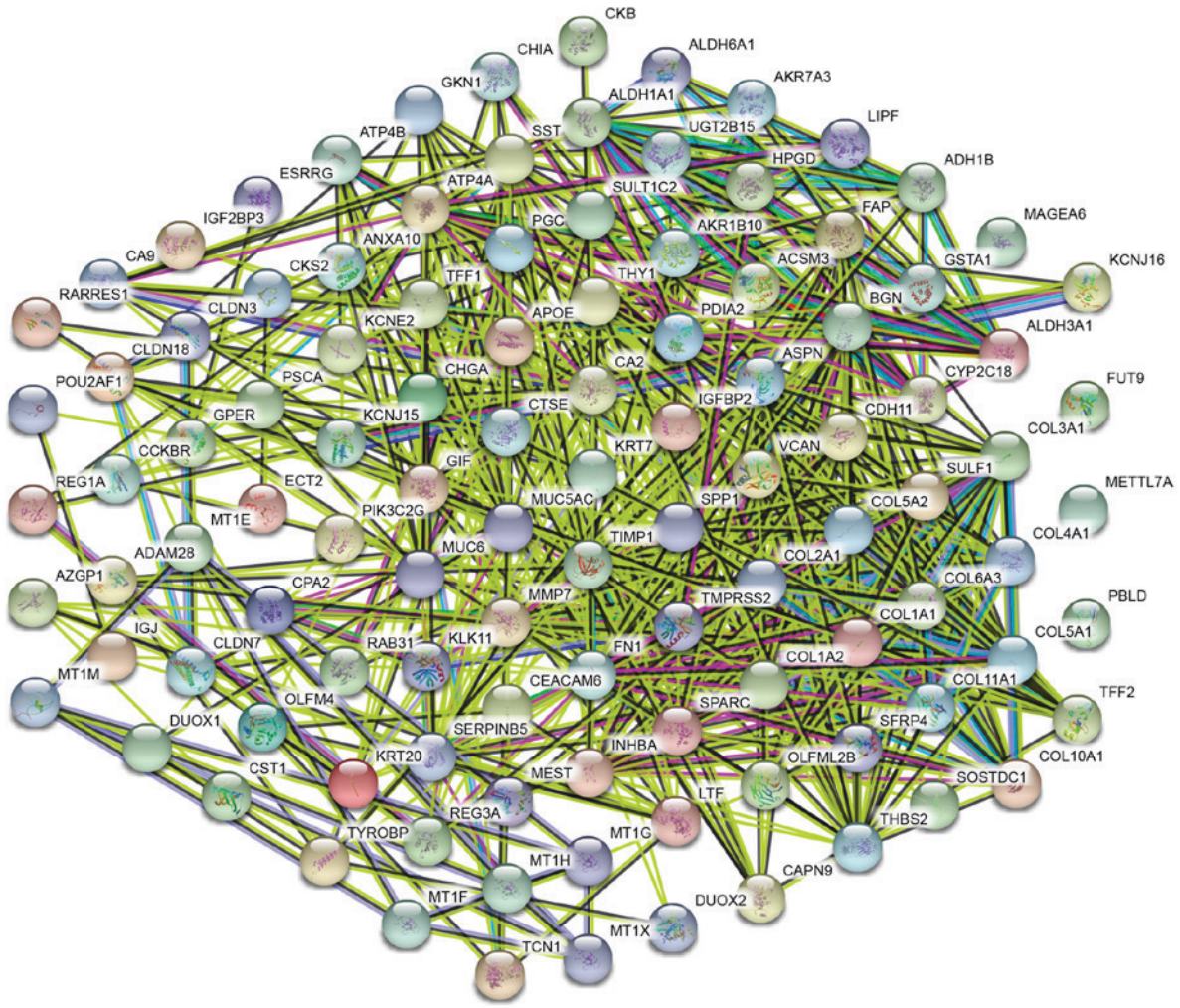


Figure 1. Protein-protein interaction network of 117 differentially expressed genes.

Enrichment analysis of tissue expression, GO terms and KEGG pathways of the 117 DEGs. After importing the 43 upregulated and 74 downregulated genes into DAVID, the enriched results of tissue expression, GO term and KEGG pathway analyses were revealed. The 43 upregulated genes were enriched in tissues including colon endothelium, saliva, placenta, bone, plasma, fibroblasts, skin, cartilage, peripheral nervous system, liver and endometrial tumours. The 74 downregulated genes were enriched in tissues including stomach, liver, colon, pancreas, small intestine, stomach mucosa, prostate, foetal liver and erythrocytes (Table II).

The top GO terms of the 43 upregulated genes were extracellular, including 'proteinaceous extracellular matrix', 'extracellular matrix', 'collagen', 'extracellular matrix part', 'extracellular region', 'extracellular matrix structural constituent', 'fibrillar collagen', 'platelet-derived growth factor binding' and 'collagen fibril organization'. The top GO terms of the 74 downregulated genes were 'digestion', 'cadmium ion binding', 'extracellular region', 'copper ion binding', 'response to inorganic substance', 'cellular aldehyde metabolic process', 'oxidation-reduction', 'extracellular space', 'response to metal ion' and 'aldo-keto reductase activity' (Table III). Detailed results of the enriched GO terms included various terms from the BP, CC and MF domains (data not shown).

With regards to the KEGG pathway analysis of the 43 upregulated genes, three pathways were enriched: 'ECM-receptor interaction', 'focal adhesion' and 'leukocyte transendothelial migration'. With regards to the KEGG pathway analysis of the 74 downregulated genes, four pathways were enriched: 'metabolism of xenobiotics by cytochrome P450', 'drug metabolism', 'retinol metabolism' and 'nitrogen metabolism'. Detailed results are displayed in Table IV.

PPI network of hub genes and modules using MCODE. Following import of the 15 hub genes, the STRING website highlighted an interaction network. This network contains known interactions from curated databases and those that were experimentally determined; predicted interactions containing gene neighbourhood, gene fusions and gene co-occurrence; and text-mining, co-expression and protein homology (Fig. 2).

After importing the PPI network of 117 DEGs, Cytoscape displayed modules in the default MCODE settings. A total of seven modules were presented in the results (Fig. 3). Genes in these modules were then assembled for enrichment analysis using DAVID. Only two gave rise to KEGG pathways (Fig. 3B and G). Two pathways, 'the ECM-receptor interaction' and 'focal adhesion' pathways, were enriched by genes presented in Fig. 3A. Four pathways, 'metabolism of

Table II. Enrichment analysis of 117 differentially expressed genes in different tissues.

A, Upregulated genes

Term	Count	Percent (%)	P-value	FDR
Colon endothelium	3	7.317073	$1.99x10^{-04}$	0.217355
Saliva	3	7.317073	0.005754	6.109932
Placenta	15	36.58537	0.005972	6.334224
Bone	3	7.317073	0.007801	8.200374
Plasma	4	9.756098	0.023135	22.56379
Fibroblast	3	7.317073	0.030255	28.51154
Skin	9	21.95122	0.031734	29.69333
Cartilage	2	4.878049	0.054281	45.64904
PNS	3	7.317073	0.056776	47.19527
Liver	9	21.95122	0.06303	50.89721
Endometrial tumour	2	4.878049	0.088208	63.53566

B, Downregulated genes

Term	Count	Percent (%)	P-value	FDR
Stomach	15	23.07692	$1.02x10^{-14}$	$1.10x10^{-11}$
Liver	22	33.84615	$2.40x10^{-06}$	0.002588
Colon	14	21.53846	$1.32x10^{-04}$	0.141746
Pancreas	11	16.92308	0.001617	1.729784
Small intestine	6	9.230769	0.005447	5.720101
Stomach mucosa	3	4.615385	0.006518	6.809155
Prostate	7	10.76923	0.013697	13.81931
Foetal liver	4	6.153846	0.033717	30.9166
Erythrocyte	2	3.076923	0.083086	60.75539

FDR, false discovery rate; PNS, peripheral nervous system; UP_TISSUE, tissue expression.

xenobiotics by cytochrome P450', 'drug metabolism', 'tyrosine metabolism', and 'glycolysis/gluconeogenesis', were enriched by the genes presented in Fig. 3F.

Survival curves, expression levels and correlation analysis of hub genes. All aforementioned 15 hub genes were analysed using the prognostic values of OS and DFS via the GEPIA website. *ASPN* and *VCAN* were significantly associated with OS and DFS (log-rank P=0.025, 0.038, 0.0014 and 0.015, respectively, Fig. 4A-D). *COL1A1* and *FN1* were significantly associated with OS (Log-rank P=0.013 and 0.05) (Fig. 4E and F). *MUC5AC* was significantly associated with DFS (Log-rank P=0.027, Fig. 4G). The analysis of these five genes revealed that low expression levels led to better survival status. The other hub genes did not exhibit statistical significance.

The genes *ASPN*, *VCAN, COL1A1*, *FN1* and *MUC5AC* were then subjected to further analysis. Expression levels of these five genes are displayed in Fig. 5A-E. With the exception of *MUC5AC*, which exhibited low expression levels in GC tissues, the other four genes presented high expression levels in GC tissues. Furthermore, *ASPN*, *VCAN, COL1A1* and *FN1* had lower expression levels in normal gastric tissues. In addition, Pearson correlation analyses between the genes are presented in Fig. 5F-O. Results revealed that *MUC5AC* was negatively correlated with the four other genes: *ASPN* (R=-0.14, P=0.0042); *COL1A1* (R=-0.092, P=0.062); *FN1* (R=-0.15, P=0.0029); *VCAN* (R=-0.12, P=0.017). However, among the four other genes, each gene was positively correlated with the three other genes (all R>0, P<0.05).

Discussion

In the present study, the potential prognostic associations between GC and DEGs in GSE29272 were investigated. The results highlighted 117 DEGs, including 43 upregulated and 74 downregulated genes, between the 134 gastric normal tissues and the 134 GC tissues. A total of 15 hub genes were selected and seven modules were identified from the 117 DEGs. Some of these hub genes exhibited potential prognostic values for patients with GC.

GC is one of the most common types of tumour and ranks sixth among all tumours (24). Approximately 60% of newly diagnosed cases originate from Eastern Asia, particularly from

Table III. Top 10 enriched Gene Ontology terms of upregulated and downregulated genes.

A, Upregulated genes

Category	Term	Count	Percent (%)	P-value	FDR
GOTERM_CC_FAT	Extracellular region part	23	56.09756	4.38E-16	4.88E-13
GOTERM_CC_FAT	Proteinaceous extracellular matrix	16	39.02439	3.89E-15	4.27E-12
GOTERM_CC_FAT	Extracellular matrix	16	39.02439	1.19E-14	1.31E-11
GOTERM_CC_FAT	Collagen	9	21.95122	4.88E-14	5.37E-11
GOTERM_CC_FAT	Extracellular matrix part	11	26.82927	7.91E-13	8.69E-10
GOTERM_CC_FAT	Extracellular region	26	63.41463	1.92E-12	2.11E-09
GOTERM_MF_FAT	Extracellular matrix structural constituent	9	21.95122	1.85E-11	2.07E-08
GOTERM_CC_FAT	Fibrillar collagen	6	14.63415	1.20E-10	1.32E-07
GOTERM_MF_FAT	Platelet-derived growth factor binding	5	12.19512	8.67E-09	9.68E-06
GOTERM_BP_FAT	Collagen fibril organization	6	14.63415	9.77E-09	1.46E-05

B, Downregulated genes

Category	Term	Count	Percent (%)	P-value	FDR
GOTERM_BP_FAT	Digestion	12	18.46154	6.44E-14	9.14E-11
GOTERM_MF_FAT	Cadmium ion binding	6	9.230769	4.04E-10	5.11E-07
GOTERM_CC_FAT	Extracellular region	23	35.38462	1.89E-06	0.002038
GOTERM_MF_FAT	Copper ion binding	6	9.230769	1.47E-05	0.018561
GOTERM_BP_FAT	Response to inorganic substance	7	10.76923	1.76E-04	0.249357
GOTERM_BP_FAT	Cellular aldehyde metabolic process	4	6.153846	1.94E-04	0.274926
GOTERM_BP_FAT	Oxidation-reduction	10	15.38462	0.001002	1.412983
GOTERM_CC_FAT	Extracellular space	10	15.38462	0.001199	1.282203
GOTERM_BP_FAT	Response to metal ion	5	7.692308	0.001848	2.59269
GOTERM_MF_FAT	Aldo-keto reductase activity	3	4.615385	0.002052	2.565994

BP, biological process; CC, cellular component; MF, molecular function; FDR, false discovery rate.

China (25). There is currently a lack of sensitive biomarkers for the early diagnosis of GC. Therefore, many patients are in an advanced stage or have distant metastases at the time of diagnosis, resulting in poor prognosis (26). With a median OS of <1 year and a poor 5-year OS the prognosis remains unsatisfactory for patients with advanced stages of GC, and surgical resection is a common palliative treatment (27). Many studies have focused on the identification of novel biomarkers for early diagnosis and recurrence prediction of GC (28-31). However, no widely accepted biomarkers have yet been discovered. Therefore, identifying novel and effective biomarkers for GC is crucial.

The present study revealed that some genes were differentially expressed between GC and normal tissues. A total of 43 genes were upregulated and 74 genes were downregulated. The DEGs then underwent tissue expression, GO term and KEGG pathway analyses. Both upregulated and downregulated genes were enriched in multiple organs. Notably, the stomach was the first organ highlighted in the enrichment analysis of the downregulated genes, and 15 genes were associated with this result. These genes were ATPase H+/K+ transporting subunit α, cholecystokinin B receptor, progastricsin, calpain 9, gastric intrinsic factor, mucin 6, aldehyde dehydrogenase 3 family member A1, chromogranin A, trefoil factor 2, cathepsin E, sulfotransferase family 1C member 2, *MUC5AC*, trefoil factor 1, gastrokine 1 and lipase F.

The majority of GO terms were enriched in CC terms in the top 10 upregulated genes. The enriched CC terms contained the 'extracellular region part', 'proteinaceous extracellular matrix', 'extracellular matrix', 'collagen', extracellular matrix part', 'extracellular region' and 'fibrillar collagen'. Furthermore, the majority of GO terms were enriched in BP terms in the top 10 downregulated genes. The enriched BP terms contained 'digestion', 'response to inorganic substance', 'cellular aldehyde metabolic process', 'oxidation-reduction' and 'response to metal ions'. In the KEGG pathway analysis, upregulated and downregulated genes were enriched in four pathways. In the module analysis, a total of seven modules were identified by MCODE analysis of the 117 DEGs. The genes in these seven modules were then analysed for KEGG pathway enrichment. Six KEGG pathways were highlighted in only two modules. Notably, four pathways were presented in the results of both DEG enrichment and module analyses; these were 'ECM-receptor interaction', 'focal adhesion',

Table IV. Enriched KEGG pathways of differentially expressed genes.

A, Upregulated genes

Term	Count	Percent (%)	P-value	Genes	FDR
ECM-receptor interaction	11	26.82927	$2.66x10^{-13}$	*COL4A1*, *COL6A3*, *COL3A1*, *COL1A2*, *COL1A1*, *COL5A2*, *THBS2*, *COL11A1*, *COL5A1*, *SPP1*, *FN1*	$1.89x10^{-10}$
Focal adhesion	11	26.82927	$1.80x10^{-09}$	*COL4A1*, *COL6A3*, *COL3A1*, *COL1A2*, *COL1A1*, *COL5A2*, *THBS2*, *COL11A1*, *COL5A1*, *SPP1*, *FN1*	$1.28x10^{-06}$
Leukocyte transendothelial migration	3	7.317073	0.084213	*CLDN7*, *CLDN3*, *THY1*	46.45857

B, Downregulated genes

Term	Count	Percent (%)	P-value	Genes	FDR
Metabolism of xenobiotics by cytochrome P450	5	7.692308	$9.02x10^{-05}$	*GSTA1*, *CYP2C18*, *ADH1C*, *ADH1B*, *UGT2B15*, *ALDH3A1*	0.082042
Drug metabolism	5	7.692308	$1.03x10^{-04}$	*GSTA1*, *CYP2C18*, *ADH1C*, *ADH1B*, *UGT2B15*, *ALDH3A1*	0.093347
Retinol metabolism	4	6.153846	0.001315	*ALDH1A1*, *CYP2C18*, *ADH1C*, *ADH1B*, *UGT2B15*	1.189785
Nitrogen metabolism	2	3.076923	0.090981	*CA9*, *CA2*	57.99834

FDR, false discovery rate.

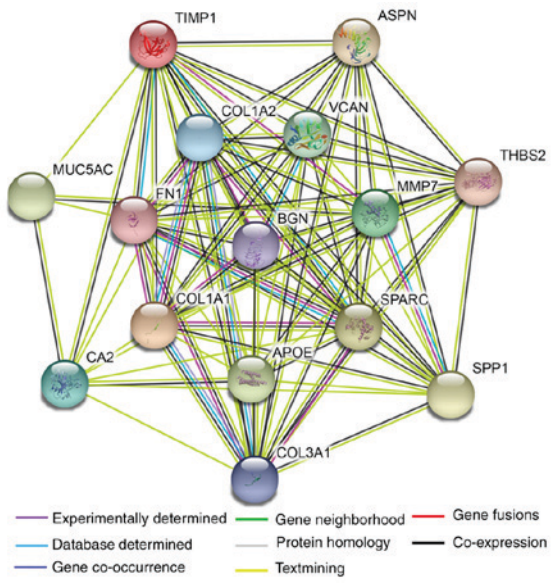
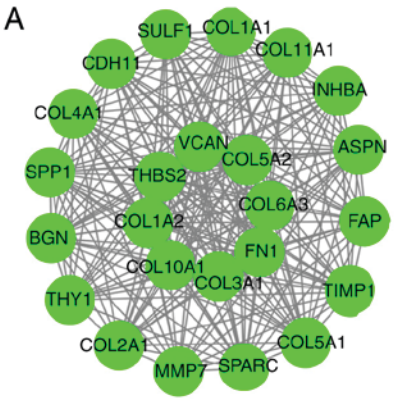


Figure 2. Protein-protein interaction network of 15 hub genes.

'metabolism of xenobiotics by cytochrome P450' and 'drug metabolism'.

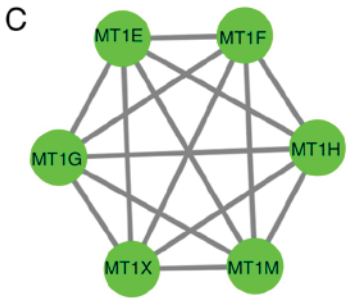
In the prognostic survival analysis, five of the 15 hub genes had prognostic values. *ASPN* and *VCAN* were associated with OS and DFS. *COL1A1* and *FN1* were associated with OS, whereas *MUC5AC* was associated with DFS. With the exception of *MUC5AC*, which exhibited low expression in GC, the four other genes exhibited high expression in GC tissues, and low expression in normal gastric tissues. These results suggest that *MUC5AC*, *ASPN*, *COL1A1*, *FN1* and *VCAN* may serve oncogenic roles in gastric cancer. These genes also serve numerous functions, possibly via BP, CC and the aforementioned four pathways.

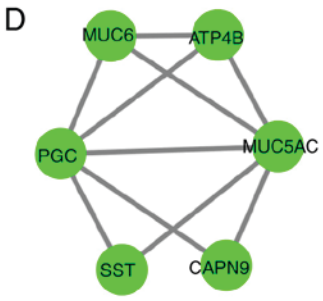
Secreted MUC5AC is commonly expressed in microsatellite instability (MSI) cancers (32). Expression of *MUC5AC* usually occurs in mucinous and MSI carcinomas (33). Renaud *et al* (34) reported that abnormal expression levels of *MUC5AC* in high CpG island methylation phenotype/MSI colorectal cancer (CRC) is closely associated with altered methylation of their promoters. Notably, *MUC5AC* is associated with the absence of tumour protein 53 mutations, and when combined with mucin 2, is associated with poor differentiation and MSI status (34). In addition, the hypomethylation of the proximal region of the *MUC5AC* promoter (MUC5AC-I) is not associated with MUC5AC protein expression (14,32,34). *MUC5AC* hypomethylation is a highly predictive biomarker and a specific regulatory mechanism of MSI cancers (34), whereas the determination of *MUC5AC* methylation status may be important for understanding and predicting the natural history of CRC (34). Renaud *et al* (35), suggested that *MUC5AC* hypomethylation can serve as a biomarker to identify serrated pathway neoplasia-associated precursors. Numerous studies strongly suggested that *MUC5AC* generates the major receptor for *Helicobacter pylori* in the human stomach (36,37) and that infection with *H. pylori* can modify expression levels of *MUC5AC* (38). Zhou *et al* (39) reported

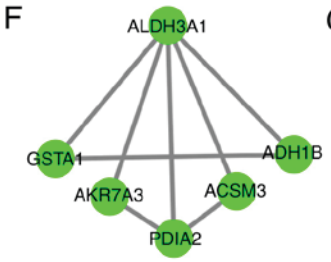


B

Term	Count	%	P value	FDR	Genes
ECM-receptor interaction	12	50	1.64E-17	1.02E-14	COL4A1, COL6A3, COL3A1, COL1A2, COL2A1, COL1A1, COL5A2, THBS2, COL11A1, COL5A1, SPP1, FN1
Focal adhesion	12	50	3.34E-13	2.08E-10	COL4A1, COL6A3, COL3A1, COL1A2, COL2A1, COL1A1, COL5A2, THBS2, COL11A1, COL5A1, SPP1, FN1

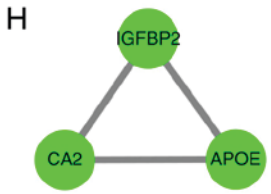






G

Term	Count	%	P value	FDR	Genes
Metabolism of xenobiotics by cytochrome P450	3	6.976744	4.08E-04	0.244546	GSTA1, ADH1B, ALDH3A1
Drug metabolism	3	6.976744	4.35E-04	0.261176	GSTA1, ADH1B, ALDH3A1
Tyrosine metabolism	2	4.651163	0.02574	14.49398	ADH1B, ALDH3A1
Glycolysis/Gluconeogenesis	2	4.651163	0.034989	19.25402	ADH1B, ALDH3A1



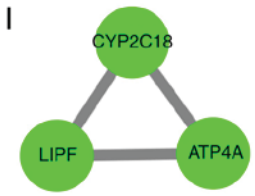


Figure 3. Modules obtained from the protein-protein interaction network and enriched pathways for genes in the modules. (A, C-F and H-I) Seven modules generated by Molecular Complex Detection. (B and G) Enriched pathways in the modules of (A) and (F), respectively. FDR, false discovery rate.

that common polymorphisms of *MUC5AC* are associated with the risk of non-cardia GC in a Chinese population. *MUC5AC* expression is also associated with Notch3 signalling, which provides an encouraging prognosis in patients with small intestine adenocarcinomas (40). The present study hypothesized that *MUC5AC* may serve a oncogenic role, which was inconsistent with the findings of Kim *et al* (41), who stated that the decreased expression of *MUC5AC* is associated with poor prognosis in GC. This inconsistency may be due to the small sample size of the present study; therefore, further investigations regarding to the role of *MUC5AC* are required.

ASPN has been widely explored in osteoarthritis, and is finely regulated in cartilage (42). *ASPN* is also implicated in the mechanisms of local invasion of breast ductal carcinoma (43). In addition, *ASPN* is overexpressed in pancreatic ductal adenocarcinoma, suggesting that it is a good candidate for diagnostic and therapeutic application (44). *ASPN* also participates in GC cell growth and migration by influencing epidermal growth factor receptor (EGFR) receptor signalling (45). The present study hypothesized that *ASPN* may have an oncogenic role in gastric tumours, which is consistent with a previous study reporting that *ASPN* serves an oncogenic role in GC progression and metastasis via the EGFR signalling pathway (45).

Two mutations (c.3235G>A and c.3247G>A) occur simultaneously in *COL1A1* and lead to type IV osteogenesis imperfecta (46). In addition, a novel mutation in the start codon of *COL1A1* causes osteogenesis imperfecta type I in a Korean family (47). *COL1A1* C-propeptide cleavage site mutation also

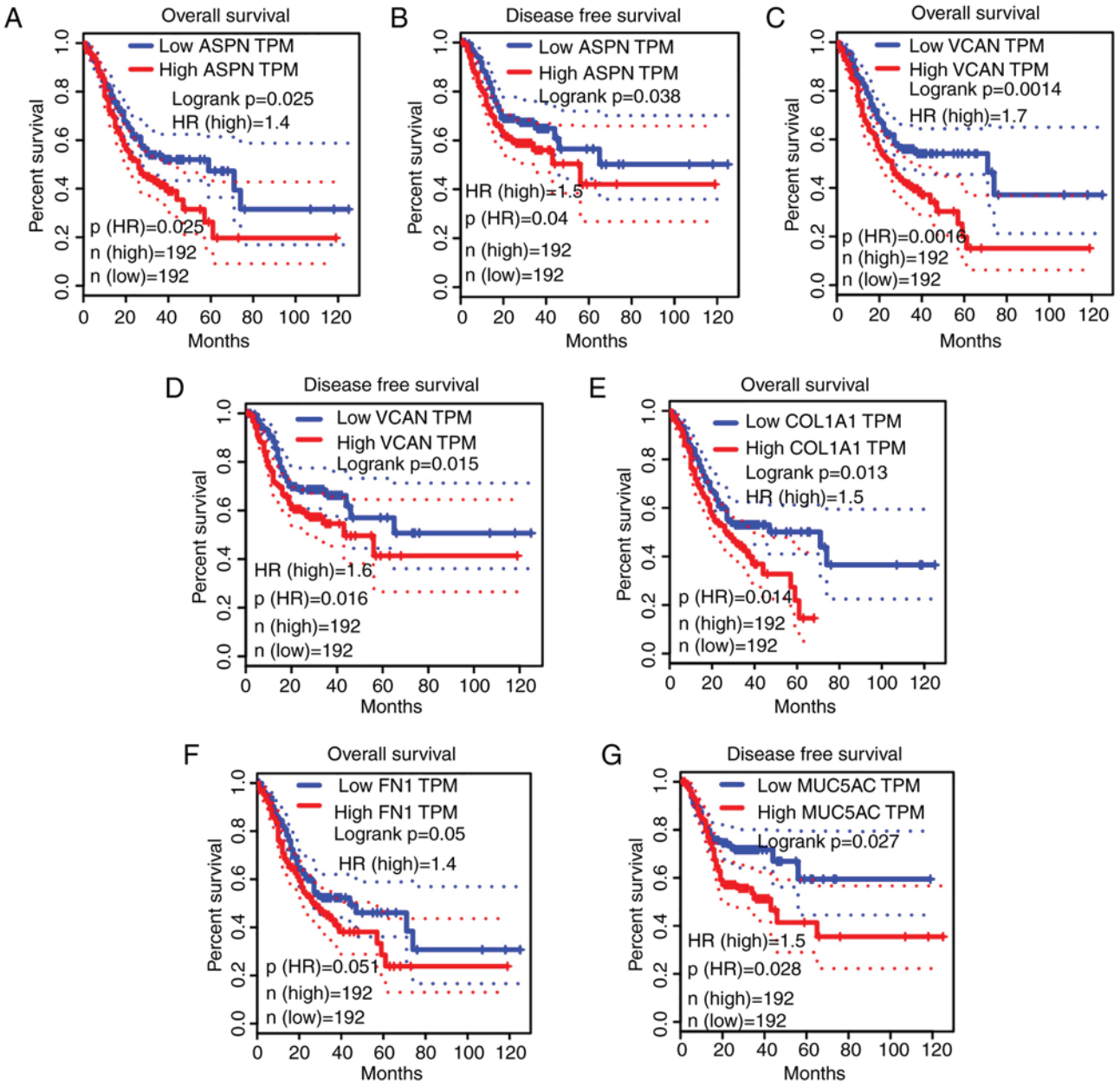


Figure 4. Prognostic survival analysis of *ASPN, COL1A1, FN1, VCAN* and *MUC5AC* genes. (A, C, E and F) (A)Overall survival of ASPN. (B) Disease free survival of ASPN. (C) Overall survival of VCAN. (D) Disease free survival of VCAN. (E) Overall survival of COL1A1. (F) Overall survival of FN1. (G) Disease free survival of MUC5AC, respectively. *ASPN*, asporin; *COL1A1*, collagen type I α1 chain; *FN1*, fibronectin 1; *MUC5AC*, mucin 5AC; *THBS2*, thrombospondin 2; *VCAN*, versican; TPM, transcripts per million.

leads to high bone mass, bone fragility and jaw lesions (48). Furthermore, *COL1A1* has been incorporated in fibroblasts as a molecular signature by hCellMarkerPlex, which indicates *COL1A1* is associated with fibroblasts and functions as a molecular signature (49). Previous studies have suggested that *COL1A1* is a candidate survival-related factor in hepatocellular carcinoma (50). In addition, *COL1A1* polymorphism is associated with an elevated risk of osteosarcoma susceptibility and mortality (51).

FN1 is a novel fusion partner of anaplastic lymphoma kinase in inflammatory myofibroblastic tumours (52). *FN1-EGF* gene fusions are recurrent in calcifying aponeurotic fibroma (53). In addition, the *FN1*-fibroblast growth factor receptor 1 genetic fusion is a frequent event in phosphaturic mesenchymal tumours (54). A single nucleotide polymorphism in *FN1* has also been reported to be associated with tumour shape in CRCs (55). In addition, *FN1* may interact with vascular endothelial growth factor A and serve important roles in non-small cell lung cancer (NSCLC), and the corresponding proteins can serve as targets for the diagnosis or treatment of patients with NSCLC (56). The overexpression of *FN1* is also associated with latent membrane protein 1 expression and has an independent prognostic value for nasopharyngeal cancer (57).

VCAN has potential prognostic value in multiple myeloma (58). *VCAN* expression levels are also associated with OS in CRC (59), and it has been identified as a potential biomarker for oral squamous cell carcinoma (60). The present study hypothesized that

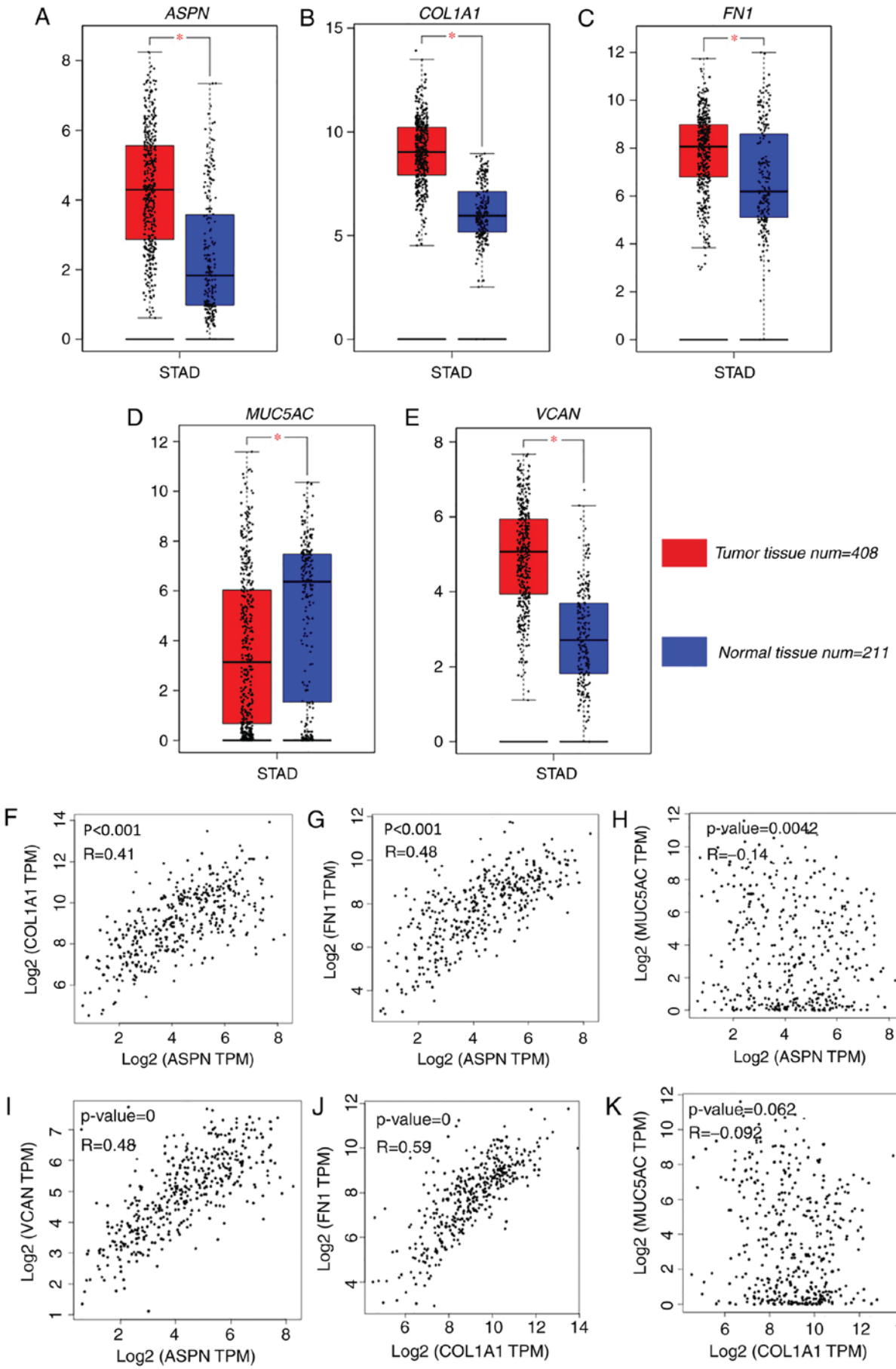


Figure 5. Expression analysis and Pearson correlation analyses of *ASPN*, *COL1A1*, *FN1*, *VCAN* and *MUC5AC* genes. Expression analysis of (A) *ASPN*, (B) *COL1A1*, (C) *FN1*, (D) *MUC5AC* and (E) *VCAN* genes in gastric normal and tumour tissues. (F-K) Pearson correlation analyses of *ASPN*, *COL1A1*, *FN1*, *MUC5AC* and *VCAN* genes.

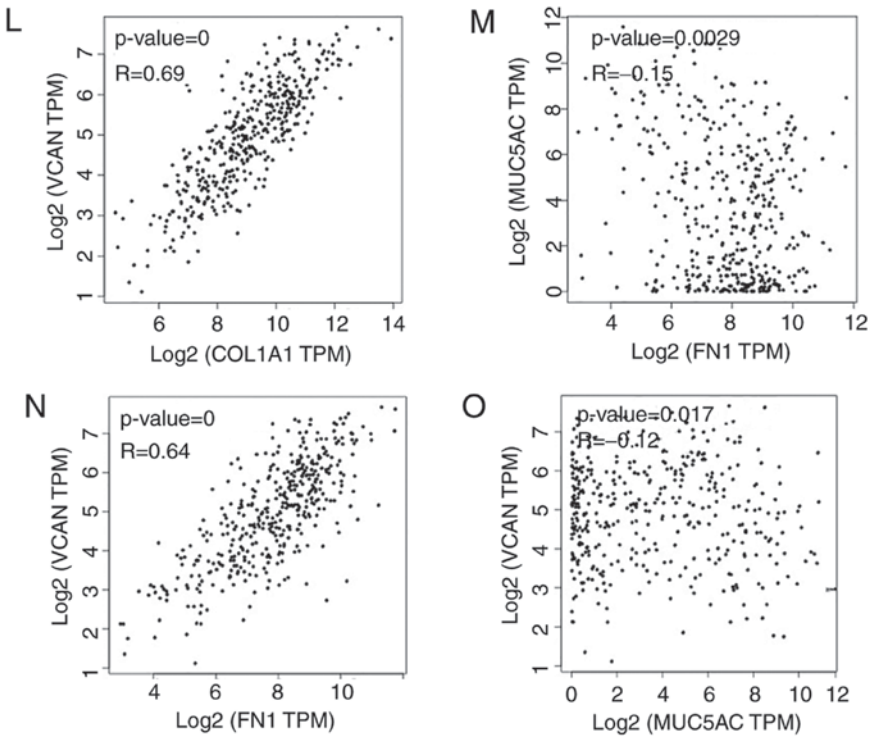


Figure 5. Continued. (I-O) Pearson correlation analyses of *ASPN, COL1A1, FN1, MUC5AC* and *VCAN* genes. *P<0.05. *ASPN*, asporin; *COL1A1*, collagen type I α1 chain; *FN1*, fibronectin 1; *MUC5AC*, mucin 5AC; *THBS2*, thrombospondin 2; *VCAN*, versican; STAD, stomach ademocarcinoma.

VCAN may have an oncogenic role in GC, which is not consistent with a report by Kim *et al* (61), stating that *VCAN* expression predicts a good prognosis for patients with GC.

The present study presented limitations. Firstly, a larger population is required in order to increase the credibility of the present findings. Secondly, other influencing factors, including pathological types, tumour size, tumour numbers and microvascular invasion, should be included, in order to better evaluate the association between DEGs and GC prognosis. Thirdly, a better-designed study focusing on the functional validation of these genes and including more ethnicities is required, combined with a greater number of research centres. Finally, future studies should increase the amount of data obtained from databases, include pathological types of GC and analyze the correlation of clinical stage classification.

In the present study, the plugins cytoHubba and MCODE of Cytoscape were used to obtain hub genes, which may represent predictive biomarkers for GC. Furthermore, DAVID and STRING were used to determine the biological processes and metabolic pathways in which these hub genes were involved. The expression levels and prognostic values of the hub genes were eventually analyzed. The present study aimed to determine potential predictive biomarkers for GC. The results demonstrated that some hub genes possessed some prognostic value for GC. Further studies focusing on the functional validation of these genes are required (via reverse transcription-quantitative polymerase chain reaction and western blotting) and should include a larger number of medical centres and more ethnicities.

In conclusion, the present study identified 117 DEGs in patients with GC and identified 15 hub genes. In addition, some hub genes had prognostic value for patients with GC. The present study suggested that *ASPN*, *COL1A1*, *FN1*, *VCAN* and *MUC5AC* may represent potential prognostic biomarkers for GC. In addition, *ASPN*, *COL1A1*, *FN1* and *VCAN* may serve oncogenic roles in gastric tumours, whereas *MUC5AC* may act as a tumour suppressor. The genes may act via BP and CC domains, and via 'ECM-receptor interaction', 'focal adhesion', 'metabolism of xenobiotics by cytochrome P450' and 'drug metabolism' pathways. In the present study, some hub genes were differentially expressed and had prognostic value for GC. Further studies are required to explore the functional roles of these genes, particularly in the development of metastases and cancer progression, in order to guide clinical direction.

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Availability of data and materials

The datasets generated and/or analyzed during the present study are available in the GEO (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29272) and GEPIA (http://gepia.cancer-pku.cn/index.html) repositories.

Authors' contributions

KJ and QX were responsible for study design. KJ, HL and DX conducted the study and analysed the data. KJ wrote the manuscript and QX guided the writing.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Conflicts of interest

The authors declare they have no competing interests.

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