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ORIGINAL RESEARCH

Analysis of Staged Features of Gastritis-Cancer Transformation and Identification of Potential Biomarkers in Gastric Cancer

Ruikang Jia[1,2,*], Xiaohui Guo[3,*], Huiyun Liu[2], Feiyue Zhao[2], Zhibin Fan[2], Menglei Wang[2], Jianliang Sui[1,2], Binghua Yin[3], Zhihong Wang[4], Zhen Wang[1,5]

[1]The Affiliated Hospital and the Medical College, Hebei University of Engineering, Handan, Hebei Province, People's Republic of China; [2]Key Laboratory of Chinese Medicine for Gastric Medicine, Hebei Province, Handan Pharmaceutical Co. LTD, Handan, People's Republic of China; [3]Handan Central Hospital, Handan, Hebei Province, People's Republic of China; [4]People's Hospital of Huangzhou District, Huanggang City, People's Republic of China; [5]Key Laboratory of Metabolism and Molecular Medicine, Ministry of Education, and Department of Biochemistry and Molecular Biology, Fudan University Shanghai Medical College, Shanghai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhen Wang, The Affiliated Hospital and the Medical College, Hebei University of Engineering, Handan, Hebei Province, People's Republic of China, Tel +8619903200632, Email wangzhen@hebeu.edu.cn

Purpose: This work aims to elucidate the staged characteristics during gastritis-cancer transformation based on the transcriptome and use bioinformatics to identify potential biomarkers.

Patients and Methods: We collected blood samples from healthy controls, patients with non-atrophic gastritis, atrophic gastritis, and gastric cancer, and tissue samples from patients with gastric cancer, respectively. RNA-seq was then performed. Differentially expressed genes, weighted gene co-expression network analysis and functional enrichment analysis were used to illustrate the staged characteristics of gastritis-cancer transformation. Genes with diagnostic potential were further identified in combination with ROC analysis. Additionally, for the gastric cancer stage, the gene expression of the collected tissue transcriptome was validated using the Cancer Genome Atlas and combined with survival analysis to identify potential biomarkers.

Results: The 279 overlapping genes among the differentially expressed genes of NAG, AG and CA indicated that the expression characteristics of different stages were different. However, the 2243 overlapping genes of differential genes between adjacent stages indicated a certain consistency in the expression characteristics of stage development. The core functions of different stages have strong stage specificity and basically have no similarities. Twenty genes with diagnostic potential for AG or CA were obtained, respectively, and no gene could effectively differentiate NAG samples. Thirty-four potential biomarkers for gastric cancer were identified, of which 14 genes have not been reported, including ACTG2, C1QTNF2, NCAPH and SORCS1.

Conclusion: There may be a stable development mechanism in the process of gastritis-carcinoma transformation, resulting in the differences in the performance of each stage. The newly discovered staging features and potential biomarkers in this work can provide references for related research.

Keywords: gastritis-cancer transformation, transcriptome, bioinformatics, biomarker

Introduction

Chronic inflammatory disease has been considered as an important cause of death in the world today, and its long-term existence is inseparable from the occurrence and development of cancer.[1–3] According to pathological and epidemiological studies, the gastritis-cancer transformation is divided into the following successive stages: chronic non-atrophic gastritis (CNAG), chronic atrophic gastritis (CAG) with or without intestinal metaplasia (IM) and dysplasia.[4] CAG, a stage before gastric cancer, carries the risk of developing gastric cancer (GC) if left uncontrolled.[5] Currently, endoscopy and histopathological biopsy are mainly used for the clinical diagnosis of atrophic gastritis (AG) and GC, which is an

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invasive diagnostic method, and the main treatment methods for gastric cancer include surgery, chemotherapy, radiotherapy and targeted therapy.[6,7]

Early detection of GC risk groups is critical for improving the survival rate of GC, and to address the large at-risk population, non-invasive tests have been used to predict precancerous conditions to minimize the need for invasive testing.[8] Pepsinogen, pepsinogen I/II ratio, and gastrin serum levels have been used to screen high-risk subjects for gastric precancerous lesions, and a meta-analysis showed their diagnostic value.[9] Compared with traditional GC biomarkers such as CEA, CA19-9 and CA72-4, the biomarker panel composed of PGI, PGII, PGI/PGII, G-17 and IGg has great potential for screening high-risk GC populations.[10] At the same time, various diagnostic biomarkers such as plasma proteins, miRNAs, lncRNAs, and circRNAs have shown good sensitivity and specificity in the research cohort, but have not been effectively extended to clinical applications.[11–14] Based on the molecular characteristics of gastric cancer, different targeted therapy strategies can be selected, such as ramucirumab, trastuzumab and dovitinib targeting VEGFR2, HER2, and FGFR-2, respectively.[15–17] However, patients with the same subtype of GC failed to achieve the expected curative effect due to tumor heterogeneity and drug resistance.[18] Therefore, understanding the gastritis-cancer transformation and mining potential biomarkers are of great significance for the diagnosis and treatment of GC.

Due to the limitation of tissue samples at various stages of gastritis, most of the existing biomarker screening work focuses on GC.[19–21] Several recent studies have shown that whole blood, as a non-invasive biomaterial, has great advantages in the field of liquid biopsy, and its transcriptome can also be applied in inflammatory disease analysis and biomarker screening.[22,23] Therefore, this work used blood and tissue samples for transcriptome sequencing, combined with bioinformatics methods, to analyze the expression characteristics of gastritis-cancer transformation process, reveal the pathway functions involved, and screen for staged biomarkers, which can provide reference for diagnosis and treatment-related research.

Materials and Methods

Sample Processing

The status of the healthy control group was examined by professional physicians through gastroscopy. Patients were diagnosed with gastroscopy and histopathological examination by professional physicians. Whole blood samples were obtained from healthy controls (HC) and patients with non-atrophic gastritis (NAG), AG, and GC. Cancerous (CA) and paracancerous (PA) tissues were obtained from GC patients. The obtained whole blood sample was quickly mixed with trizol reagent and left to stand for 5 minutes. Ex vivo tissue samples were cut on ice and sub-packed into containers. After the above treatment, whole blood samples and tissue samples were snap-frozen in liquid nitrogen and then stored at −80°C. This study was conducted with the approval of the Ethics Committee of the Affiliated Hospital of Hebei Engineering University (AF/SC-08/02.0), and complies with the Declaration of Helsinki. Sampled informed consent was provided to all participants. A total of 185 samples were obtained (Supplementary File 1).

RNA-Sequencing and Data Preprocessing

Total RNA was extracted from the samples using a Trizol-based method and quality-controlled by an Agilent 2100 bioanalyzer instrument. The DNA library was constructed using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® according to the instructions. The transcriptome data of the samples were obtained based on the Illumina NovaSeq 6000 platform.

TCGA Data and Preprocessing

Gene expression data and clinical data of GC patients were downloaded from the Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov). Samples with missing clinical information were removed from the dataset. A total of 427 samples were obtained, including 392 CA tissue samples and 35 PA tissue samples.

Data Analysis

The DESeq2[24] package was used to analyze gene expression differences. The log fold change (LFC) of genes was calculated, and a threshold ($|LFC| > 1$, $P < 0.05$) was set to screen out differentially expressed genes (DEGs).

The WGCNA[25] package was used to perform weighted gene co-expression network analysis (WGCNA). Co-expression networks and modules were obtained. The genes at the core position in the co-expression module were screened out by calculating the correlation between the gene and the module vector (MM) and setting a threshold ($|MM| > 0.8$, $P < 0.05$).

The ClusterProfiler[26] package was used to implement the functional enrichment analysis. The KEGG[27] database is used to reveal the related pathways involved in genes, and the GO[28] database describes the functions of genes from three perspectives: biological process (BP), cellular component (CC), and molecular function (MF).

The pROC[29] package was used to draw receiver operator characteristic curve (ROC curve) to measure the ability of gene expression to discriminate between different types of samples. Genes with better discriminative power were selected by setting a threshold ($AUC \geq 0.85$).

The Survival (https://CRAN.R-project.org/package=survival) package was used to perform survival analysis. GC patients were divided into high expression group and low expression group according to gene expression. The survival curve was drawn, and the significance of the difference was tested.

The UpSetR[30] and VennDiagram[31] packages were used to analyze the overlap between gene sets. All data-related work in this work is based on the R language (https://www.r-project.org/).

Results

Features of Gene Expression Changes in Gastritis-Cancer Transformation

Whole blood samples at different stages of gastritis-cancer transformation were compared with HC, and 2984, 1036, and 3011 DEGs were obtained from NAG, AG, and CA, respectively (Figure 1A, Supplementary File 2). Further analysis revealed that there were only 278 overlapping genes in DEGs at different stages, of which only 8 genes showed consistent up/down-regulation trends (Figure 1B). This suggests that the expression status of different stages is not consistent, and each stage may have unique parts. Based on this finding, differential gene expression analysis between any two stages was performed to screen out DEGs that were up-regulated or down-regulated relative to any other stage, and finally 394, 952 and 787 stage-specific DEGs were obtained from NAG, AG and CA, respectively (Figure 1C–E, Supplementary File 2).

At the same time, an attempt was made to perform differential analysis of gene expression between adjacent stages to characterize the development process of gastritis-cancer transformation. 2984, 5686 and 5658 DEGs were obtained, respectively, and a gene set consisting of 2241 DEGs was unexpectedly found in the subsequent gene overlap analysis (Supplementary Figure 1, Supplementary File 2). These genes maintained significant expression changes as stages progressed, suggesting their close association with gastritis-cancer transformation.

Staged Functional Features of Gastritis-Cancer Transformation

WGCNA was performed to characterize interactions between genes and reveal functions from a polygenic perspective. Through outlier removal, soft threshold selection, dynamic shearing, and module merging, 1, 10, and 4 co-expression modules were obtained from NAG, AG, and CA, respectively (Figure 2A, Supplementary Figure 2). Based on the co-expression modules, MM was calculated, and 7176, 3256 and 4434 genes with high correlation with the module vector were screened from NAG, AG and CA, respectively (Figure 2B, Supplementary File 3). The overlap with the staged DEGs obtained above was considered as the core gene for each stage (Figure 2C, Supplementary File 3). The expression of the core genes is stage-specific and occupies a central position in the co-expression module, and its expression changes have a greater impact on the module, which can reflect staged functional characteristics.

Based on KEGG and GO, functional enrichment analysis of core genes in different stages of gastritis-cancer transformation was performed. NAG is mainly involved in biological processes such as lymphocyte differentiation and nuclear transport (Figure 3A). The corresponding products are mainly located in the nuclear membrane and fibrillar

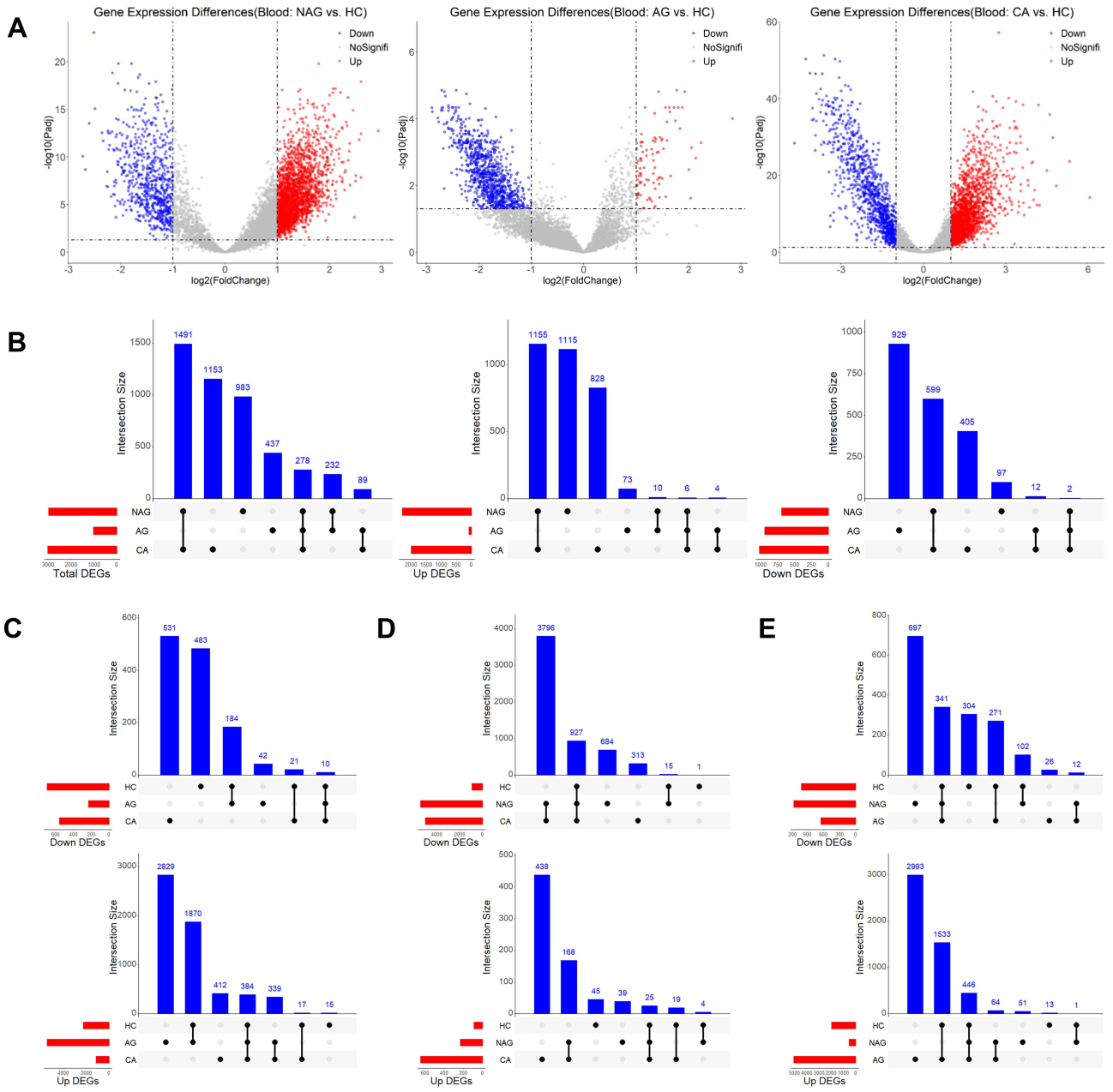

Figure 1 Gene expression changes in gastritis-cancer transformation. (**A**) DEGs obtained by comparing NAG, AG and CA with HC, respectively. (**B**) Overlap analysis between DEGs from different stages. (**C**) Staged DEGs in NAG. (**D**) Staged DEGs in NAG. (**E**) Staged DEGs in CA.

center (Figure 3B) and perform the functions of snoRNA binding and structural constituent of nuclear pore (Figure 3C). Nucleocytoplasmic transport and aldosterone synthesis and secretion pathways were found to be significantly enriched (Figure 3D). AG is mainly involved in mRNA processing-related biological processes. The corresponding products are mainly located in nuclear speck and focal adhesion and perform functions such as GTP binding and ATP hydrolysis activity. Pathways such as PD-L1 expression and PD-1 checkpoint were found to be significantly enriched (Supplementary Figure 3A). CA mainly involves regulation of actin filament polymerization and superoxide anion generation biological process. The corresponding products were mainly located in secretory granule lumen and cytoplasmic vesicle lumen. Diabetic cardiomyopathy and leukocyte transendothelial migration pathways were found to be significantly enriched (Supplementary Figure 3B). These results reveal a staged functional change. See appendix for details (Supplementary File 4).

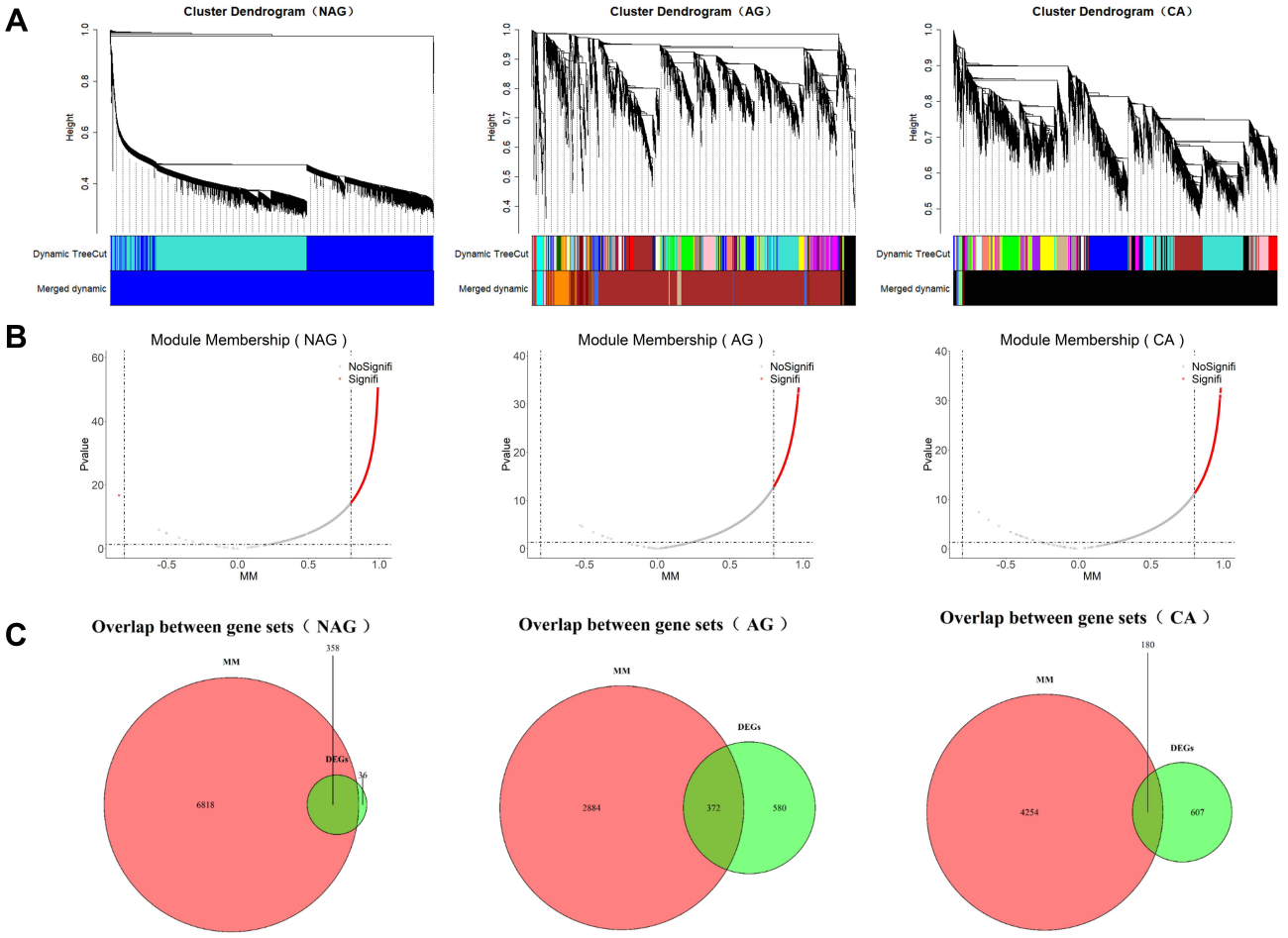

Figure 2 Screening of core genes at each stage. (**A**) Construction of weighted gene co-expression network and division of co-expression modules. (**B**) Screen for genes at core positions in co-expression modules. (**C**) The intersection of staged DEGs and genes at core positions was regarded as core genes.

Identification of Genes with Diagnostic Potential in Gastritis-Cancer Transformation

ROC was used to obtain genes that could effectively identify samples of specific stages. The samples of a certain stage were regarded as positive examples, the remaining samples were regarded as negative examples, and the ROC curve was drawn (Figure 4A–C). The results showed that 351 genes could effectively distinguish AG samples, 151 genes could effectively distinguish CA samples, and no genes were found that could effectively distinguish NAG samples (Supplementary File 5). The top 20 genes with AUC values were selected for display. The results showed that the expression level of top 20 gene in AG was lower than that of other stages (Figure 4D), while the expression level of Top 20 gene in CA was higher than that of other stages (Figure 4E). The expression differences of these genes are stage-specific and can effectively distinguish the corresponding samples and were identified as genes with diagnostic potential.

Expression and Functional Features of GC Revealed in Tissues

The expression and functional features of GC were also analyzed using the tissue transcriptome. CA tissue samples were compared with the PA tissue samples, and 765 DEGs were obtained (Figure 5A, Supplementary File 6). By constructing a weighted gene co-expression network and dynamic shearing, 13 co-expression modules were obtained (Figure 5B). By calculating the MM between the gene and its module, 6785 genes located at the core of the co-expression module were screened out (Figure 5C, Supplementary File 6), and 269 core genes were obtained by intersecting them with the DEGs (Figure 5D, Supplementary File 6). Details of core gene mining can be found in Supplementary File 6. Functional enrichment analysis based on GO and KEGG showed that core genes were mainly involved in biological processes related to cell division, such as nuclear division, organelle division and chromosome segregation (Figure 6A). These core

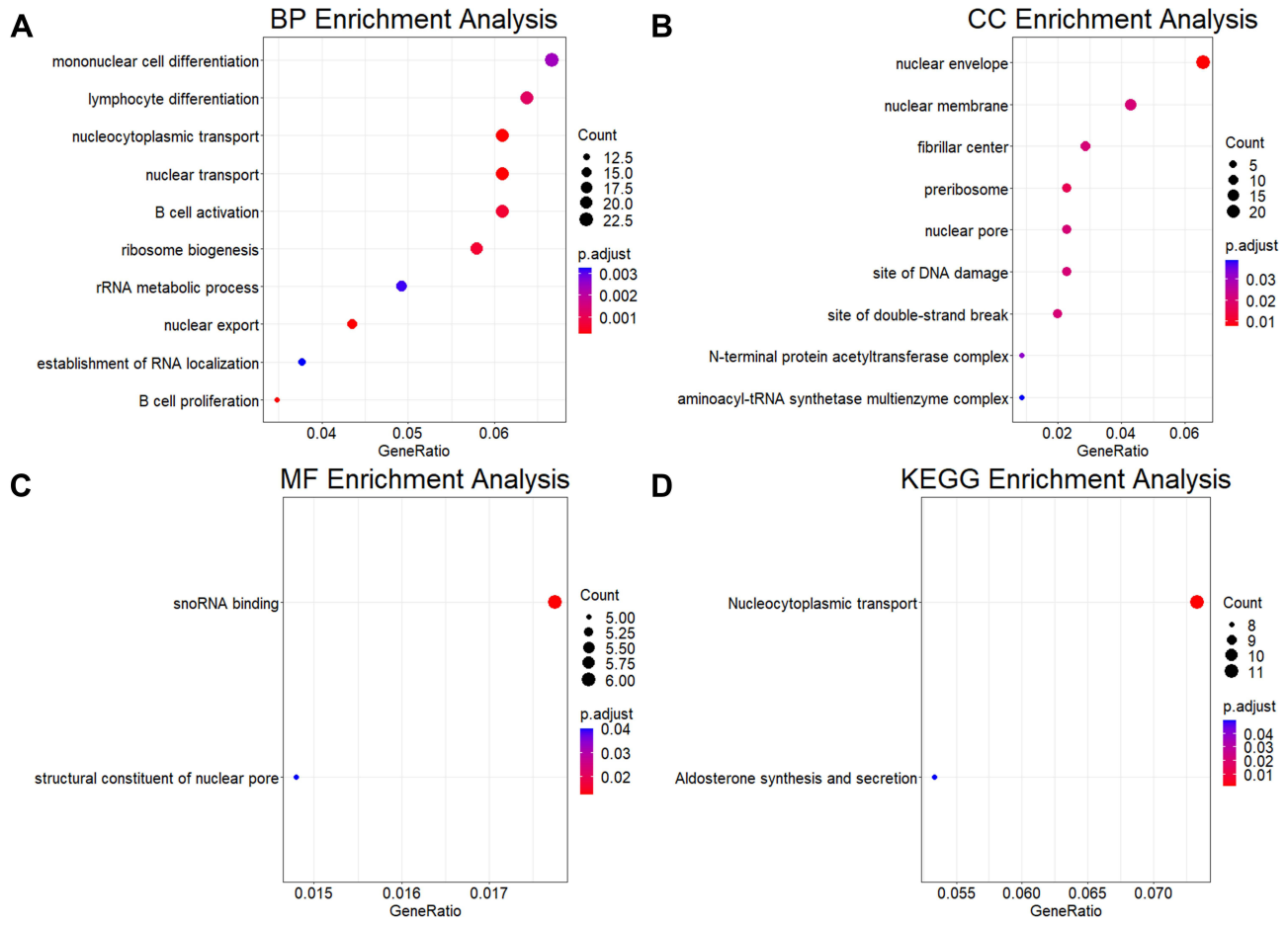

Figure 3 Functional enrichment analysis of the core genes of NAG. (**A**–**C**) Functional description of the core genes of NAG in BP, CC, and MF. (**D**) Pathways involved in core genes of NAG.

genes are mainly located in chromosomal regions and spindle microtubules and mainly function as histone kinase activity and inward rectifier potassium channel activity (Figure 6B and C). The pathways involved are gastric acid secretion and protein digestion and absorption (Figure 6D). See appendix for details (Supplementary File 7). These results also indicate that the normal cell cycle behavior of gastric cancer tissue has been difficult to maintain, and the function of nutrient absorption has been severely affected.

Identification of Potential Biomarkers for GC

Further, the identification of potential biomarkers of gastric cancer was carried out in combination with the GC tissue transcriptome dataset in TCGA. By comparing CA tissue samples with PA tissue samples, 4709 DEGs were obtained (Figure 7A). The expression changes of the core genes obtained above were verified using these differential genes, and 70 up-regulated genes and 79 down-regulated genes with consistent changes in the two datasets were obtained, respectively (Figure 7B). Subsequently, these 149 genes were subjected to survival analysis using the clinical information of TCGA, from which 34 genes significantly correlated with patient survival were obtained (Figure 7C). See appendix for details (Supplementary File 8). These 34 genes were identified as potential biomarkers for gastric cancer based on the following characteristics: gene expression changes were significant and consistent in both datasets; genes were at the core of co-expression modules; gene expression was significantly associated with gastric cancer patient survival. Nine of these genes were up-regulated in CA, and 25 genes were down-regulated in CA (Figure 7D and E). These biomarkers can provide reference for gastric cancer-related research.

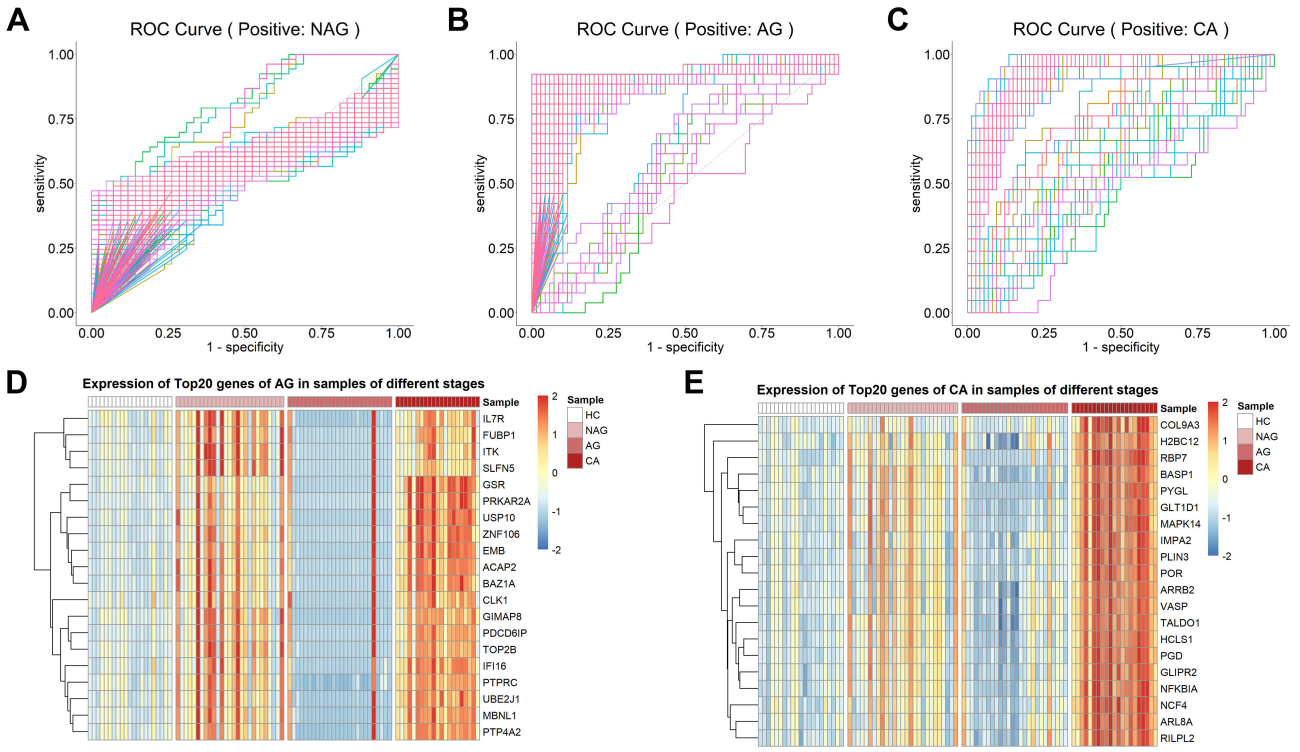

Figure 4 Screening of genes with diagnostic potential. (**A**–**C**) The discriminative ability of genes in NAG, AG and CA for corresponding stage samples was analyzed by ROC curve. (**D** and **E**) The expression of the top 20 genes ranked by AUC value in AG and CA.

Discussion

Gastric cancer is a very common disease worldwide, which seriously threatens human health.[32] The cure rate of early gastric cancer is much higher than that of advanced gastric cancer, so early diagnosis of gastric cancer is very important.[9,10] The gastritis-cancer transformation model expands the perspective of gastric cancer prevention to the gastritis stage. Biomarkers still need to be identified to address the heterogeneity of gastric cancer as treatment moves into the individualized phase.[18,33,34] Compared with tissue samples, blood samples have the natural advantage of being easy to obtain and can be used for diagnosis and testing.[35,36] Therefore, this study used the whole blood transcriptome to dissect the developmental characteristics of gastritis-cancer transformation and screen for genes with diagnostic potential. At the same time, the tissue transcriptome was used to enrich the analysis of gastric cancer stage and identify potential gastric cancer biomarkers.

Staged analysis of the gastritis-cancer transformation process provides some new insights. We first compared the samples of each stage of gastritis-cancer transformation with healthy controls to describe the "static characteristics" exhibited by each stage. Few overlapping DEGs were found for the various stages of gastric cancer-carcinogenesis, suggesting specificity between stages. We then compared the samples of each stage of gastritis-cancer transformation with the samples of the previous stage to describe the "dynamic characteristics" of the development of the stage. A total of 2241 overlapping DEGs were found during gastritis-cancer transformation, indicating a coherence in stage development. We surveyed related work and found that its analysis and discussion only involve "static characteristics", so we believe that this part of the work can provide richer reference information. Based on "static characteristics", we obtained genes with diagnostic potential that can effectively differentiate GC and even AG. For "dynamic characteristics" in gastritis-cancer transformation, whether the expression changes of these 2241 overlapping DEGs promote stage development or just follow stage development is a question worthy of exploration in follow-up research. Compared with similar work, we absorbed the information on stage development and successfully obtained genes with diagnostic potential for AG and GC, respectively, and these genes were more staged in the gastritis-cancer transformation process and were less affected by adjacent types of samples. The

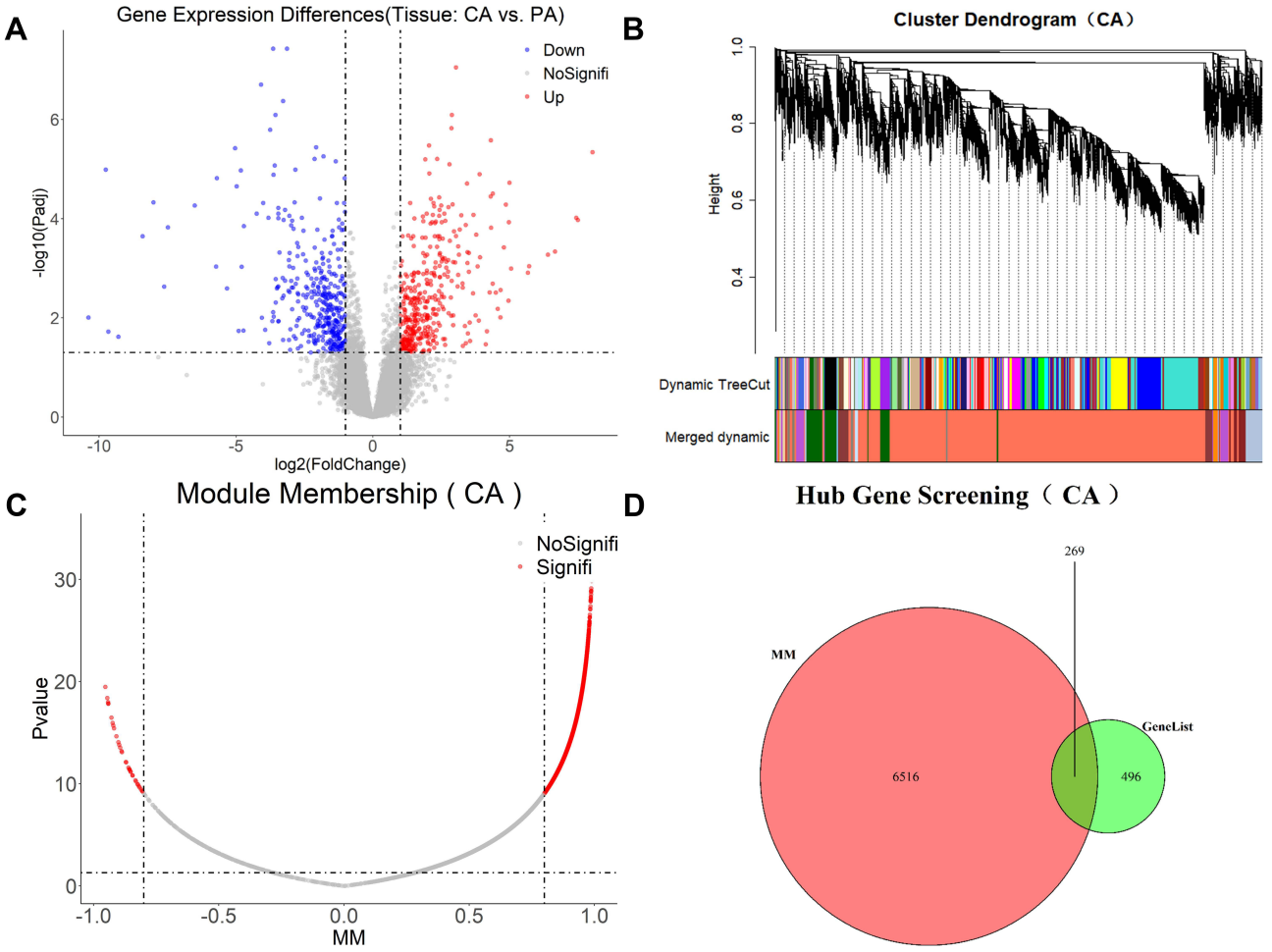

Figure 5 Gene expression features and core gene mining in GC tissues. (**A**) DEGs obtained by comparing CA with PA. (**B**) Construction of weighted gene co-expression network and division of co-expression modules. (**C**) Screen for genes at core positions in co-expression modules. (**D**) The intersection of DEGs and genes at core positions was regarded as core genes.

treatment and control of AG is far less difficult than that of GC.[37,38] The detection and treatment of patients with AG is beneficial to reduce the incidence of GC.[5,7,39] Therefore, these results can provide reference for the related research on the diagnosis and detection of AG or GC.

In the functional analysis, we found that the functional changes of differentiation, activation and proliferation of various immune cells mainly occur in NAG, while the functional changes related to immune cell migration and secretion mainly occur in CA. These results suggest that although the immune response is critical in gastritis, it may focus on different functions during different stages of gastritis development. At the same time, aldosterone secretion in NAG changes, and previous studies have shown that aldosterone can activate various innate and adaptive immune cells and stimulate pro-inflammatory transcription factors, adhesion molecules, inflammatory cytokines and chemokines through the activation of mineralocorticoid receptors.[40] This is consistent with the above-mentioned characteristics of the biological process, and also suggests that aldosterone may also play a role as a key molecule in NAG. When it comes to AG, the T-cell receptor pathway and the PD-1 checkpoint pathway in cancer are significantly changed, suggesting a potential cancer risk, which is consistent with the general view of current research.[41,42] Furthermore, it was observed that the defense response to symbiont changed only at this stage, most likely caused by H. pylori infection, which is clinically highly associated with AG.[37] If *Helicobacter pylori* infection is an external cause, then the corresponding change in the body is an internal response, compared to detecting an external cause. The detection of internal factors should better reflect the true state of the body than the detection of

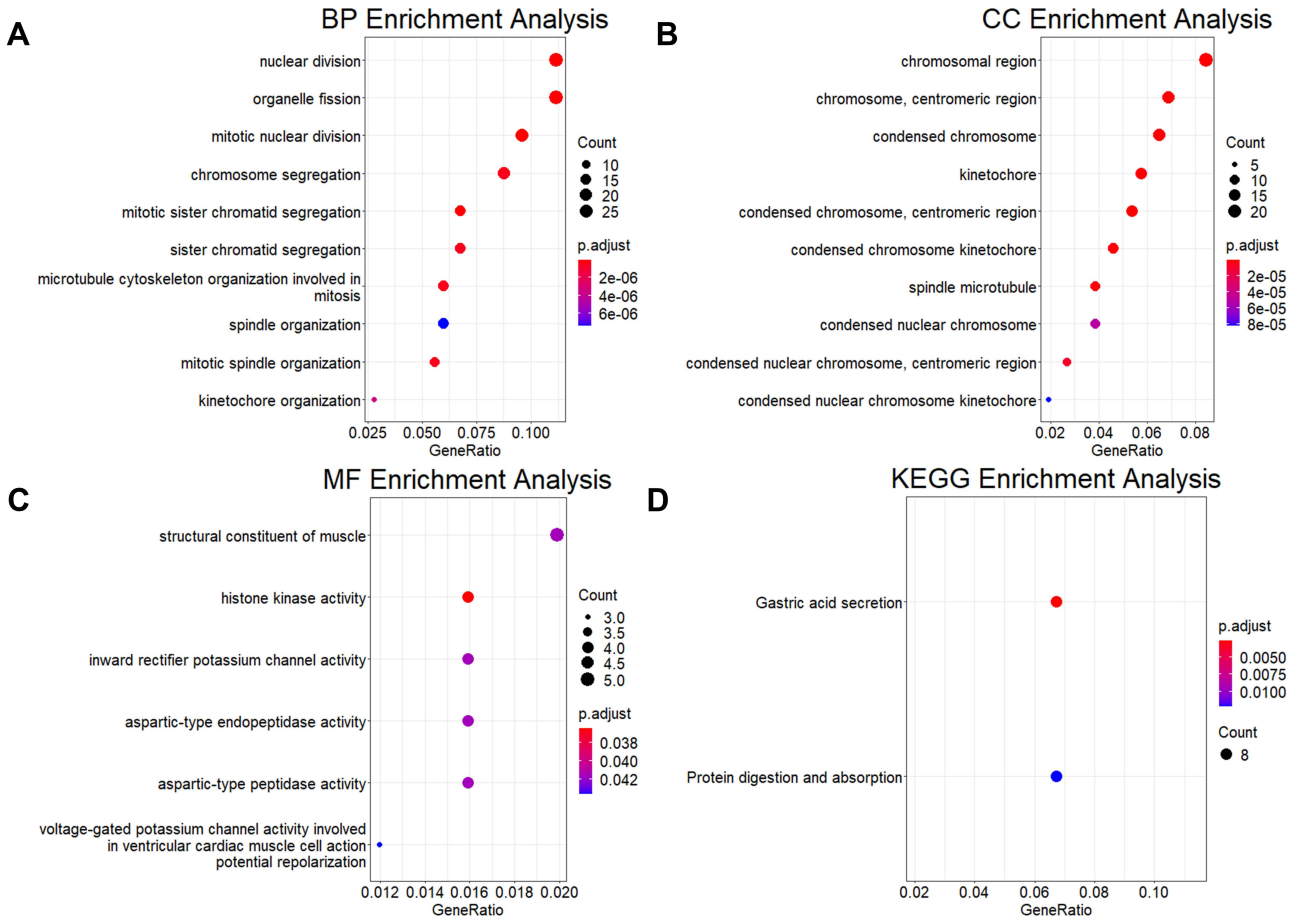

Figure 6 Functional enrichment analysis of the core genes of CA tissue samples. (**A**–**C**) Functional description of the core genes of CA tissue samples in BP, CC, and MF. (**D**) Pathways involved in core genes of CA tissue samples.

external factors. This part of the work provides a more detailed delineation of the staged features of function in gastro-inflammatory cancer transformation.

In the transcriptome analysis of gastric cancer tissues, we obtained 34 potential biomarkers. Through searching these genes one by one on the web of science, it was found that 5 genes, *ASCL2, ESRRG, MMP7, MYB* and *MYLK*, have been studied in gastric cancer-related experiments. High expression of *ASCL2* is necessary for the stemness and tumor formation of gastric cancer cells.[43] *ESRRG* has been identified as a tumor suppressor with good potential for therapeutic development.[44] *MMP7* promotes the invasion and metastasis of gastric cancer cells by directly acting on *SOX12*.[45] *MYB* affects gastric cancer tumor growth by regulating angiogenesis.[46] *MYLK* as a downstream site of androgen receptor variant 12 regulates gastric cancer migration and invasion.[47] These results verify the reliability of our identification results to a certain extent. Fifteen genes such as *AQP4, ATP1B2, CEP55* and *CNN1* have been studied in other cancer-related experiments, and the roles of these genes in gastric cancer remain to be explored. These results verify the reliability of our identification results to a certain extent. There are very few experimental studies related to 14 genes such as *ACTG2, C1QTNF2, NCAPH* and *SORCS1*, which may affect the state of gastric tissue by potential mechanisms. The protein expression levels of these 14 genes were analyzed by protein atlas. It was found that the changes of protein expression levels of *ACTG2, CDCA5, DNAJB5* and *NCAPH* were consistent with the changes of mRNA expression levels, which were of great exploratory value.

In summary, our work elucidates the staged characteristics of gastritis-cancer transformation, screened genes with diagnostic potential for atrophic gastritis and gastric cancer, and identified potential biomarkers for gastric cancer. These results can provide reference for gastritis-cancer transformation-related research and subsequent development and application.

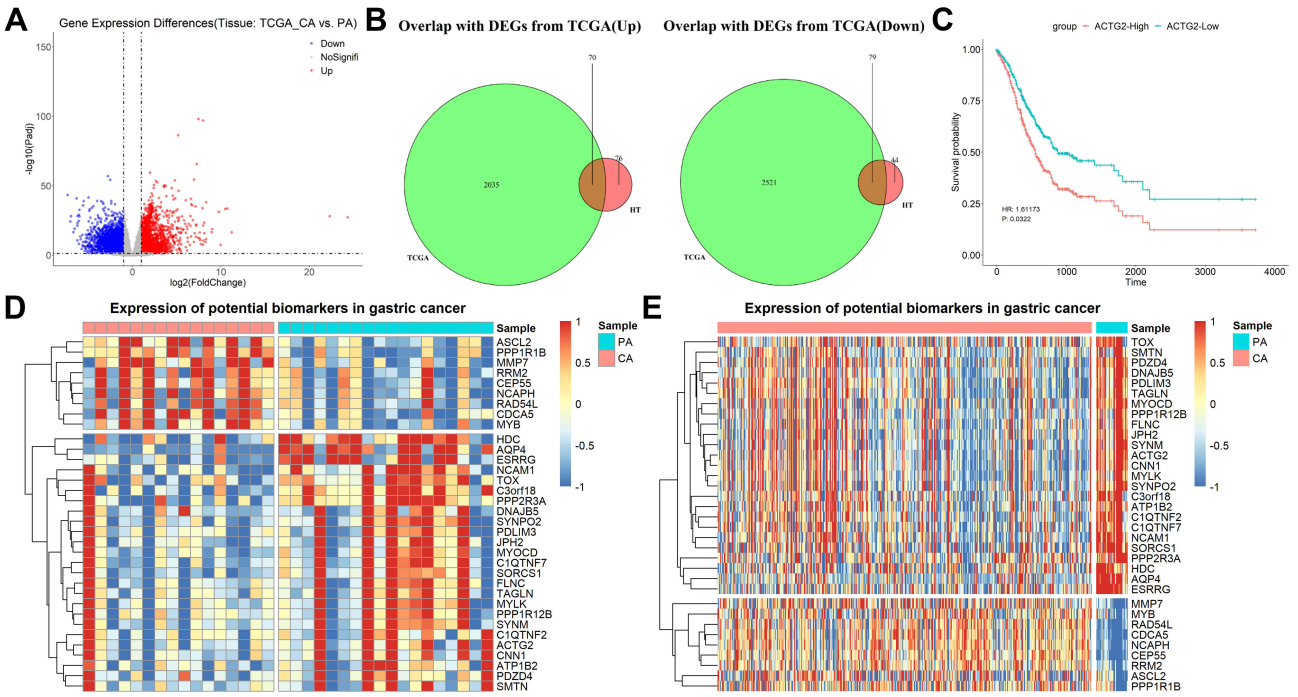

Figure 7 Combined transcriptome data in TCGA to identify potential biomarkers of GC. (**A**) DEGs obtained by comparing CA with PA in TCGA. (**B**) Screening of genes with consistent differential changes in the two datasets. (**C**) Association between genes and survival in GC patients. (**D** and **E**) Expression of potential biomarkers in self-collected datasets and TCGA datasets.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials and Supplementary Files.

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Author Contributions

Conception and design: Ruikang Jia, Xiaohui Guo, Zhen Wang. Development of methodology: Jianliang Sui, Binghua Yin. Acquisition of data (provided samples, acquired and managed patients, provided facilities, etc.): Xiaohui Guo, Huiyun Liu, Feiyue Zhao, Zhihong Wang, Zhen Wang. Analysis and interpretation of data (eg, statistical analysis, biostatistics, computational analysis): Ruikang Jia, Zhibin Fan, Menglei Wang, Zhen Wang. Writing, review, and/or revision of the manuscript: Ruikang Jia, Huiyun Liu, Feiyue Zhao, Zhen Wang. Administrative, technical, or material support (ie, reporting or organizing data, constructing databases): Huiyun Liu, Menglei Wang. Study supervision: Zhen Wang.

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no competing interests in this work.

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