Celecoxib exhibits an anti-gastric cancer effect by targeting focal adhesion and leukocyte transendothelial migration-associated genes

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Abstract. Gastric cancer (GC) is a prevalent cancer, which remains incurable, and therefore requires an alternative treatment method. Celecoxib is a nonsteroidal anti-inflammatory drug that targets cyclooxygenase-2, and exhibits anticancer effects. The present study aimed to investigate the anti-GC mechanism of celecoxib using bioinformatics methods. Gene expression datasets GSE56807 (GC tissues and normal gastric tissues) and GSE54657 (celecoxib-treated and non-treated human GC epithelial AGS cells) were downloaded from the Gene Expression Omnibus database. Two groups of differentially expressed genes (DEGs) were identified using limma package in R language. The criterion for GSE56807 was a false discovery rate of <0.05, while that for GSE54657 was P<0.01. Overlapping DEGs from the two datasets were screened out. Subsequently, pathway enrichment analysis was performed using Database for Annotation, Visualization and Integrated Discovery software (P<0.1; gene count ≥2). In addition, the protein-protein interactions (PPIs) among the overlapped DEGs were obtained based on IntAct, Database of Interacting Proteins, Biomolecular Interaction Network Database and Human Protein Reference Database. Finally, a PPI network was visualized using Cytoscape software. A total of 137 overlapped DEGs were obtained, and DEGs with opposite regulation directions in the two datasets were significantly enriched in focal adhesion and leukocyte transendothelial migration. Subsequently, a PPI network of overlapped DEGs was constructed. Comprehensively, a total of 8 key DEGs [cysteine and glycine rich protein 1 (*CSRP1*), thrombospondin 1 (*THBS1*), myosin light chain 9 (*MYL9*), filamin A (*FLNA*), actinin alpha 1 (*ACTN1*), vinculin (*VCL*), laminin subunit gamma 2 (*LAMC2*) and claudin 1 (*CLDN1*)] were upregulated in GC tissues and downregulated in celecoxib-treated cells.

In conclusion, celecoxib may exhibit anti-GC effects by suppressing the expression of *CSRP1*, *THBS1*, *MYL9*, *FLNA*, *ACTN1*, *VCL*, *LAMC2* and *CLDN1*, and inhibiting leukocyte transendothelial migration and focal adhesion. However, relevant experiments are required to confirm the conclusion of the present study.

Introduction

Gastric cancer (GC) is the fifth most prevalent cancer and the third leading cause of cancer-associated mortality (1). The prognosis of GC is generally poor, since GC metastasize frequently from gastric glands to other parts of the body. The 5-year survival rate of GC is <10% (2). Therefore, study concerning the treatment of GC is of great importance.

Treatments for GC include surgery, radiation therapy and chemotherapy (3). Drugs used in GC treatment primarily consist of fluorouracil or its analog capecitabine, carmustine, mitomycin C, semustine, doxorubicin, cisplatin and taxotere (3,4). However, the results of these treatments are unsatisfactory, and GC remains incurable; therefore, requiring an alternative treatment method (2).

Celecoxib is a classic nonsteroidal anti-inflammatory drug (NSAID) targeting cyclooxygenase-2 (COX-2). Previous epidemiological studies have demonstrated that prolonged treatment with NSAIDs may reduce the risk of GC (5,6). Reportedly, celecoxib possesses anticancer effects, since it downregulates AKT serine/threonine kinase 1, glycogen synthase kinase 3β and forkhead box O1, and upregulates caspase-9 in the mitochondrial apoptotic pathway (7). In addition, celecoxib regulates cell cycle arrest, mitochondrial cytochrome C release and caspase activation in cancer cells (8). Furthermore, celecoxib suppresses the invasion of GC by affecting the expression of E-cadherin, vascular endothelial growth factor and COX-2, and interfering with nuclear factor-κB signaling, Snail signaling and microvessel density (9,10). However, the anti-GC mechanism of celecoxib remains unclear and requires further study.

The present study aimed to improve the understanding of the anti-GC mechanism of celecoxib using bioinformatics methods. Two gene expression datasets (GSE56807, GC vs. normal gastric tissues; GSE54657, celecoxib-treated vs. non-treated GC cells) were downloaded from the Gene Expression Omnibus (GEO) database. GSE56807 was uploaded by Wang *et al* (11), who investigated

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hypoxia inducible factor-1α-regulated transcription factors and regulatory signaling pathways in GC. The present study identified two sets of differentially expressed genes (DEGs) and their overlapped DEGs from the two datasets. Furthermore, pathway enrichment analysis was performed, and a protein-protein interaction (PPI) network was constructed to predict the targets of celecoxib.

Materials and methods

Microarray data. Two gene expression datasets, GSE56807 (11) and GSE54657 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE54657), were downloaded from the GEO database (www.ncbi.nlm.nih.gov/geo/) (12). Dataset GSE56807 consisted of 10 samples, which included 5 pairs of GC and normal gastric tissues. Its corresponding platform is GPL5175 [HuEx-1_0-st] Affymetrix Human Exon 1.0 ST Array [transcript (gene) version]. Dataset GSE54657 consisted of 6 samples, which included 3 celecoxib-treated human GC epithelial AGS cell line samples and 3 non-treated AGS cell line samples. The celecoxib-treated AGS samples were harvested following incubation with 20 μM celecoxib for 24 h in triplicate. The corresponding platform for GSE54657 is GPL6244 [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version].

Data preprocessing and DEGs screening. The downloaded raw gene expression data were preprocessed based on R language (13). Data in different chips were normalized using the Robust Multichip Averaging algorithm (14). Subsequently, limma version 3.22.1 software (www.bioconductor.org/pack-ages/release/bioc/html/limma.html) (15) in R language was applied to identify DEGs. For DEGs between GC tissues and normal gastric tissues (GSE56807), the P-value was adjusted using the Benjamini-Hochberg method (16). Adjusted P<0.05, also known as false discovery rate (FDR), was set as the criterion for DEG screening. For DEGs between celecoxib-treated AGS samples and non-treated AGS samples (GSE54657), P<0.01 was set as the cut-off criterion. DEGs shared by the two DEG groups were defined as overlapped DEGs, which exhibited the same or opposite regulation directions in the two datasets.

Hierarchical clustering analysis. In order to determine the sample-specificity of the overlapped DEGs, bidirectional hierarchical clustering analysis (BHCA) (17) was performed using pheatmap version 1.0.8 package (https://cran.r-project.org/web/packages/pheatmap/) in R language. DEGs with similar expression patterns were clustered.

Pathway enrichment analysis. Pathway enrichment analysis of the overlapped DEGs was performed using Database for Annotation, Visualization and Integrated Discovery software version 6.7 (david.abcc.ncifcrf.gov/) (18) based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (19). P<0.1 and gene count ≥2 were set as the cut-off criteria.

Construction of a PPI network. Based on the PPI data downloaded from IntAct (www.ebi.ac.uk/intact/) (20), Database of Interacting Proteins (dip.doe-mbi.ucla.edu/dip/Main.cgi) (21), Biomolecular Interaction Network Database (http://bind.ca) (22)
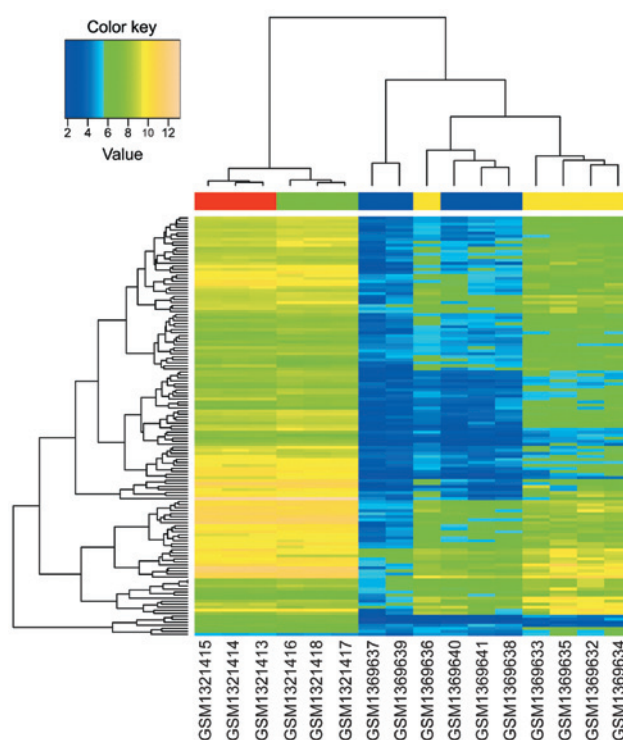

Figure 1. Bidirectional hierarchical clustering analysis of overlapped differently expressed genes. Blue terms, normal gastric tissues; yellow terms, gastric cancer samples; red terms, non-treated human gastric cancer epithelial AGS cell samples; green terms, celecoxib-treated AGS samples.

and Human Protein Reference Database (http://www.hprd.org/) (23), and the studies by Rual *et al* (24), Stelzl *et al* (25) and Ramani *et al* (26), a PPI set was established, which investigated the PPIs among the overlapped DEGs. Subsequently, the PPI network of DEGs was visualized using Cytoscape version 3.2.0 software (www.cytoscape.org/) (27).

Results

DEG screening and BHCA. Based on the aforementioned analysis, 5190 DEGs (FDR <0.05) between GC tissues and normal gastric tissues, and 540 DEGs (P<0.01) between celecoxib-treated AGS samples and non-treated AGS samples were identified. In total, 137 overlapped DEGs were obtained by investigating the intersection of the two DEG groups. Furthermore, BHCA was performed, and the genes with similar expression patterns are shown in Fig. 1. The two datasets used in the present study were based on different platforms; therefore, gene expression levels exhibited a large difference between the two datasets. However, qualitative conclusions were obtained from Fig. 1: The overlapped DEGs could differentiate between GC and normal gastric tissues, and between celecoxib-treated and non-treated AGS samples; and celecoxib did affect the gene expression patterns of AGS cells during treatment.

Pathway enrichment analysis. To investigate the mechanism underlying the effects of celecoxib on GC cells, KEGG pathway enrichment analysis of the 137 overlapped DEGs was performed. As shown in Table I, the overlapped DEGs were primarily enriched in four pathways (P<0.1; gene

Table I. Significantly enriched pathways in overlapped DEGs between two datasets used by the present study.

Pathway ID	Pathway	Gene count	DEGs	RD	P-value
hsa04142	Lysosome	6	*NEU1, GLB1, FUCA1, CLN5, ATP6AP1, CTSD*	Same	0.002834
hsa00511	Other glycan degradation	3	*NEU1, GLB1, FUCA1*	Same	0.007776
hsa04510	Focal adhesion	6	*THBS1, MYL9, FLNA, ACTN1, VCL, LAMC2*	Opposite	0.026095
hsa04670	Leukocyte transendothelial migration	4	*MYL9, VCL, ACTN1, CLDN1*	Opposite	0.076932

Datasets, GSE56807 and GSE54657. DEGs, differently expressed genes; RD, regulation direction of the two datasets used; NEU1, neuraminidase 1; GLB1, galactosidase beta 1; FUCA1, fucosidase, alpha-L- 1; CLN5, ceroid-lipofuscinosis, neuronal 5; ATP6AP1, ATPase H+ transporting accessory protein 1; CTSD, cathepsin D; THBS1, thrombospondin 1; MYL9, myosin light chain 9; FLNA, filamin A; ACTN1, actinin alpha 1; VCL, vinculin; LAMC2, laminin subunit gamma 2; CLDN1, claudin 1.
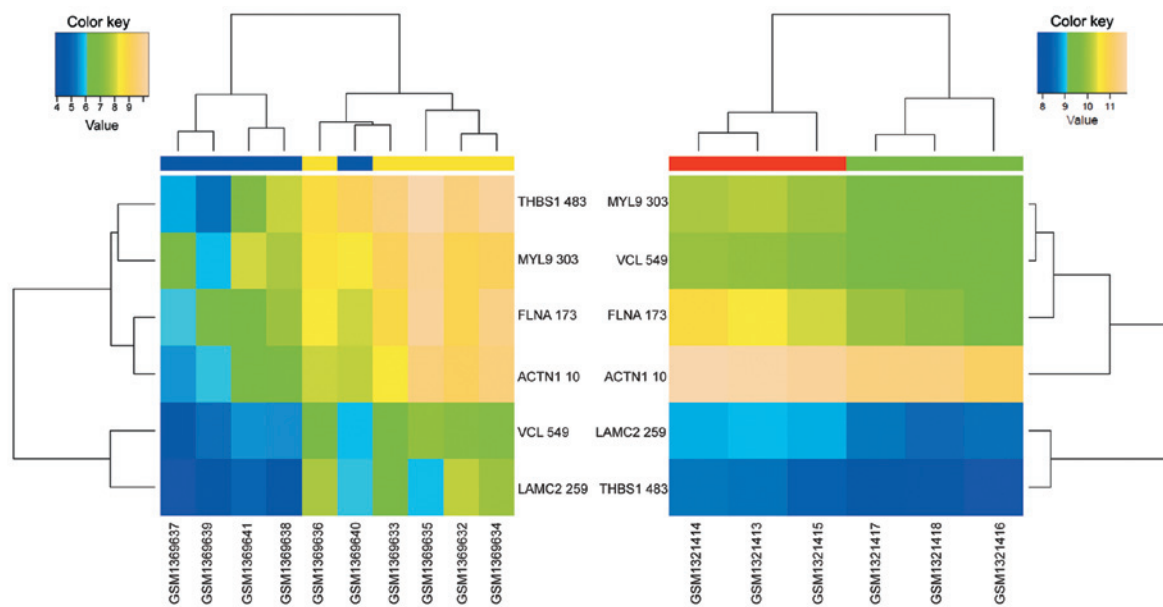

Figure 2. Bidirectional hierarchical clustering analysis of differently expressed genes enriched in focal adhesion pathways. Blue terms, normal gastric tissues; yellow terms, gastric cancer samples; red terms, non-treated human gastric cancer epithelial AGS cell samples; green terms, celecoxib-treated AGS samples.

count ≥2), including lysosome, other glycan degradation, focal adhesion and leukocyte transendothelial migration pathways. Among these pathways, focal adhesion (Fig. 2) and leukocyte transendothelial migration (Fig. 3) were enriched by DEGs that exhibited opposite regulation directions in the two datasets. In fact, 6 DEGs enriched in focal adhesion [thrombospondin 1 (*THBS1*), myosin light chain 9 (*MYL9*), filamin A (*FLNA*), actinin alpha 1 (*ACTN1*), vinculin (*VCL*), laminin subunit gamma 2 (*LAMC2*)] and 4 DEGs enriched in leukocyte transendothelial migration [*MYL9*, *VCL*, *ACTN1* and claudin 1 (*CLDN1*)] were significantly upregulated in GC tissues compared with normal gastric tissues, and significantly downregulated in celecoxib-treated AGS cells compared with non-treated AGS cells.

Construction of PPI network. To further investigate the underlying mechanism of celecoxib in GC treatment, a PPI network of the 137 overlapped DEGs was constructed, consisting of 8 DEGs and 5 PPIs (Fig. 4). Cysteine and glycine

rich protein 1 (*CSRP1*), *VCL* and *ACTN1* exhibited opposite regulation directions in the two datasets. In fact, *CSRP1*, *VCL* and *ACTN1* were significantly upregulated in GC tissues compared with normal gastric tissues, while significantly downregulated in celecoxib-treated AGS cells compared with non-treated AGS cells. Comprehensively, a total of 8 key DEGs (*CSRP1*, *THBS1*, *MYL9*, *FLNA*, *ACTN1*, *VCL*, *LAMC2* and *CLDN1*) were upregulated in GC tissues and downregulated in celecoxib-treated cells.

Discussion

GC is a prevalent and malignant cancer, which remains incurable. As a NSAID, celecoxib is a potentially effective chemotherapy for GC. However, the anti-GC mechanism of celecoxib remains unclear. To gain insight into the anti-GC mechanism of celecoxib, the present study systematically analyzed the gene expression data of gastric tissue and human gastric cancer epithelial AGS cells using bioinformatics
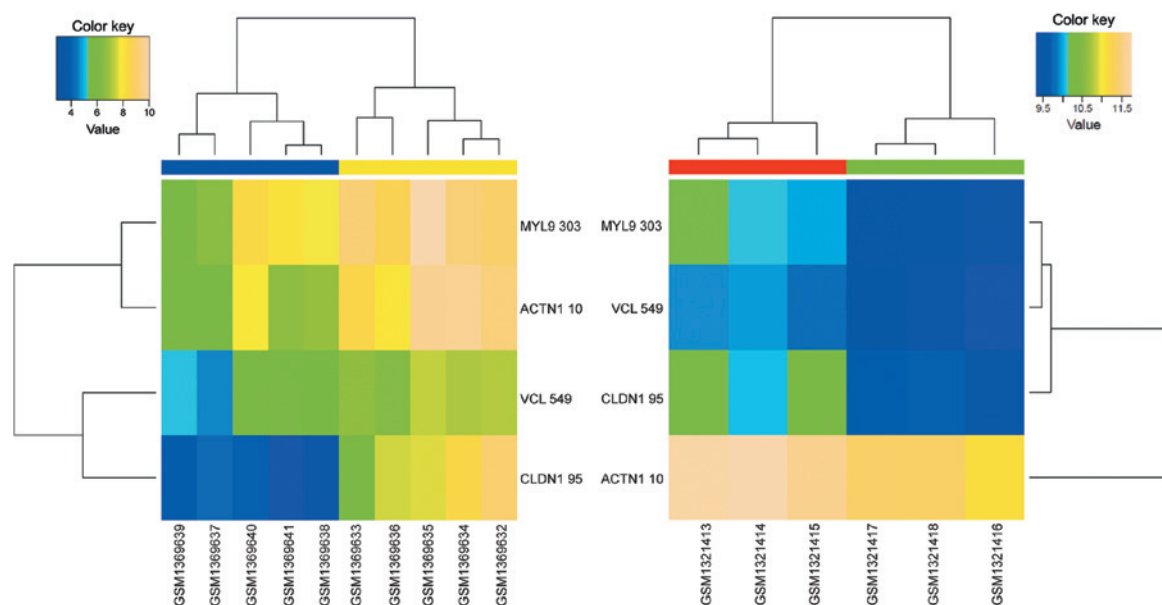
Figure 3. Bidirectional hierarchical clustering analysis of differently expressed genes enriched in leukocyte transendothelial migration pathways. Blue terms, normal gastric tissues; yellow terms, gastric cancer samples; red terms, non-treated human gastric cancer epithelial AGS cell samples; green terms, celecoxib-treated AGS samples.

methods. Consequently, a total of 137 genes were identified to be differentially expressed between GC and normal gastric tissues, and between celecoxib-treated and non-treated AGS cells. Following pathway enrichment analysis and PPI network construction, 8 key DEGs (*CSRP1*, *THBS1*, *MYL9*, *FLNA*, *ACTN1*, *VCL*, *LAMC2* and *CLDN1*) were identified, which were upregulated in GC tissues and downregulated in celecoxib-treated cells, and enriched in focal adhesion and leukocyte transendothelial migration pathways.

Among the 8 DEGs, *THBS1*, *MYL9*, *FLNA*, *LAMC2* and *CLDN1* have been reported to participate in the development of GC, as follows: *THBS1* encodes thrombospondin-1, and its expression is a prognostic factor in advanced GC (28); *MYL9* encodes myosin light chain 9, which is an upregulated apoptosis-associated protein and biomarker in GC (29); *FLNA* encodes filamin A, which is aberrantly regulated in GC tissue and regulates migration and invasion of GC cells *in vitro* (30); *LAMC2* encodes the γ2 chain of laminin-5, which is a major component of the basement membrane, and the expression of *LAMC2* is frequently upregulated by promoter demethylation in GC (31); *CLDN1* encodes claudin 1, a tight junction protein that is critical in the maintenance of epithelial integrity. The overexpression of *CLDN1* has been identified in GC patients with lymph node metastasis, and is associated with decreased overall survival (32). In the present study, *THBS1*, *MYL9*, *FLNA*, *LAMC2* and *CLDN1* were aberrantly upregulated in GC tissue. This is consistent with previous studies, particularly studies concerning *MYL9*, *LAMC2* and *CLDN1* (29,31,32). In addition, these genes were significantly downregulated in celecoxib-treated cells, indicating that celecoxib may exhibit an anti-GC effect by targeting these genes.

Following PPI network construction by the present study, it was revealed that *ACTN1* interacted with *CSRP1* and *VCL*. *VCL* encodes vinculin, an important focal
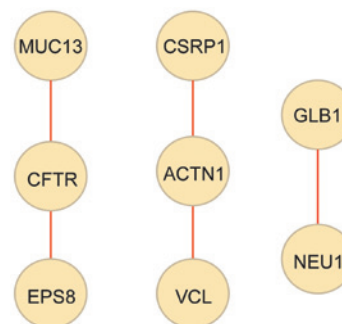

Figure 4. Protein-protein interaction network of the overlapped DEGs. Yellow nodes, DEGs (proteins); red lines, interactions between DEGs (proteins). DEGs, differently expressed genes; MUC13, mucin 13, cell surface associated; CSRP1, cysteine and glycine rich protein 1; GLB1, galactosidase beta 1; CFTR, cystic fibrosis transmembrane conductance regulator; ACTN1, actinin alpha 1; NEU1, neuraminidase 1; EPS8, epidermal growth factor receptor pathway substrate 8; VCL, vinculin.

adhesion protein that is responsible for cell-matrix junction and signal transduction on the membrane. Vinculin forms a vinculin-talin-actin scaffolding complex and promotes the malignancy and invasiveness of various cancers, including breast (33), pancreatic (34) and prostate (35) cancer. Additionally, the dephosphorylation of vinculin in gastric epithelial cells results in altered cell-matrix adhesion, contractility, motility and wound repair (36). In the present study, the gene expression value of *VCL* was significantly upregulated in GC tissues [$\log_2$ fold change (FC)=1.59; FDR=0.0043], indicating that *VCL* may participate in GC progression by promoting tumor malignancy and invasiveness.

ACTN1 encodes actinin α1, which triggers the unmasking of vinculin, allowing for F-actin binding and vinculin activation. In this way, the transmission of extracellular or intracellular forces and integrin-mediated mechano-chemical signaling are enabled (37). In the present study, the gene

expression value of *ACTN1* was significantly upregulated in GC tissues (log$_2$FC=2.43; FDR=0.0044), indicating that ACTN1 may participate in GC progression by activating VCL and promoting tumor malignancy and invasiveness.

CSRP1 is a member of the *CSRP* family, which encodes a group of proteins with LIM (Lin11, Isl-1 and Mec-3) domains. CSRPs are generally transcription regulators associated with gene regulation, cell growth and somatic differentiation (38). In the present study, *CSRP1* was upregulated in GC tissues (log$_2$FC=2.88; FDR=0.0061), and interacted with *ACTN1*, indicating that the upregulation of *CSRP1* may promote GC progression by regulating the expression of *ACTN1*. It should be noted that *CSRP1* (log$_2$FC=-0.53; P=0.0002), *VCL* (log$_2$FC=-0.48; P=0.0011) and *ACTN1* (log$_2$FC=-0.39; P=0.0058) were significantly downregulated in celecoxib-treated AGS cells, indicating that celecoxib may exhibit an anti-GC effect by targeting *CSRP1*, *VCL* and *ACTN1*.

Furthermore, *THBS1*, *MYL9*, *FLNA*, *ACTN1*, *VCL* and *LAMC2* were identified to be significantly enriched in focal adhesion pathways by the present study, while *MYL9*, *VCL*, *ACTN1*, and *CLDN1* were significantly enriched in leukocyte transendothelial migration pathways. Focal adhesion is essential in various important biological processes, including cell survival and apoptosis (39), and leukocyte transendothelial migration is generally activated in cancer progression, which hampers the anti-tumour responses of the host (40). In the present study, the DEGs enriched in focal adhesion and leukocyte transendothelial migration were significantly upregulated in GC tissues and downregulated in celecoxib-treated AGS cells, suggesting that these pathways were activated in GC progression, and celecoxib may exhibit an anti-GC effect by suppressing these pathways.

Overall, the present study proposes that celecoxib may exhibit an anti-GC effect by suppressing the expression of *CSRP1*, *THBS1*, *MYL9*, *FLNA*, *ACTN1*, *VCL*, *LAMC2* and *CLDN1*, and inhibiting leukocyte transendothelial migration and focal adhesion. Although limitations exist in the present study, including the small sample size and lack of validation, the predictions proposed, based on bioinformatics analysis, provide novel directions for the understanding of the anti-GC mechanism of celecoxib. Future studies by the present authors may focus on enlarging the sample size and validating the conclusions *in vitro* and *in vivo*.

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