Int J Clin Exp Med 2019;12(10):12132-12140
www.ijcem.com /ISSN:1940-5901/IJCEM0098924

Original Article

High expression of CEP55 in tumor tissues predicts better overall survival in gastric carcinoma patients

Yongpan Xu, Jiehong Wang

Department of Gastroenterology, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China

Received June 25, 2019; Accepted September 3, 2019; Epub October 15, 2019; Published October 30, 2019

Abstract: Objective: Potential key genes and molecular mechanisms of gastric cancer (GC) remain poorly understood. The current study aimed to screen key genes related to GC prognosis employing comprehensive bioinformatic tools. Methods: GSE26942 and GSE63089 were downloaded from the Gene Expression Omnibus (GEO) database. Differentially-expressed genes (DEGs) between GC samples and control ones were analyzed using the limma package. Gene ontology (GO) functional annotation and Kyoto encyclopedia of genes and genomes (KEGG) pathway analysis were also carried out. GSE84437 and GEPIA (Gene Expression Profiling Interactive Analysis) online tools were utilized to confirm prognostic value of the top genes. Results: GO enrichment analysis indicated that these DEGs were significantly enriched in cell adhesion, cell division, cell proliferation, mitotic nuclear division, and regulation of cell proliferation. The genes were enriched in KEGG pathway-termed ECM-receptor interaction, cell cycle, protein digestion and absorption, and p53 signaling pathways. The top ten hub genes, with a high degree of connectivity, were identified from the PPI network. They were all significantly increased in GC tissues. However, only CEP55 was related to better overall survival in GC. Conclusion: Present results suggest that 10 hub genes may be potential core genes related to gastric carcinogenesis. CEP55 could be used as a new biomarker for diagnosis and prediction of better overall survival, confirmed in GEO and TCGA databases. However, future clinical studies are required to validate present findings.

Keywords: Gene expression omnibus, biomarker, gastric cancer, overall survival

Introduction

Gastric cancer (GC) is the fifth most common cancer and second leading cause of cancer-related deaths worldwide [1]. It has been reported that more than 677,000 cases have been diagnosed in developing countries, with half occurring in Eastern Asia (mainly in China) [2]. It has been estimated that approximately 26,370 new individuals will be diagnosed in 2016 in the United States [3]. These statistics indicate a great threat to worldwide human health. GC patients are usually asymptomatic in early stages. However, when obvious symptoms occur, this disease generally reaches advanced stages and metastasizes before its discovery [4]. Recent studies have demonstrated that an increasing number of young people suffer from gastric cancer, due to a diversity of diet structure, higher working pressure, and potential related pathological factors [5, 6]. Although incidence of GC has declined recently, prognosis for GC remains overwhelmingly negative [7]. Five-year overall survival rates have rarely exceeded 10% since numerous patients have reached advanced stages of GC [8]. Therefore, aiming to improve the survival rate and quality of life in GC patients, early detection of GC is vitally important. Although extensive progress has been made in pathologic diagnosis and pathogenesis of GC, the underlying molecular mechanisms regarding GC malignancy and progression require further exploration.

In recent years, progress in high-throughput technologies in biomedical research has been widely used to explore potential biomarkers related to cancer diagnosis, treatment, and prognosis. The Gene Expression Omnibus (GEO) database is a public repository that stores high-throughput gene expression and other related data sets [9]. Microarray analysis is a common-

ly used high-throughput technology for exploration of gene expression changes on a worldwide scale. Furthermore, because of limited sample sizes and inconsistent results, caused by different detection platforms, integrated bioinformatics technology has been used in tumor research. A large amount of significant biological information has been revealed [10-12]. Microarray technology, along with integrated bioinformatics analysis, can provide a new and effective method of examining molecular mechanisms of various diseases [13, 14]. Therefore, the current study was designed to screen potential core genes related to GC prognosis, employing comprehensive bioinformatic tools.

Materials and methods

Microarray data

Microarray data of GSE26942 and GSE63089 gene expression profile matrix files was selected and downloaded from the GEO database. The limma package in R was used to determine DEGs between GC tissues and normal tissues. The combined datasets contained 307 samples, including 57 normal gastric tissues and 250 gastric cancer samples. The platform of the GSE26942 dataset is the GPL6947 Illumina HumanHT-12 V3.0 expression beadchip. This dataset contains 12 gastric surrounding normal tissues and 205 gastric tumor tissues. GSE63089, the platform with the GPL5175 [HuEx-1_0-st] Affymetrix Human Exon 1.0 ST Array [transcript (gene) version], contains 45 normal gastric tissues and 45 gastric cancer tissues. Chip probe ID was converted to a gene symbol using a Perl language command. Shared genes in both databases were merged via a Perl language command. R language software was further applied to normalize the merged datasets. Batch effects are the most commonly identified potential factors in different genomic experiments. They may influence the statistical or biological validity of a study [15, 16]. Several tools have been employed to remove batch effects. SVA and ComBat are the most widely used approaches for batch correction [16, 17]. In this study, batch correction was performed using the SVA package in bio-conductor.

Identification of DEGs in GC

The limma package in R software, with multiple testing corrections based on the Benjamini & Hochberg method, was employed to screen for DEGs in the merged dataset. Criteria for screening of DEGs were defined as $|\log_2 FC| > 1$ and adjusted P values < 0.05. The heat map of DEGs was generated using the gplots package for R (http://cran.r-project.org/web/packages/gplots/; version 3.4.3). TXT files of all DEGs in the merged dataset were saved for further integration analysis.

PPI network construction and hub genes identification

Search Tool for the Retrieval of Interacting Genes (STRING) is a freely accessible biological database. It was used to construct the PPI network of all identified DEGs. STRING is a widely used online tool that provides information on protein co-expression relationships. To assess interactive relationships of all DEGs, the DEGs were put into STRING with a combined score > 0.4. PPI was then visualized by Cytoscape. Nodes with a high degree of connectivity (proteins encoded by certain genes interact with other proteins encoded by other genes) were selected as hub genes.

Functional and pathway enrichment analyses of DEGs

Gene Ontology (GO) includes biological function (BP), molecular function (MF), and cellular components (CC). This study used DEGs annotation by R package from the latest version of bioconductor (library “affy”, “limma” and “hgu133plus2.db”). Thus, GO and KEGG analysis were performed with STRING. GO and KEGG analysis results are regarded as statistically significant if P values are less than 0.05.

Survival analysis and expression levels of hub genes

Prognostic roles of hub genes determined in gastric cancer samples were first evaluated using the GSE84437 dataset. This included 433 gastric cancer patients. They were then validated using the online Gene Expression Profiling Interactive Analysis (GEPIA) database. GEPIA consisted of 9,736 tumors and 8,587 normal samples, based on the TCGA database. Expression levels of hub genes between GC and control groups were compared. Next, boxplots were conducted to visualize association levels. Survival curves of samples with high gene expression and low gene expression levels were compared using log rank tests. $P < 0.05$ indicates statistical significance.

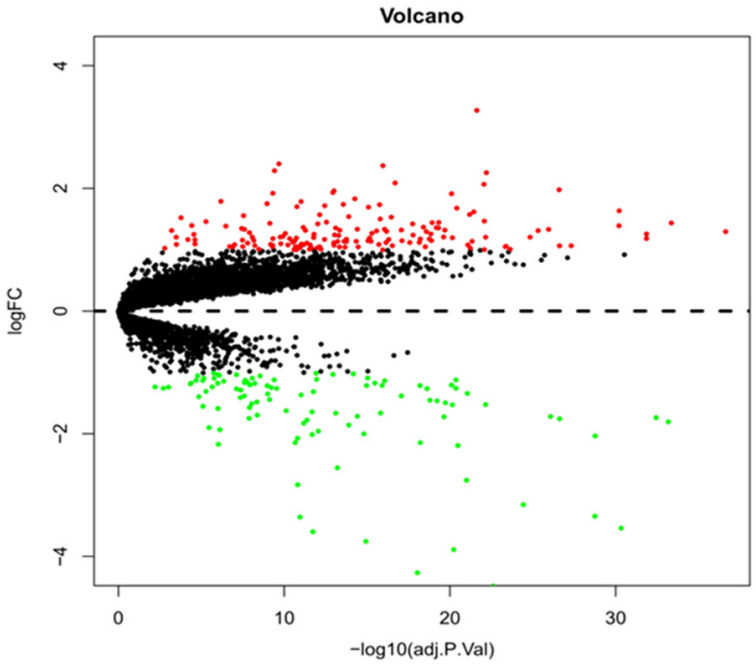

Figure 1. Volcano plot of all DEGs. Red plot indicates upregulated genes. Green plot indicates downregulated genes. Black plot indicates the remaining genes without expression changes.

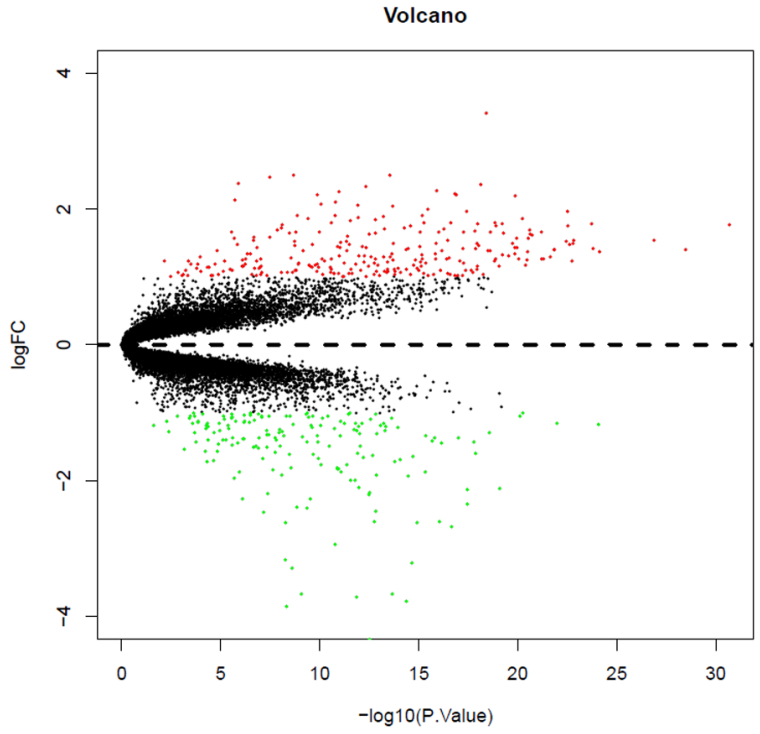

Figure 2. Heatmap of all DEGs. The diagram shows the results of a bidirectional hierarchical clustering of all DEGs and samples. Each row indicates a gene and each column indicates a sample.

Results

Identification of DEGs and hub genes

A total of 260 DEGs were determined, including 158 upregulated and 102 downregulated DEGs in GC tissues, compared to normal tissues. All DEGs were plotted using a volcano plot (**Figure 1**). Expression levels of DEGs were visualized on a heatmap. The DEGs were well clustered between GC tissues and normal tissues, as presented in **Figure 2**. A PPI network of all identified DEGs was performed using the STRING tools online database. Placing the 260 DEGs into the STRING database, a PPI network, with required interaction scores > 0.7, was constructed with 259 nodes and 1,114 edges. The number of links between genes was calculated. The top 10 outstanding nodes, with high degree of connectivity, were identified (**Table 1**). The top 10 hub genes (CCNA2, CDC20, TOP2A, BUB1, ASPM, KIF11, MELK, NCAPG, RRM2, and CEP55) were then selected as hub genes.

Go function and KEGG pathway analysis of DEGs

Investigating the biological function of identified DEGs, KEGG pathway enrichment and GO functional analyses were performed. GO function analysis revealed that the DEGs were mainly involved in cell adhesion, cell division, cell proliferation, mitotic nuclear division, and regulation of cell proliferation (**Figure 3A**). The genes were enriched in KEGG pathway-termed ECM-receptor interaction, cell cycle, gastric acid secretion, protein digestion

Table 1. Top 10 hub genes ranked by the degree method

Rank	Name	Gene symbol	Degree	Expression alteration
1	CCNA2	cyclin A2	48	Up
2	CDC20	cell division cycle 20	47	Up
3	TOP2A	DNA topoisomerase II alpha	47	Up
4	BUB1	budding uninhibited by benzimidazoles 1	46	Up
5	MELK	maternal embryonic leucine zipper kinase	45	Up
6	RRM2	ribonucleotide reductase regulatory subunit M2	45	Up
7	KIF11	kinesin family member 11	45	Up
8	ASPM	abnormal spindle microtubule assembly	45	Up
9	NCAPG	non-SMC condensin I complex subunit G	45	Up
10	CEP55	centrosomal protein 55	44	Up

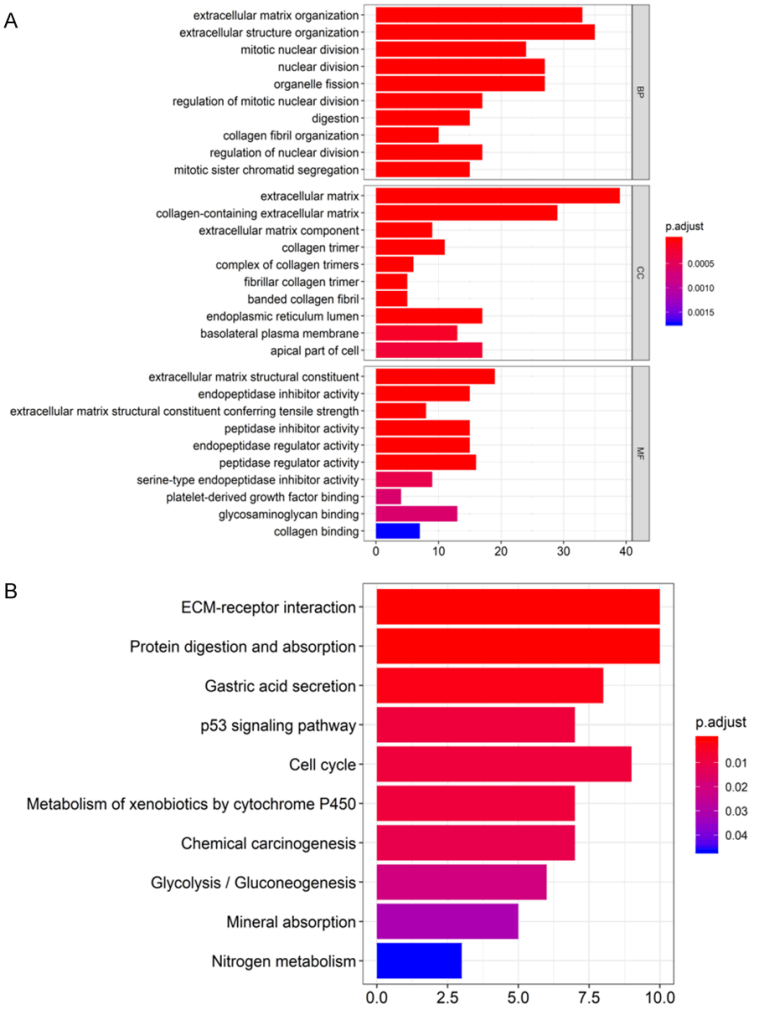

Figure 3. Top 10 most significant enriched gene ontology terms (A) and Kyoto Encyclopedia of Genes and Genomes pathways (B).

and absorption, and p53 signaling pathways (**Figure 3B**).

Survival prognosis of hub genes in a GEO dataset

The current study used the GSE84437 dataset to evaluate the prognostic value of the 10 hub genes. Result indicated that only CEP55 (P = 0.04595), CCNA2 (P = 0.01396), and MELK (P = 0.04543) were associated with better overall survival for GC patients (**Figure 4**).

Survival prognosis validation of hub genes in TCGA database

The prognostic value of the 3 hub genes revealed in the GSE84437 dataset was further analyzed according to the TCGA database using GEPIA. It was found that only expression of CEP55 (HR = 0.69, P = 0.021, **Figure 5A**) was related to better overall survival for GC patients. However, expression levels of CCNA2 (HR = 0.92, P = 0.6) and MELK (HR = 0.75, P = 0.077) were not associated with overall survival. The GEPIA database was further used to validate hub gene expression levels between GC and control samples. Expression levels of CEP55 are illustrated in **Figure 5B**. It was found that, compared with normal gastric tissues, CEP55 levels were elevated in

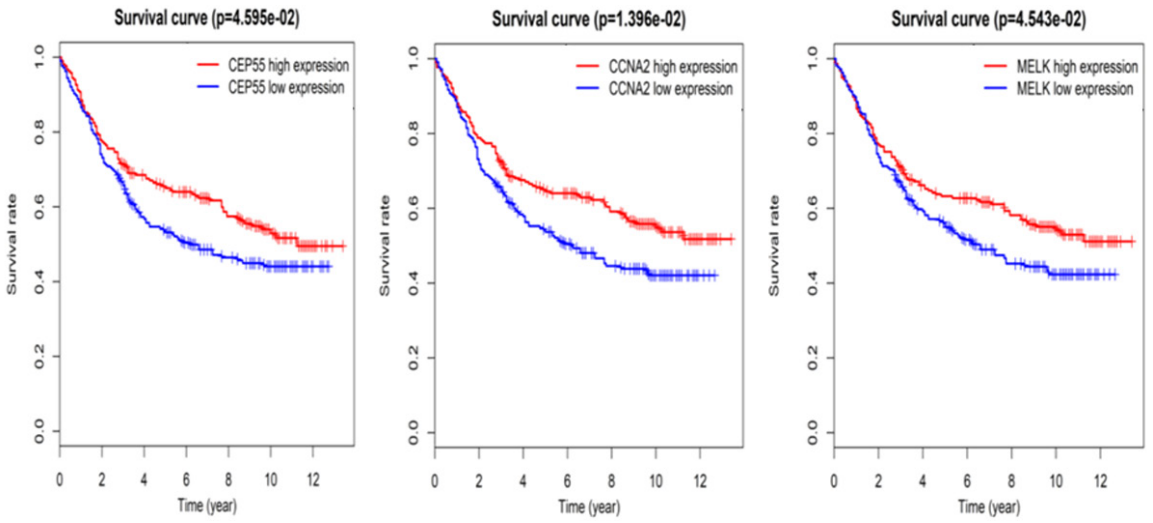

Figure 4. Prognosis roles of three hub genes associated with better overall survival in the GSE84437 dataset.

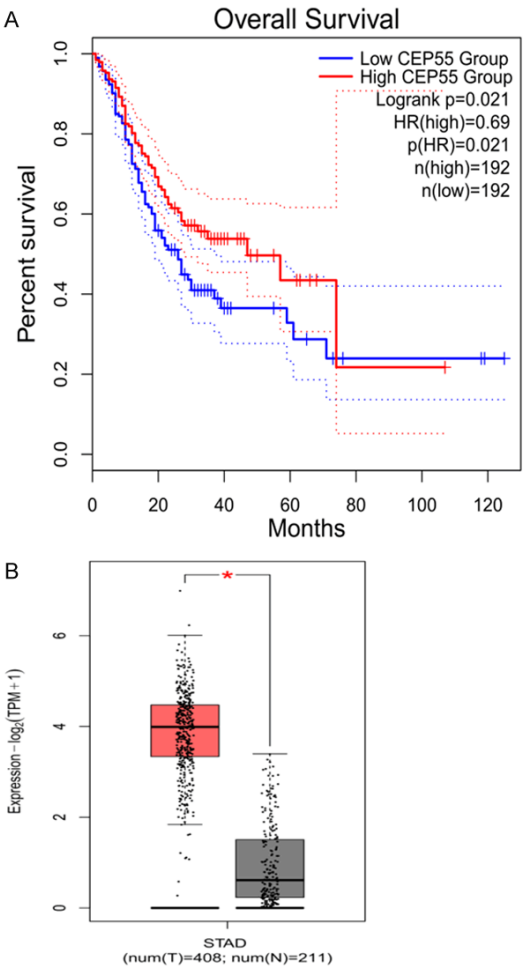

Figure 5. A. Prognosis roles of CEP55 in gastric cancer patients in Gene Expression Profiling Interactive Analysis database. B. Expression levels of CEP55 in gastric cancer and normal tissues based on Gene Expression Profiling Interactive Analysis database.

tumor tissues. Levels differed remarkably between normal and gastric cancer tissues. Therefore, CEP55 high-expression levels are associated with better prognosis in gastric cancer.

Discussion

Gastric cancer is a common malignancy involving the interaction between *Helicobacter pylori* infections and environmental and genetic risks of the host [18]. It is generally caused by uninhibited cell proliferation, invasion, and metastasis [19]. It has been generally accepted that the stage of disease at diagnosis is associated with prognosis of GC. Therefore, exploring promising diagnostic and prognostic biomarkers, as early as possible, is of great importance. GEO microarray data has obvious advantages in exploring possible biomarkers, due to vast storage of gene expression data [20, 21]. Identification of key genes associated with early diagnosis of disease, as well as clinical survival rates, might contribute to gastric cancer pathogenesis.

The current study employed a merged GEO dataset to screen potential key genes related to GC prognosis. A total of 260 DEGs were observed. GO enrichment analysis indicated that these DEGs were significantly enriched in cell adhesion, cell division, cell proliferation, mitotic nuclear division, and regulation of cell proliferation. Present results were consistent with previous findings, which were mostly en-

riched in cell adhesion and extracellular matrix interactions [22]. Moreover, KEGG pathway enrichment results revealed that they were involved with PI3K-Akt signaling pathways, cell cycle, gastric acid secretion, oocyte meiosis, protein digestion and absorption, and p53 signaling pathways. Based on the PPI network, 10 hub genes were determined. CEP55 presented a favorable prognosis. GEPIA was used to validate hub gene expression levels between gastric tissues and normal tissues. It was found that CEP55 was highly expressed GC and normal tissues. Thus, high expression of CEP55 was associated with better prognosis in gastric cancer. Cell cycle regulators are closely involved in cancer. Disorders of the cell cycle pathway are related to progression of gastric cancer development [23]. CCNA2 belongs to one of the mammalian A-type cyclin family in humans, mainly involved in regulation of the cell cycle [24]. It regulates the transition from G1 to S phase. CCNA2 has been reported to be overexpressed and to present poor overall survival in several human cancers [24-26]. However, the prognostic value of CCNA2 in gastric cancer patients remains unclear. A recent bioinformatics study demonstrated CCNA2 was related to worse overall survival [27], while the present study failed to find an association. This divergence may perhaps arise from the heterogeneity of the GEO database included. BUB1 belongs to the BUB gene family which encodes proteins. This is vital in the regulation of the mitotic spindle assembly checkpoint [28]. It has been confirmed that a high expression level of BUB1 is related to well survival rates in gastric cancer patients [29]. However, a previous study revealed that the prognosis value of ASPM in gastric cancer yielded controversial results [30, 31]. CDC20, the cell division cycle 20 homolog, is a regulatory protein that plays a vital role in regulatory of the cell-cycle checkpoint [32]. Previous studies have reported that CDC20 plays an important role in the process of human carcinogenesis. High expression of CDC20 has been closely related to a worse prognosis in non-small cell lung cancer, gastric cancer, colorectal cancer, and pancreatic cancer [33-35]. High expression of TOP2A has been found in some types of cancer, usually accepted as a cancer target in clinical practice [36-38]. For patients with advanced gastric cancer, TOP2A levels are associated with high risk of peritoneum recurrence, as well as hematogenous recurrence [39]. RRM2 plays an essential role in cancer progression and is usually overexpressed in numerous cancers. It is mainly implicated in the regulation process of cell invasiveness, cell migration, and cancer metastasis. Increased expression of RRM2 has been revealed to be closely related to adverse prognosis of gastric cancer by activating RRM2/AKT/NF-κB signaling pathways [40]. KIF11 is highly expressed in bladder, breast, ovarian, lung, and pancreatic cancer. It probably contributes to gastric cancer stem cell formation and is over-expressed in gastric cancer [41]. MELK is mainly involved in several cellular processes, including cell cycle, cell proliferation, apoptosis, and cell migration [42-44]. MELK is commonly over-expressed in gastric cancer and is associated with lymph node involvement, distant metastasis, and poor clinical outcomes [45]. Expression of NCAPG is considerably raised in gastric cancer tissues and is enriched in the cell cycle term, in line with a previous study [46]. CEP55 belongs to the centrosomal relative protein family, which plays a significant role in cell-cycle regulation. It is highly expressed in lung cancers, colon cancer, and several tumor cell lines [47-49]. A previous study disclosed that CEP55 expression must be closely regulated to guarantee that the final stages of cell division evolve precisely [49]. CEP55 was found to be highly expressed in gastric cancer tissues and CEP55-knockdown cell proliferation was inhibited due to cell cycle arrest at the G2/M phase. Convincing evidence has indicated that CEP55 can be used as a potential therapeutic target in gastric cancer [50]. However, the prognosis value of CEP55 has not been well-studied. The present study revealed that CEP55 could be used as a novel biomarker for diagnosis, as well as prediction of better overall survival, confirmed both in GEO and TCGA databases.

Conclusion

In conclusion, it was hypothesized that CCNA2, CDC20, TOP2A, BUB1, ASPM, KIF11, MELK, NCAPG, RRM2, and CEP55 may be potential core genes related to gastric carcinogenesis. Results suggest that CEP55 could be used as a novel biomarker for diagnosis, as well as prediction, of better overall survival. Present conclusions are based on comprehensive bioinformatic tools. Future clinical studies are required to validate these findings. However, the current

study validated the prognostic value of CEP55 in GEO and TCGA databases, increasing the reliability of results.

Data availability

All the data in this study are available from GEO database (https://www.ncbi.nlm.nih.gov/geo/) and GEPIA (http://gepia.cancer-pku.cn/).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yongpan Xu, Department of Gastroenterology, Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712000, Shaanxi Province, China. E-mail: dw1976-06@163.com

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