ISSN 2307-8960 (online)

World Journal of *Clinical Cases*

World J Clin Cases 2020 February 26; 8(4): 652-853

Published by Baishideng Publishing Group Inc

World Journal of Clinical Cases

Contents

Semimonthly Volume 8 Number 4 February 26, 2020

OPINION REVIEW

ORIGINAL ARTICLE

Clinical and Translational Research

Case Control Study

Retrospective Study

Contents

World Journal of Clinical Cases

Volume 8 Number 4 February 26, 2020

Observational Study

SYSTEMATIC REVIEW

CASE REPORT

ABOUT COVER

Editor-in-Chief of *World Journal of Clinical Cases*, Sandro Vento, MD, Dean, Full Professor, Faculty of Medicine, University of Puthisastra, Phnom Penh, Cambodia

AIMS AND SCOPE

The primary aim of *World Journal of Clinical Cases* (*WJCC*, *World J Clin Cases*) is to provide scholars and readers from various fields of clinical medicine with a platform to publish high-quality clinical research articles and communicate their research findings online.

WJCC mainly publishes articles reporting research results and findings obtained in the field of clinical medicine and covering a wide range of topics, including case control studies, retrospective cohort studies, retrospective studies, clinical trials studies, observational studies, prospective studies, randomized controlled trials, randomized clinical trials, systematic reviews, meta-analysis, and case reports.

INDEXING/ABSTRACTING

The *WJCC* is now indexed in PubMed, PubMed Central, Science Citation Index Expanded (also known as SciSearch®), and Journal Citation Reports/Science Edition. The 2019 Edition of Journal Citation Reports cites the 2018 impact factor for *WJCC* as 1.153 (5-year impact factor: N/A), ranking *WJCC* as 99 among 160 journals in Medicine, General and Internal (quartile in category Q3).

RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Ji-Hong Liu*

Proofing Production Department Director: *Xiang Li*

NAME OF JOURNAL
World Journal of Clinical Cases

ISSN
ISSN 2307-8960 (online)

LAUNCH DATE
April 16, 2013

FREQUENCY
Semimonthly

EDITORS-IN-CHIEF
Dennis A Bloomfield, Bao-Gan Peng, Sandro Vento

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/2307-8960/editorialboard.htm

EDITORIAL OFFICE
Jin-Lei Wang, Director

PUBLICATION DATE
February 26, 2020

INSTRUCTIONS TO AUTHORS
https://www.wjgnet.com/bpg/gerinfo/204

GUIDELINES FOR ETHICS DOCUMENTS
https://www.wjgnet.com/bpg/GerInfo/287

GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH
https://www.wjgnet.com/bpg/gerinfo/240

PUBLICATION MISCONDUCT
https://www.wjgnet.com/bpg/gerinfo/208

ARTICLE PROCESSING CHARGE
https://www.wjgnet.com/bpg/gerinfo/242

STEPS FOR SUBMITTING MANUSCRIPTS
https://www.wjgnet.com/bpg/GerInfo/239

ONLINE SUBMISSION
https://www.f6publishing.com

World Journal of Clinical Cases

Submit a Manuscript: https://www.f6publishing.com

World J Clin Cases 2020 February 26; 8(4): 658-669

DOI: 10.12998/wjcc.v8.i4.658

ISSN 2307-8960 (online)

ORIGINAL ARTICLE

Clinical and Translational Research

Identification of key genes and pathways in gastric signet ring cell carcinoma based on transcriptome analysis

Zi-Tong Zhao, Yang Li, Hong-Yu Yuan, Fu-Hai Ma, Yong-Mei Song, Yan-Tao Tian

ORCID number: Zi-Tong Zhao (0000-0001-9279-0924); Yang Li (0000-0002-4549-7087); Hong-Yu Yuan (0000-0002-3086-3582); Fu-Hai Ma (0000-0003-2437-6881); Yong-Mei Song (0000-0002-7789-0158); Yan-Tao Tian (0000-0001-6479-7547).

Author contributions: Zhao ZT and Li Y contributed equally to this work; Zhao ZT, Li Y, Yuan HY, Ma FH, Song YM, and Tian YT contributed to the design of the study and the writing of the manuscript; all the authors reviewed and approved the final version to be published.

Supported by National Key R&D Program of China, No. 2018YFC1313101; Wu Jieping Medical Foundation, No. 320.6750.15276.

Institutional review board statement: This study was approved by the institutional review board of the National Cancer Center of China.

Informed consent statement: The institutional review board of the National Cancer Center of China waived informed consent due to the retrospective nature of this research.

Conflict-of-interest statement: We have no financial relationships to disclose.

Data sharing statement: No additional data are available.

Zi-Tong Zhao, Hong-Yu Yuan, Yong-Mei Song, State Key Laboratory of Molecular Oncology, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Yang Li, Fu-Hai Ma, Yan-Tao Tian, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Corresponding author: Yan-Tao Tian, MD, Professor, Department of Pancreatic and Gastric Surgery, National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, No. 17, Panjiayuan Nanli, Chaoyang District, Beijing 100021, China. tyt67@163.com

Abstract

BACKGROUND

Gastric signet ring cell carcinoma (GSRCC) is one of the most malignant tumors. It has the features of high invasiveness, rapid progression, and resistance to chemotherapy. However, systematic analyses of mRNAs have not yet been performed for GSRCC.

AIM

To identify key mRNAs and signaling pathways in GSRCC.

METHODS

A transcriptome analysis of two GSRCC and two non-GSRCC samples was performed in this study. Differentially expressed mRNAs and pathways were identified based on the KEGG and PANTHER pathway annotations. The interactive relationships among the differential genes were mapped with the STRING database. Quantitative real-time polymerase chain reaction was used to validate the key gene expression in GSRCC.

RESULTS

About 1162 differential genes (using a 2-fold cutoff, $P < 0.05$) were identified in GSRCC compared with non-GSRCC. The enriched KEGG and PANTHER pathways for the differential genes included immune response pathways, metabolic pathways, and metastasis-associated pathways. Ten genes (*MAGEA2*, *MAGEA2B*, *MAGEA3*, *MAGEA4*, *MAGEA6*, *MUC13*, *GUCA2A*, *FFAR4*, *REG1A*, and *REG1B*) were identified as hub genes in the protein-protein interaction network. The expression levels of five genes (*MAGEA2*, *MAGEA3*, *MAGEA4*,

Manuscript source: Invited manuscript

Received: January 2, 2020
Peer-review started: January 2, 2020
First decision: January 19, 2020
Revised: January 20, 2020
Accepted: February 15, 2020
Article in press: February 15, 2020
Published online: February 26, 2020

P-Reviewer: Kim SE, Lee ST, Park SJ
S-Editor: Wang JL
L-Editor: Wang TQ
E-Editor: Liu JH

MAGEA6, and *REG1B*) showed potential clinical value.

CONCLUSION
We have identified the potential key genes and pathways in GSRCC, and these hub genes and pathways could be diagnostic markers and therapeutic targets for GSRCC.

Key words: Signet ring cell; Transcriptome sequencing; Gastric carcinoma; Bioinformatical analysis; Pathway; Gene

Core tip: Gastric signet ring cell carcinoma (GSRCC) is one of the most malignant tumors. It has the features of high invasiveness, rapid progression, and resistance to chemotherapy. However, systematic analyses of mRNAs have not yet been performed for GSRCC. We have identified the potential key genes and pathways of GSRCC, and these hub genes and pathways could be diagnostic markers and therapeutic targets for GSRCC.

Citation: Zhao ZT, Li Y, Yuan HY, Ma FH, Song YM, Tian YT. Identification of key genes and pathways in gastric signet ring cell carcinoma based on transcriptome analysis. *World J Clin Cases* 2020; 8(4): 658-669
URL: https://www.wjgnet.com/2307-8960/full/v8/i4/658.htm
DOI: https://dx.doi.org/10.12998/wjcc.v8.i4.658

INTRODUCTION

Gastric cancer (GC) is the 5th most common carcinoma in the world, with a morbidity of over 1000000 new patients in 2018, and is the 3rd principal cause of death by cancer worldwide[1]. Recently, different pathohistological standard methods have been established for GC. The massive majority of GCs are adenocarcinomas, which can be further divided into intestinal and diffuse types by Lauren classification[2,3]. An alternative classification, proposed by the World Health Organization, divides gastric cancer into tubular, mucinous, papillary, and poorly cohesive carcinomas[4]. Diffuse type is generated by poorly cohesive cells without gland formation and is frequently referred to as gastric signet ring cell carcinoma (GSRCC). GSRCC is a histologic diagnosis based on microscopic characteristics as the presence of signet ring cells in over 50% of the cancer cells by the World Health Organization[5]. These classification methods have little clinical value. An important priority of proper classifiers should be studied to guide therapeutics. Considering the epidemiology of GSRCC, it happens more frequently in females than non-GSRCC, and occurs among patients aged between 55 to 61 years, 7 years ahead of those with non-GSRCC[6,7]. Since the beginning of treatment to eradicate *Helicobacter pylori*, the incidence of gastric adenocarcinoma has decreased. Conversely, the incidence of GSRCC is rising. The GSRCC is found in 8% to 30% of GCs[8].

The prognosis of GSRCC in entire mentioned reports has been described as un-inferior than the other gastric cancer subtypes in the early stage[9,10]. Contrariwise, the prognosis of GSRCC in advanced stages is quite poor, and most studies showed a significantly worse 5-year survival rate in patients with GSRCC than in those with non-GSRCC. Furthermore, Lemoine *et al*[11] showed that patients with advanced GSRCC appeared to benefit less from chemotherapy. Pathological response rate to chemotherapy was significantly lower in GSRCC patients (5.3% *vs* 28.1%, $P = 0.0004$). Another German analysis confirmed that a worse histopathological response (16.3% *vs* 28.9%, $P < 0.001$) in patients was affected by preoperative chemotherapy. An ongoing PRODIGE19 trial (NCT01717924) tends to randomize patients with resectable GCs with signet ring cells receiving perioperative chemotherapy with ECF *vs* an upfront surgery followed by adjuvant chemotherapy with the same regimen[12]. However, the results are still uncertain. The effects of chemotherapy on GSRCC are still in controversy. According to its potential drug resistance, current studies have mainly focused on the pathogenesis study of GSRCC with regard to some disease-related genes.

To find out the molecular mechanisms for tumorigenesis and heterogeneity of GSRCC at the molecular level, large studies have been taken to characterize the comprehensive genomic features through transcriptome sequencing, and multiple driver alterations have been recognized. The exploration of the molecular mechanism of GSRCC is important to improve the recognition of GSRCC and find the effective therapeutics to raise the survival rate of patients. To our best knowledge, systematic and comprehensive analyses of mRNAs have not yet been conducted for GSRCC. In this study, transcriptome sequencing and comprehensive analysis were performed to identify key mRNAs and signaling pathways in GSRCC, with an aim to provide new insights into the treatment and feature of GSRCC.

MATERIALS AND METHODS

Gastric signet ring cell cancer tissue samples

A total of 60 patients who underwent surgery for GSRCC at National Cancer Center, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China were selected for inclusion in the study. All clinicopathological data including age, sex, histological type, and lymph node metastasis were obtained from the database. Four of samples were sent to conduct transcriptome sequencing, and 56 of samples were taken for validation.

Transcriptome sequencing

The transcriptome sequencing was used for mRNA expression profiling (CapitalBio).

KEGG and PANTHER pathway analyses

The functional enrichment of the differential genes was assessed based on the KEGG and PANTHER pathway annotations.

Protein-protein interaction network construction

To evaluate the interactive relationships among the differential genes, we mapped the differential genes to the STRING database (http://string-db.org).

RNA extraction, RT-PCR, and quantitative real-time PCR

Total RNA was extracted from frozen fresh tissues with the RNAExpress Total RNA Kit (New Cell & Molecular Biotech Co., Ltd). cDNA was synthesized with the First-Strand cDNA Synthesis Kit (Applied Biological Materials). Quantitative real-time polymerase chain reaction (qPCR) was performed with EvaGreen 2X qPCR MasterMix (Applied Biological Materials). And the primers used are listed in Table 1. The mRNA sequence of *MAGEA2/3/6* is too similar to design primers for separately verifying and validating these three genes, so we designed three pairs of primers for *MAGEA2/3/6* to validate the expression of these genes together.

Statistical analysis

The data are presented as the mean ± SE. The differences were assessed by the two-tailed Student's *t*-test for group comparisons using the GraphPad Prism 5 software package. Differences were considered significant when *P* values were less than 0.05.

RESULTS

Clinical characteristics of SRCC samples

In this study, four primary GC patients underwent transcriptome sequencing, consisting of two patients with GSRC and two with adenocarcinoma. All of them are male with a median age of 49 years old and poorly differentiated carcinomas. The positive lymph node rate was separately 26/70 and 5/26 in the GSRC group, and 0/45 and 27/34 in the adenocarcinoma group. The two GSRC samples pathologically consisted of over 90% of signet ring cells in the tumor.

Further, 56 samples were chosen for validation. The analysis of the 60 samples showed that there were not significant differences between the GSRCC group and adenocarcinoma group in baseline characteristics such as age, gender, TNM stages, lymphovascular invasion, or nerve invasion, although there were significant differences in Lauren type ($P < 0.001$) and histology differentiation ($P = 0.038$, Table 2).

Identification of differential genes between GSRCC and non-GSRCC

Transcriptome sequencing was used to profile the mRNA expression in four patients

Table 1 Primers used for quantitative real-time polymerase chain reaction

Gene	Primer sequence (5′-3′)
MAGEA2/3/6; primer 1	F: CAGAGGAGTCAGCACTGCAA
	R: TAGTCGAGAAGCTGGAGGCT
MAGEA2/3/6; primer 2	F: CAGAGGAGTCAGCACTGCAA
	R: TAGTCGAGAAGCTGGAGGCT
MAGEA4	F: CTGCTGTCTCCTCCTCCTCT
	R: GGAACAAGGACTCTGCGTCA
MAGEA2/3/6; primer 3	F:CAGAGGAGTCAGCACTGCAA
	R:GACTCTGGGGAGGATCTGGT
REG1A	F: TGACCCCAAAAAGAACCGCC
	R: AGAACTTGTCTTCACAAGGCAC
REG1B	F: GATCCCCGAGCAGTGCTAAT
	R: CTTCTCACAAGATTCATCCTTCCA
GAPDH	F: TGTTGCCATCAATGACCCCTT
	R: CTCCACGACGTACTCAGCG

F: Forward; R: Reverse.

with GC (two with GSRCC and two with non-GSRCC) who underwent surgical treatment (Figure 1). There were 1162 differential genes (using a 2-fold cutoff, $P < 0.05$), which included 682 upregulated and 480 downregulated genes in GSRCC compared with non-GSRCC. The top 10 up-regulated genes were *MAGEA2*, *MAGEA6*, *MAGEA3*, *MAGEA2B*, *ISY1-RAB43*, *MAGEA9*, *MAGEA9B*, *MAGEA12*, *KRT16P6*, and *NEUROD2*. The top 10 down-regulated genes were *REG1B*, *REG1A*, *SPINK4*, *GIF*, *ATOH1*, *IGHV4-4*, *GUCA2A*, *DMBT1*, *SOSTDC1*, and *ATP4B*. The fold changes and *P* values of these differential genes are listed in Table 3.

KEGG and PANTHER pathway enrichment analyses

KEGG pathway enrichment analyses were performed on the differential genes. The enriched KEGG pathways (Figure 2A) for the differential genes included Rap1 signaling pathway, epidermal growth factor receptor tyrosine kinase inhibitor resistance, antigen processing and presentation, HIF-1 signaling pathway, calcium signaling pathway, PI3K-Akt signaling pathway, inflammatory mediator regulation of TRP channels, phagosome, Ras signaling pathway, gastric acid secretion, metabolic pathways, chemokine signaling pathway, peroxisome proliferator-activated receptors signaling pathway, cytokine-cytokine receptor interaction, and endocytosis. For the differential genes, the enriched PANTHER pathways (Figure 2B) included angiogenesis, apoptosis signaling pathway, epidermal growth factor receptor signaling pathway, Notch signaling pathway, endothelin signaling pathway, inflammation mediated by chemokine and cytokine signaling pathway, vascular endothelial growth factor signaling pathway, glycolysis, fibroblast growth factor signaling pathway, integrin signaling pathway, insulin/insulin like growth factor pathway-protein kinase B signaling cascade, fructose galactose metabolism, CCKR signaling map, interleukin signaling pathway, and Toll receptor signaling pathway. Both KEGG and PANTHER pathways mainly focused on immune response pathways, metabolic pathways, and metastasis-associated pathways.

Construction and analysis of protein-protein interaction network

As shown in Figure 3, a regulatory network of differential genes (a 20-fold cutoff, $P < 0.05$) was constructed. There were 141 nodes and 171 edges among these 310 genes. The top 10 hub genes were *MAGEA2*, *MAGEA2B*, *MAGEA3*, *MAGEA4*, *MAGEA6*, *MUC13*, *GUCA2A*, *FFAR4*, *REG1A*, and *REG1B*.

Validated hub genes in GSRCC with TCGA database and tissue samples

According to the difference of gene expression and pathway and the protein-protein interaction result, we chose *MAGEA2*, *MAGEA3*, *MAGEA4*, *MAGEA6*, *REG1A*, and *REG1B* for validation. The data of TCGA and GTEx (GEPIA, http://gepia.cancer-pku.cn/index.html) revealed that the expression of *MAGEA2*, *MAGEA3*, *MAGEA4*, and *MAGEA6* was upregulated, but the expression of *REG1B* was downregulated, in GC compared to the normal gastric tissue, and there was no difference in *REG1A* between GC and normal gastric tissues (Figure 4A). The correlation analysis of gene

Table 2 Characteristics of 30 gastric signet ring cell carcinoma and 30 adenocarcinoma patients, *n* (%)

Characteristic	Overall (*n* = 60)	GSRCC (*n* = 30)	Adenocarcinoma (*n* = 30)	*P* value
Age (yr)				0.052
≤ 65	41 (68.3)	24 (80.0)	17 (56.7)	
> 65	19 (31.7)	6 (20.0)	13 (43.3)	
Gender				0.584
Male	40 (66.7)	21 (70.0)	19 (63.3)	
Female	20 (33.3)	9 (30.0)	11 (36.7)	
Tumor size (cm)				0.584
≤ 5	40 (66.7)	21 (70.0)	19 (63.3)	
> 5	20 (33.3)	9 (30.0)	11 (36.7)	
Lauren type				0
Intestinal	12 (20.0)	0 (0)	12 (40.0)	
Diffuse	35 (58.3)	26 (86.7)	9 (30.0)	
Mixed	12 (20.0)	4 (13.3)	8 (26.7)	
Unknown	1 (1.67)	0 (0)	1 (3.3)	
Histology differentiation				0.038
Poorly/un-differentiated	56 (93.3)	30 (100.0)	26 (86.7)	
Well/moderately differentiated	4 (6.7)	0 (0)	4 (13.3)	
Unknown				
T stage				0.725
T1	5 (8.3)	3 (10.0)	2 (6.7)	
T2	9 (15.0)	3 (10.0)	6 (20.0)	
T3	12 (20.0)	6 (20.0)	6 (20.0)	
T4	34 (56.7)	18 (60.0)	16 (53.3)	
N stage				0.639
N0	16 (26.7)	8 (26.7)	8 (26.7)	
N1	6 (10.0)	3 (10.0)	3 (10.0)	
N2	20 (33.3)	8 (26.7)	12 (40.0)	
N3	18 (30.0)	11 (36.7)	7 (23.3)	
M stage				0.15
Metastasis	2 (3.3)	2 (6.7)	0 (0)	
No metastasis	58 (96.7)	28 (93.3)	30 (100.0)	
Positive lymph nodes				0.39
≤ 8	43 (71.7)	20 (66.7)	23 (76.7)	
> 8	17 (28.3)	10 (33.3)	7 (23.3)	
Lymphovascular invasion				0.355
Yes	31 (51.7)	15 (50.0)	16 (53.3)	
No	27 (45.0)	13 (43.3)	14 (46.7)	
Unknown	2 (3.3)	2 (6.7)	0 (0)	
Nerve invasion				0.213
Yes	42 (70.0)	22 (73.3)	20 (66.7)	
No	16 (26.7)	6 (20)	10 (33.3)	
Unknown	2 (3.3)	2 (6.7)	0 (0)	
Neoadjuvant chemotherapy				0.688
Yes	7 (88.3)	4 (13.3)	3 (10.0)	
No	53 (11.7)	26 (86.7)	27 (90.0)	

GSRCC: Gastric signet ring cell carcinoma.

expression and clinical data revealed a negative correlation between *MAGEA2*, *MAGEA3*, *MAGEA4*, and *MAGEA6*, but a positive correlation between *REG1A* and *REG1B*, and poor survival in patients with GC (GEPIA, http://gepia.cancer-pku.cn/index.html, Figure 4B).

To investigate the clinical value of these five genes in GC, qPCR was used to

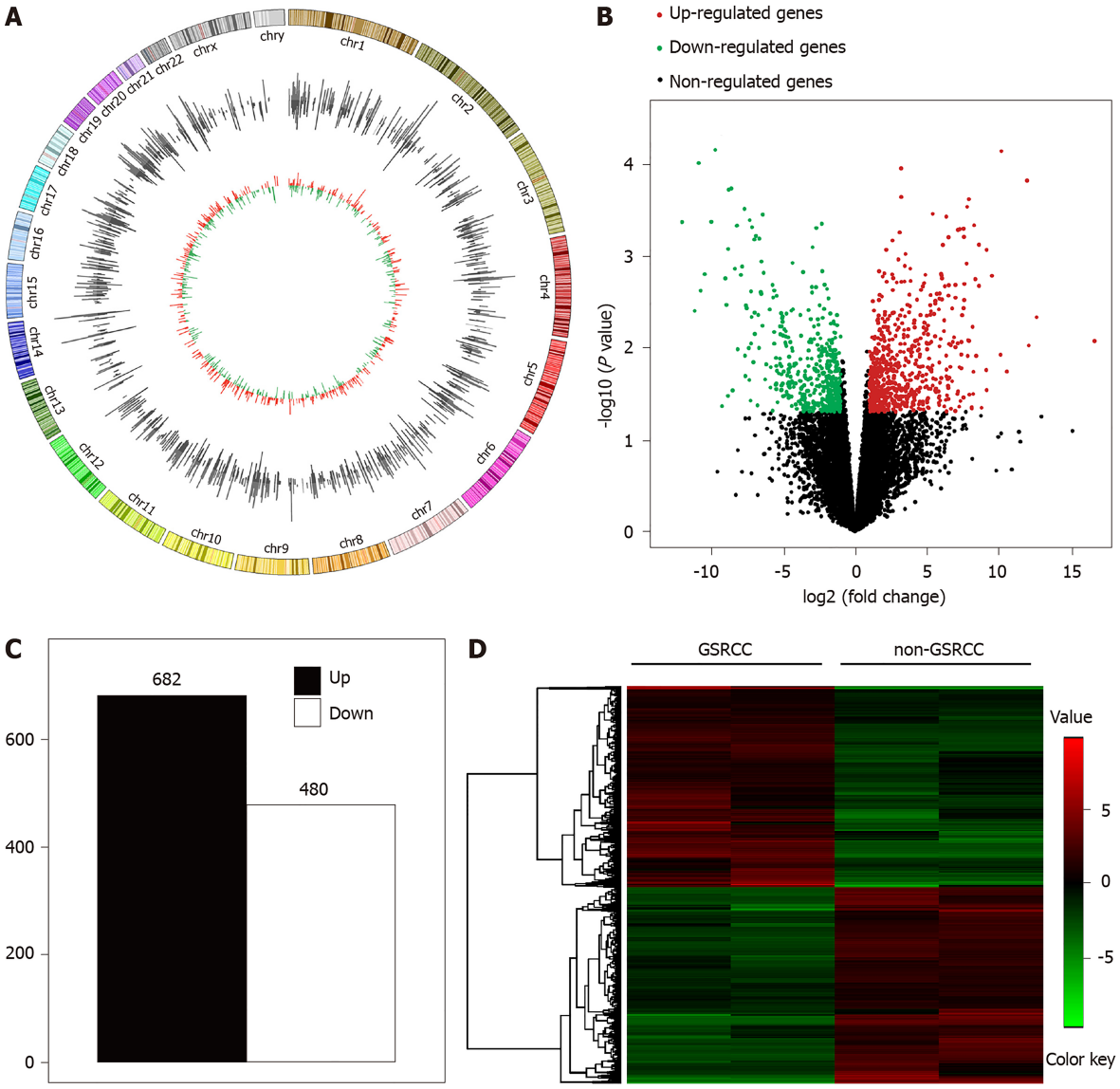

Figure 1 Summary of transcriptome sequencing results of mRNA expression in gastric carcinoma. A: Circos plot shows the differential genes on the human chromosomes. From the outside and inwards, the first layer of the Circos plot is a chromosome map of the human genome; the black and white bars represent expression, while the third layer of circus plot represents differential genes, red indicates up-regulated genes, and green indicates down-regulated genes; B: Volcano plot of the distributions of mRNAs; C: Numbers of differential genes between gastric signet ring cell carcinoma and non-gastric signet ring cell carcinoma; D: Cluster analysis of functional mRNAs in four cases of gastric carcinoma, and data are presented as a heat map ($P < 0.05$). GSRCC: Gastric signet ring cell carcinoma.

measure their expression in 27 GSRCC and 29 non-GSRCC samples. The qPCR results revealed that the expression of *MAGEA2/3/6* and *MAGEA4* was significantly higher, but the expression of *REG1B* was much lower, in patients with GSRCC than in patients with non-GSRCC (Figure 4C).

DISCUSSION

Advanced GSRCC is commonly considered to be more invasive, has a greater probability of lymph node metastasis than other gastric cancer types, has a worse prognosis[13,14], and is less sensitive to chemotherapy than non-SRCC[15-17]. Chemotherapy remains the major treatment for advanced GC. Several studies report that chemoresistance exists in GSRCC with a poor prognosis[8]. It is still unclear whether GSRCC patients can benefit from a detailed therapy.

As we know, vascular endothelial growth factor and HER-2 have been agreed as targets for molecular pathways in GC. However, SRCC cannot benefit from targeted therapy for a low expression level of HER-2[18]. Some studies have mentioned that elevated expression of *PKM2* and *E-cadherin*, and declined expression of *TMEM207* in GSRCC are associated with its biological behavior of invasion and metastasis[19].

Table 3 Fold change of differential genes

Genes	Fold change	*P* value
Top 10 up-regulated genes		
MAGEA2	9182.176	0.000031
MAGEA6	4410.054	0.000429
MAGEA3	2508.358	0.0000307
MAGEA2B	1971.738	0.0000976
ISY1-RAB43	1921.249	0.0000394
MAGEA9	1787.459	0.002399
MAGEA9B	1469.006	0.001587
MAGEA12	1064.08	0.000427
KRT16P6	633.9792	0.043469
NEUROD2	574.5342	0.000245
Top 10 down-regulated genes		
REG1B	0.00000891	0.008502
REG1A	0.000149	0.004671
SPINK4	0.000217	0.009491
GIF	0.000237	0.000152
ATOH1	0.000632	0.018238
IGHV4-4	0.000822	0.0000723
GUCA2A	0.000853	0.011982
DMBT1	0.001304	0.001651
SOSTDC1	0.001678	0.000862
ATP4B	0.001709	0.017566

Systematic analyses of mRNAs in GSRCC were rare. Chen *et al*[19] reported that hsa-miR-665 and hsa-miR-95 were found in GSRCC compared to intestinal GC, and concluded that these two mRNAs were downregulated in GSRCC but upregulated in intestinal GC, and the relatively differential expression of the miRNAs negatively regulating their target genes could be intently associated with high invasion, metastasis, and chemoresistance of GSRCC[19]. Yao *et al*[20] have described the genome features of GSRCC and they found the importance of highly frequent *CLDN18-ARHGAP26/6* fusions in chemotherapy response in GSRCC. Kakiuchi *et al*[21] reported that *RHOA* mutations happen specifically in diffused GCs, the majority of which were pathologically characterized by the presence of poorly differentiated components, with no available molecularly targeted drugs for this poor-prognosis subtype of GC. However, that study was not specific for GSRCC. Due to a very limited number of studies in this area, this present study aimed to identify specifically expressed genes and identify biological features in GSRCC.

By pathway analyses, we confirmed frequent alterations across various pathways in GSRCC, including immunological response pathways, metabolic pathways, and metastasis-associated pathways. Further protein-protein interaction network analyses identified the hub genes in GSRCC. Of the hub genes, *MAGE-A* family and *REG1B* are immune-related genes. *REG1B* has been reported to be able to upregulate the expression of interleukin (IL)-6 mRNA and protein.

MAGE-A family are the best characterized cancer-testis antigens (*CTA*) family members, which are expressed mainly, but not exclusively, in germ cells. *MAGE-A* family are infrequently expressed in the normal human placenta, but they are widely expressed in numerous human cancers. Alvi *et al*[22] concluded that the $MSI^+/CIMP^+/BRAF\ V600E^+/CD3^+/PDL1^+$ hypermethylated genotype is an ideal candidate for immune checkpoint inhibitor therapy. In addition, one-fourth of SRCC cases can potentially be targeted by KIT inhibitors. As similar as we found, MAGE-A proteins are highly immunogenic and are considered as potential targets for immunotherapy.

In conclusion, we have identified the potential key genes and pathways in GSRCC. These hub genes and pathways could be diagnostic markers and therapeutic targets for GSRCC. *MAGE-A* family as a *CTA* family member may be the potential targets for GSRCC. More research should be conducted for exploration of the mechanisms involved.

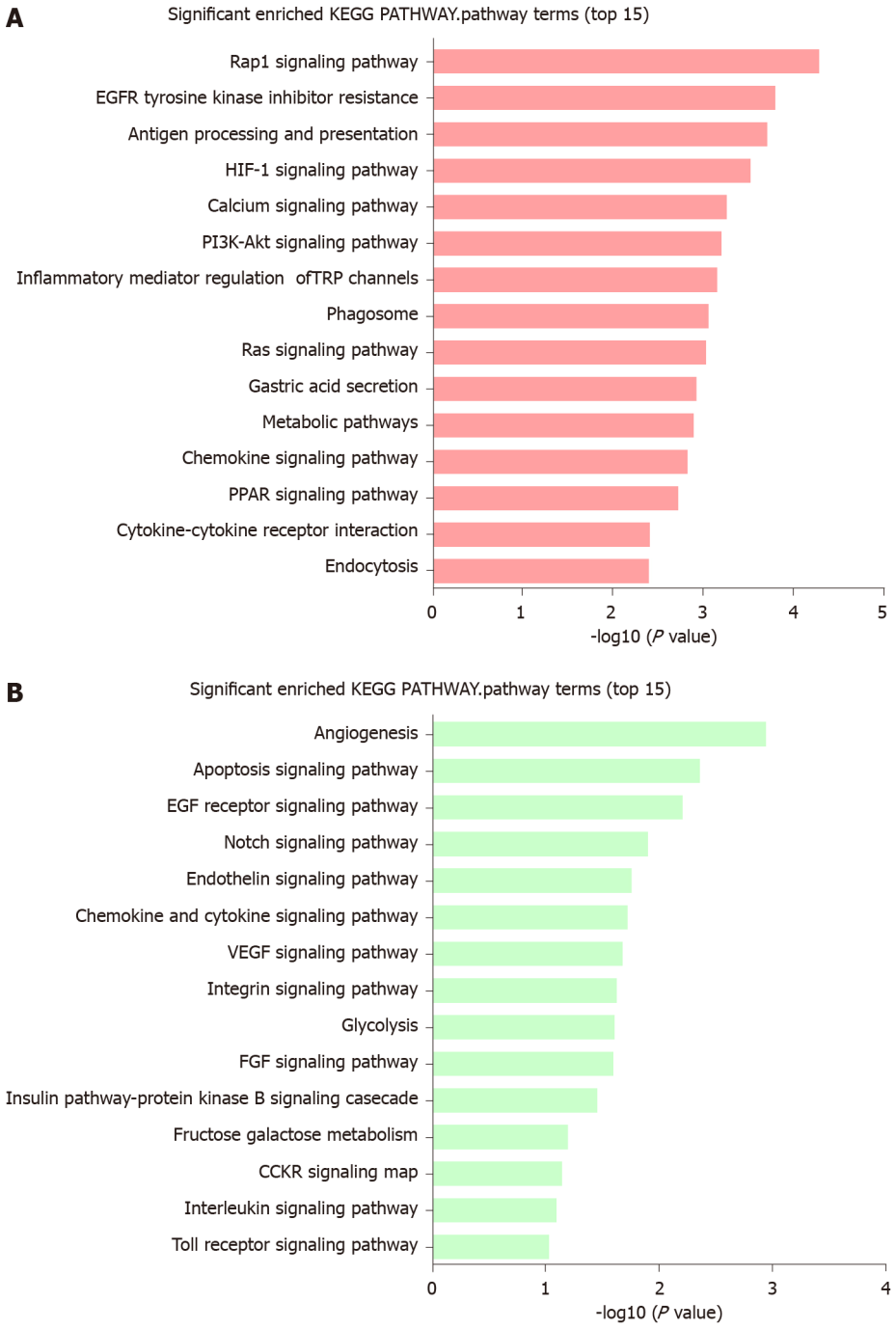

Figure 2 KEGG and PANTHER pathway analysis of differential genes. A: Top 15 enriched KEGG pathways are presented; B: Top 15 enriched PANTHER pathways are presented.

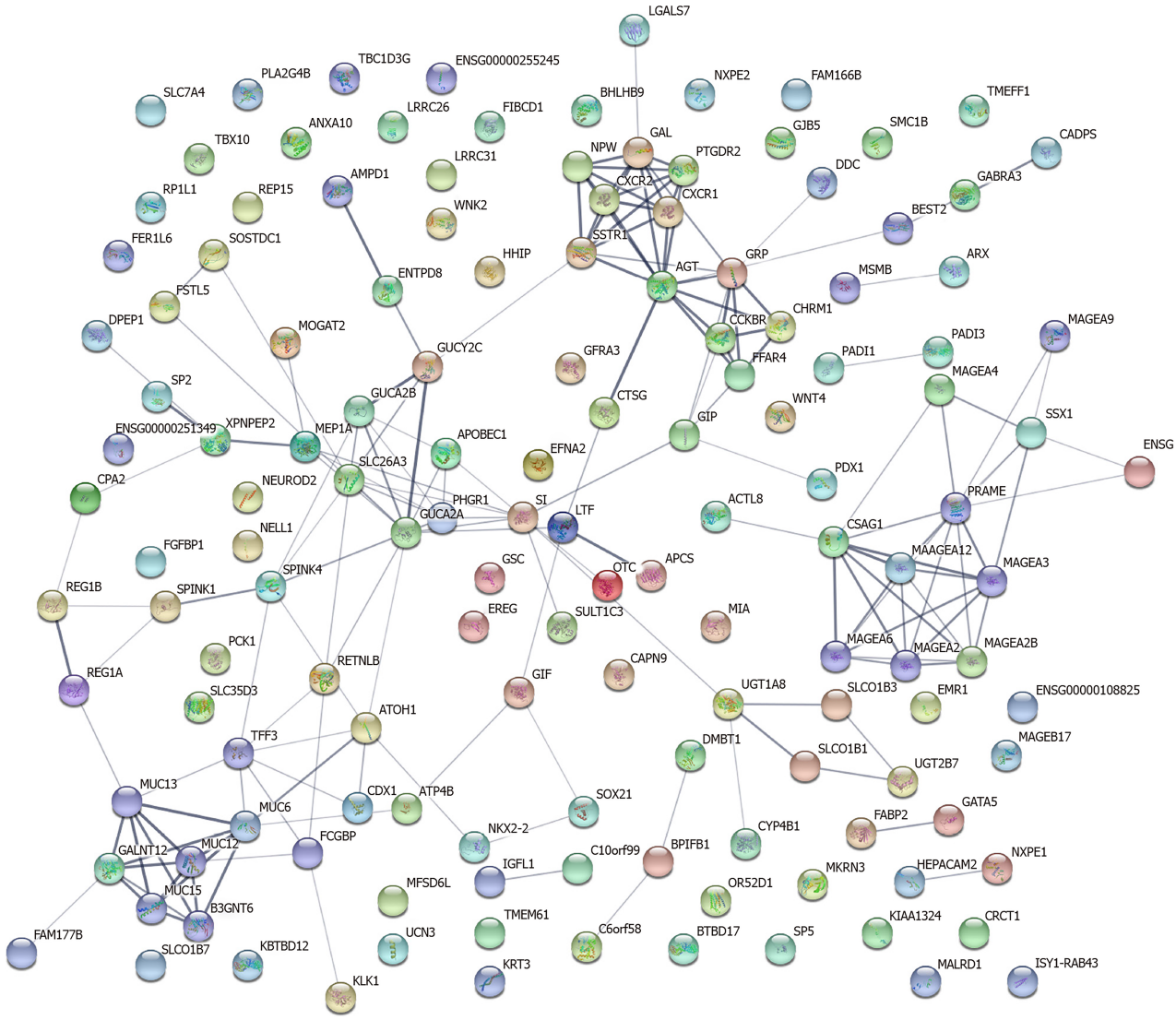

Figure 3 Protein-protein interaction network of differential genes. Using the STRING online database, 310 genes were selected and used to construct the protein-protein interaction network.

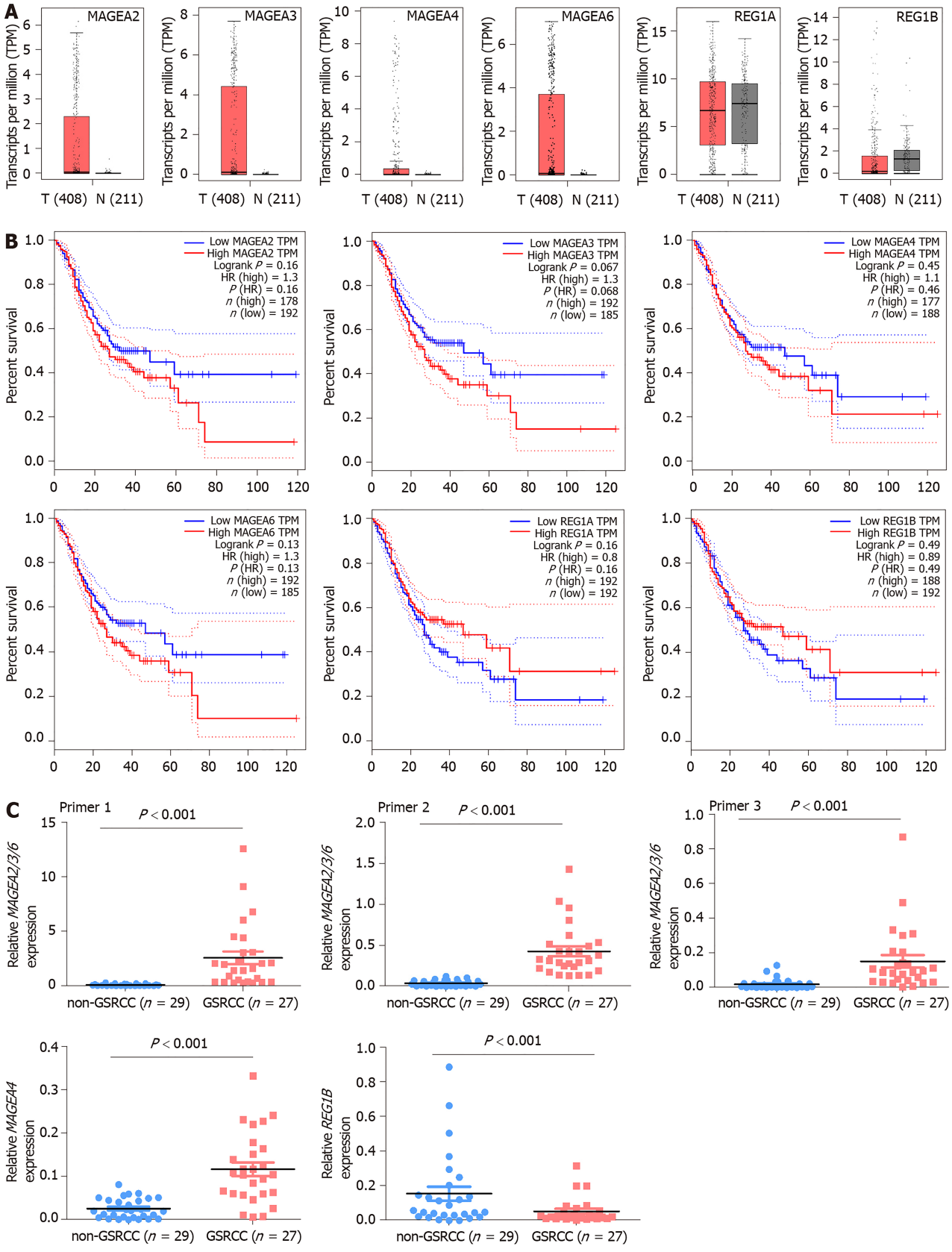

Figure 4 Expression of *MAGEA2/3/6*, *MAGEA4*, and *REG1B* in gastric signet ring cell carcinoma. Data were collected from TCGA and GTEx data using GEPIA (http://gepia.cancer-pku.cn/index.html). A: FPKM of genes in transcriptome sequencing data in 408 gastric carcinoma tissues (T) and 211 normal tissues (N); B: Survival rate calculated by the Kaplan-Meier method in patients separated according to the median expression level of each gene (95%CI); C: Quantitative real-time PCR was used to measure the relative expression levels of the indicate genes in 27 gastric signet ring cell carcinoma samples and 29 non-gastric signet ring cell carcinoma samples. The results were normalized to the endogenous *GAPDH* RNA control. GSRCC: Gastric signet ring cell carcinoma.

ARTICLE HIGHLIGHTS

Research background

Gastric signet ring cell carcinoma (GSRCC) has the features of high invasiveness, rapid progression, and resistance to chemotherapy. However, systematic analyses of mRNAs have not yet been performed.

Research motivation

The exploration of the molecular mechanism of GSRCC is important to improve the recognition of GSRCC and find the effective therapeutics to raise the survival rate of patients.

Research objectives

Transcriptome sequencing and comprehensive analysis were performed to identify key mRNAs and signaling pathways in GSRCC.

Research methods

A transcriptome analysis of two GSRCC and two non-GSRCC samples was performed. Differentially expressed mRNAs and pathways were identified. The interactive relationships among the differential genes were mapped with the STRING database.

Research results

The enriched KEGG and PANTHER pathways for the differential genes included immune response pathways, metabolic pathways, and metastasis-associated pathways. *MAGEA2*, *MAGEA2B*, *MAGEA3*, *MAGEA4*, *MAGEA6*, *MUC13*, *GUCA2A*, *FFAR4*, *REG1A*, and *REG1B* were identified as hub genes in the protein-protein interaction network. The expression levels of *MAGEA2*, *MAGEA3*, *MAGEA4*, *MAGEA6*, and *REG1B* showed potential clinical value.

Research conclusions

The potential key genes and pathways of GSRCC have been identified. These hub genes and pathways could be diagnostic markers and therapeutic targets for GSRCC.

Research perspectives

MAGE-A family as a *CTA* family member may be the potential targets for GSRCC. More research should be conducted for exploration of the mechanisms involved.

REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]

2 **Taghavi S**, Jayarajan SN, Davey A, Willis AI. Prognostic significance of signet ring gastric cancer. *J Clin Oncol* 2012; **30**: 3493-3498 [PMID: 22927530 DOI: 10.1200/JCO.2012.42.6635]

3 **Shu Y**, Zhang W, Hou Q, Zhao L, Zhang S, Zhou J, Song X, Zhang Y, Jiang D, Chen X, Wang P, Xia X, Liao F, Yin D, Chen X, Zhou X, Zhang D, Yin S, Yang K, Liu J, Fu L, Zhang L, Wang Y, Zhang J, An Y, Cheng H, Zheng B, Sun H, Zhao Y, Wang Y, Xie D, Ouyang L, Wang P, Zhang W, Qiu M, Fu X, Dai L, He G, Yang H, Cheng W, Yang L, Liu B, Li W, Dong B, Zhou Z, Wei Y, Peng Y, Xu H, Hu J. Prognostic significance of frequent CLDN18-ARHGAP26/6 fusion in gastric signet-ring cell cancer. *Nat Commun* 2018; **9**: 2447 [PMID: 29961079 DOI: 10.1038/s41467-018-04907-0]

4 **Hu B**, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: Classification, histology and application of molecular pathology. *J Gastrointest Oncol* 2012; **3**: 251-261 [PMID: 22943016 DOI: 10.3978/j.issn.2078-6891.2012.021]

5 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]

6 **Kim HW**, Kim JH, Lim BJ, Kim H, Kim H, Park JJ, Youn YH, Park H, Noh SH, Kim JW, Choi SH. Sex Disparity in Gastric Cancer: Female Sex is a Poor Prognostic Factor for Advanced Gastric Cancer. *Ann Surg Oncol* 2016; **23**: 4344-4351 [PMID: 27469120 DOI: 10.1245/s10434-016-5448-0]

7 **Ren J**, Niu G, Wang X, Song T, Hu Z, Ke C. Effect of Age on Prognosis of Gastric Signet-Ring Cell Carcinoma: A SEER Database Analysis. *Med Sci Monit* 2018; **24**: 8524-8532 [PMID: 30473583 DOI: 10.12659/MSM.911766]

8 **Pernot S**, Voron T, Perkins G, Lagorce-Pages C, Berger A, Taieb J. Signet-ring cell carcinoma of the stomach: Impact on prognosis and specific therapeutic challenge. *World J Gastroenterol* 2015; **21**: 11428-11438 [PMID: 26523107 DOI: 10.3748/wjg.v21.i40.11428]

9 **Kao YC**, Fang WL, Wang RF, Li AF, Yang MH, Wu CW, Shyr YM, Huang KH. Clinicopathological differences in signet ring cell adenocarcinoma between early and advanced gastric cancer. *Gastric Cancer* 2019; **22**: 255-263 [PMID: 30069742 DOI: 10.1007/s10120-018-0860-8]

10 **Hyung WJ**, Noh SH, Lee JH, Huh JJ, Lah KH, Choi SH, Min JS. Early gastric carcinoma with signet ring cell histology. *Cancer* 2002; **94**: 78-83 [PMID: 11815962 DOI: 10.1002/cncr.10120]

11 **Lemoine N**, Adenis A, Bouche O, Duhamel A, Heurgue A, Leteurtre E, Amela E, Salleron J, Hebbar M. Signet Ring Cells and Efficacy of First-line Chemotherapy in Advanced Gastric or Oesogastric Junction Adenocarcinoma. *Anticancer Res* 2016; **36**: 5543-5549 [PMID: 27798928 DOI: 10.21873/anticanres.11138]

12 **Piessen G**, Messager M, Le Malicot K, Robb WB, Di Fiore F, Guilbert M, Moreau M, Christophe V, Adenis A, Mariette C. Phase II/III multicentre randomised controlled trial evaluating a strategy of primary surgery and adjuvant chemotherapy versus peri-operative chemotherapy for resectable gastric signet ring

cell adenocarcinomas - PRODIGE 19 - FFCD1103 - ADCI002. *BMC Cancer* 2013; **13**: 281 [PMID: 23758655 DOI: 10.1186/1471-2407-13-281]

13 **Lee D**, Ham IH, Son SY, Han SU, Kim YB, Hur H. Intratumor stromal proportion predicts aggressive phenotype of gastric signet ring cell carcinomas. *Gastric Cancer* 2017; **20**: 591-601 [PMID: 27858181 DOI: 10.1007/s10120-016-0669-2]

14 **Liu K**, Wan J, Bei Y, Chen X, Lu M. Prognostic Impact of Different Histological Types on Gastric Adenocarcinoma: a Surveillance, Epidemiology, and End Results Database Analysis. *Pathol Oncol Res* 2017; **23**: 881-887 [PMID: 28116561 DOI: 10.1007/s12253-017-0198-2]

15 **Messager M**, Lefevre JH, Pichot-Delahaye V, Souadka A, Piessen G, Mariette C, FREGAT working group - FRENCH. The impact of perioperative chemotherapy on survival in patients with gastric signet ring cell adenocarcinoma: a multicenter comparative study. *Ann Surg* 2011; **254**: 684-93; discussion 693 [PMID: 22005144 DOI: 10.1097/SLA.0b013e3182352647]

16 **Voron T**, Messager M, Duhamel A, Lefevre J, Mabrut JY, Goere D, Meunier B, Brigand C, Hamy A, Glehen O, Mariette C, Paye F. Is signet-ring cell carcinoma a specific entity among gastric cancers? *Gastric Cancer* 2016; **19**: 1027-1040 [PMID: 26606931 DOI: 10.1007/s10120-015-0564-2]

17 **Charalampakis N**, Nogueras González GM, Elimova E, Wadhwa R, Shiozaki H, Shimodaira Y, Blum MA, Rogers JE, Harada K, Matamoros A, Sagebiel T, Das P, Minsky BD, Lee JH, Weston B, Bhutani MS, Estrella JS, Badgwell BD, Ajani JA. The Proportion of Signet Ring Cell Component in Patients with Localized Gastric Adenocarcinoma Correlates with the Degree of Response to Pre-Operative Chemoradiation. *Oncology* 2016; **90**: 239-247 [PMID: 27046280 DOI: 10.1159/000443506]

18 **Jin S**, Xu B, Yu L, Fu Y, Wu H, Fan X, Wei J, Liu B. The PD-1, PD-L1 expression and CD3+ T cell infiltration in relation to outcome in advanced gastric signet-ring cell carcinoma, representing a potential biomarker for immunotherapy. *Oncotarget* 2017; **8**: 38850-38862 [PMID: 28418918 DOI: 10.18632/oncotarget.16407]

19 **Chen J**, Sun D, Chu H, Gong Z, Zhang C, Gong B, Li Y, Li N, Jiang L. Screening of differential microRNA expression in gastric signet ring cell carcinoma and gastric adenocarcinoma and target gene prediction. *Oncol Rep* 2015; **33**: 2963-2971 [PMID: 25964059 DOI: 10.3892/or.2015.3935]

20 **Yao F**, Kausalya JP, Sia YY, Teo AS, Lee WH, Ong AG, Zhang Z, Tan JH, Li G, Bertrand D, Liu X, Poh HM, Guan P, Zhu F, Pathiraja TN, Ariyaratne PN, Rao J, Woo XY, Cai S, Mulawadi FH, Poh WT, Veeravalli L, Chan CS, Lim SS, Leong ST, Neo SC, Choi PS, Chew EG, Nagarajan N, Jacques PÉ, So JB, Ruan X, Yeoh KG, Tan P, Sung WK, Hunziker W, Ruan Y, Hillmer AM. Recurrent Fusion Genes in Gastric Cancer: CLDN18-ARHGAP26 Induces Loss of Epithelial Integrity. *Cell Rep* 2015; **12**: 272-285 [PMID: 26146084 DOI: 10.1016/j.celrep.2015.06.020]

21 **Kakiuchi M**, Nishizawa T, Ueda H, Gotoh K, Tanaka A, Hayashi A, Yamamoto S, Tatsuno K, Katoh H, Watanabe Y, Ichimura T, Ushiku T, Funahashi S, Tateishi K, Wada I, Shimizu N, Nomura S, Koike K, Seto Y, Fukayama M, Aburatani H, Ishikawa S. Recurrent gain-of-function mutations of RHOA in diffuse-type gastric carcinoma. *Nat Genet* 2014; **46**: 583-587 [PMID: 24816255 DOI: 10.1038/ng.2984]

22 **Alvi MA**, Loughrey MB, Dunne P, McQuaid S, Turkington R, Fuchs MA, McGready C, Bingham V, Pang B, Moore W, Maxwell P, Lawler M, James JA, Murray GI, Wilson RH, Salto-Tellez M. Molecular profiling of signet ring cell colorectal cancer provides a strong rationale for genomic targeted and immune checkpoint inhibitor therapies. *Br J Cancer* 2017; **117**: 203-209 [PMID: 28595259 DOI: 10.1038/bjc.2017.168]

Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-3991568
E-mail: bpgoffice@wjgnet.com
Help Desk: https://www.f6publishing.com/helpdesk
https://www.wjgnet.com