



# Down-regulated *hsa\_circ\_0067934* facilitated the progression of gastric cancer by sponging *hsa-mir-4705* to downgrade the expression of *BMPR1B*

Shen-Nan He<sup>1</sup>, Shi-Hua Guan<sup>1</sup>, Meng-Yao Wu<sup>1</sup>, Wei Li<sup>1,2,3</sup>, Meng-Dan Xu<sup>1</sup>, Min Tao<sup>1,2</sup>

<sup>1</sup>Department of Oncology, the First Affiliated Hospital of Soochow University, Suzhou 215006, China; <sup>2</sup>PREMED Key Laboratory for Precision Medicine, Soochow University, Suzhou 215021, China; <sup>3</sup>Comprehensive Cancer Center, Suzhou Xiangcheng People's Hospital, Suzhou 215000, China

**Contributions:** (I) Conception and design: SN He, M Tao, MD Xu; (II) Administrative support: W Li, M Tao; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: SN He; (V) Data analysis and interpretation: SN He, SH Guan; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

**Correspondence to:** Min Tao, MD, PhD; Meng-Dan Xu, MD. Department of Oncology, the First Affiliated Hospital of Soochow University, Suzhou, China. Email: taomin@suda.edu.cn; doctor\_xmd@163.com.

**Background:** Gastric cancer is the third most lethal cancer worldwide. Finding a novel marker is essential to targeted therapy and the diagnosis of gastric cancer. As newly discovered markers, circRNAs have aroused widespread attention on a global scale. Our research aims to understand the role of circRNAs in gastric cancer and to explore the underlying pathogenesis.

**Methods:** Raw expression data of circRNAs were obtained from the GEO database. Integrated bioinformatics analysis was used to screen differentially expressed circRNAs (DECs) by RobustRankAggreg package. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed to predict the functions of DECs. Then, the miRNAs and mRNAs at the downstream of DECs were predicted. Expression data of miRNAs and mRNAs were downloaded from The Cancer Genome Atlas (TCGA). The aberrantly expressed miRNAs and mRNAs were selected using the edgeR package.

**Results:** Four datasets (GSE78092, GSE83521, GSE89143, and GSE93541) were downloaded from the GEO database. Among them, two DECs (*hsa\_circ\_0007991* and *hsa\_circ\_0067934*) were screened. The functional analyses of DECs confirmed that they were cancer-related circRNAs. Furthermore, *hsa-mir-4705* (miRNA) and *BMPR1B* (mRNA) at the downstream of *hsa\_circ\_0067934* were found differentially expressed in gastric cancer by expression data from TCGA database.

**Conclusions:** Our study discovered the critical roles of *hsa\_circ\_0007991* and *hsa\_circ\_0067934* in the development of gastric cancer, and they could be novel markers for targeted therapy and assist the diagnosis of early-stage gastric cancer. Moreover, we discovered that the *hsa\_circ\_0067934/hsa-mir-4705/BMPRI1B* axis might be involved in the carcinogenesis of gastric cancer.

**Keywords:** Gastric cancer; circRNA; Gene Expression Omnibus (GEO) database; integrated bioinformatics; The Cancer Genome Atlas (TCGA)

Submitted May 25, 2019. Accepted for publication Oct 10, 2019.

doi: 10.21037/tcr.2019.10.32

View this article at: <http://dx.doi.org/10.21037/tcr.2019.10.32>

## Introduction

Gastric cancer, whose incidence accounts for 5.7% of total cancer cases worldwide, is the fifth most commonly diagnosed cancer, lagging just behind lung cancer (11.6%), breast cancer (11.6%), colorectal cancer (10.2%), and prostate cancer (7.1%). Moreover, up to 8.2% mortality of the total cancer-related deaths makes gastric cancer the third leading cause of cancer-related death, ranking next only to lung cancer (18.4%) and colorectal cancer (9.2%) (1). As the only curative treatment for gastric cancer, adequate surgical resection can make the overall 5-year survival rate of patients with early-stage gastric cancer reach 98%. Unfortunately, most patients with gastric cancer were diagnosed at an advanced stage. The lack of an apparent syndrome and practical screening approach at an early stage are likely responsible for the high mortality of gastric cancer (2). In the past few decades, we have also developed adjuvant and neoadjuvant therapies applied according to the stage and risk assessment of gastric cancer, such as pre-/peri- or postoperative chemotherapy and chemoradiotherapy, as well as targeted therapy. Nevertheless, the overall prognosis of advanced gastric cancer remains poor (3,4). We must find new diagnostic approaches to detect gastric cancer in the early stage along with efficient therapies to improve the prognosis of gastric cancer.

Circular RNAs, also known as circRNAs, used to be thought of as the byproducts of the normal splicing process (5), and were first discovered nearly 43 years ago (6) and found pervasive expression in eukaryotic genes in 2012 (7). The critically biological functions of circRNAs were identified in 2013 (8). In recent years, there have been new research hotspots in the field of cancer, and the understandings about circRNAs have been deepened. Most circRNAs are non-coding RNA and are located in the cytoplasm. Different from linear RNAs, the downstream 3' splice site and the upstream 5' splice site of circRNAs are complementary. The formation of the unique "backsplice" causes circRNAs to become a highly conserved and stably covalently closed RNA circle (9). Recent works have revealed that circRNAs mostly generated from exons of protein-coding genes have numerous functions. For instance, they can act as miRNA sponges to regulate the expression of miRNA; binding tumor suppressor proteins by protein-binding sites on circRNAs to inhibit oncogenesis (10); the exons of the circRNAs may translate protein to influence the function of cell; changing mRNA expression from the host gene (11). Also, increasing

reports have found that circRNAs are aberrantly expressed in various cancers, such as breast cancer (12), colorectal carcinoma (CRC) (13), ovarian cancer (14), hepatocellular carcinoma (HCC) (15) and so on. More importantly, circRNAs with a highly stable structure are enriched in blood, plasma, and platelets. Meanwhile, changes in circRNA expression level (downregulation or upregulation) were found to be tightly related to tumorigenesis, progression, invasion, and cell proliferation. Given the circRNA properties mentioned above, we speculated that circRNAs might be promising cancer biomarkers that could be detected at an early stage of cancer and a therapeutic target to improve the prognosis of cancer (11).

This study aimed to screen the significantly cancer-related circRNAs in gastric cancer and find potential mechanisms among oncogenesis. First, microarray datasets of gastric cancer tissue were downloaded from Gene Expression Omnibus (GEO) database. Secondly, integrated bioinformatics analysis methods were applied to filter markedly differently expressed circRNAs (DECs). Thirdly, the targeted miRNAs and mRNAs were predicted. Subsequently, Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses about the DECs were performed to identify the functions of DECs. To validate the predictions about DECs targets, we also downloaded the expression data of miRNAs and mRNAs from The Cancer Genome Atlas (TCGA). Aberrantly expressed miRNAs and mRNAs were obtained by the edgeR package. Overlapped miRNAs and mRNAs in predicted results and outputs from the TCGA database were obtained by taking the intersection. Lastly, we discovered the *hsa\_circ\_0067934/hsa-mir-4705/BMPRI1B* axis, which promoted the occurrence and progression of gastric cancer by regulating the Hippo pathway and the signaling pathways regulating pluripotency of stem cells.

## Methods

### *Acquisition of expression data from GEO*

We screened circRNA expression profiles from Gene Expression Omnibus (GEO), a regularly updated database storing numerous circRNAs expression profiles (16), by searching the keywords "circRNA and gastric cancer." As a result, 8 datasets were identified. Further screening criteria were set as the following: (I) the specimens must be human gastric cancer tissue; (II) the type of datasets must be expression profiling by array; (III) samples must be able to

be divided into a normal group and cancer group; (IV) no special treatments, such as radiotherapy and chemotherapy, were applied on patients; (V) the name of circRNAs in the data of platforms must be a standard name or named by Agilent Arraystar. The raw expression profiles and platforms were retrieved.

#### ***Screening of aberrantly expressed circRNAs by integrated bioinformatics analysis***

Normalization and log<sub>2</sub> transformation for raw expression data of all datasets were performed. Then, the limma package in R software was used to analyze differentially expressed circRNAs (DECs) in each dataset (17).  $|\log_2\text{FoldChange}| > 1$  and P value  $< 0.05$  were set as cut-off criteria. In order to find more significant DECs, RobustRankAggreg was applied to integrate and rank all DECs (18). Statistical significance was defined as  $|\log_2\text{FoldChange}| > 1$  and adj. P value  $< 0.05$ .

#### ***Prediction of miRNAs interacted with circRNAs***

Cancer-specific circRNA database (CSCD) is a powerful web tool to annotate circRNAs functions, including the prediction of microRNA response element sites (MRE), RNA-binding protein sites, and potential open reading frames (19). Hence, we made use of CSCD to forecast the miRNAs which bind to differentially expressed circRNAs.

#### ***Prediction of miRNA target genes***

The public databases, such as miRDB (20), miRTar Base (21), Target Scan (22), are practical online resources that can carry on functional annotations of miRNAs and predict miRNA-targeted genes. We took miRNAs overlapped in the three databases as significant targets.

#### ***Functional enrichment analysis***

Database for Annotation, Visualization, and Integrated Discovery (DAVID), an online software that can conduct Gene Ontology (GO) analysis, can perform concretely functional annotations [the molecular function (MF), biological process (BP), and cellular component (CC)] of genes (23). Then, we took DAVID to implement GO analysis about predicted genes, and the P value  $< 0.01$  was statistically significant. As an R software package that enables us to carry on biological-term classification and the

enrichment analysis of gene clusters, the clusterProfiler package was run for the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis to discover the potential pathways that DECs may participate in (24). P value  $< 0.01$  was set as the cut-off criterion. The R package ggplot2 was utilized to present the results.

#### ***Collection of miRNAs and genes expression data from TCGA***

TCGA is a publicly accessible database with thousands of tumor samples and contains a variety of molecular data such as gene expression, gene methylation, copy number variation (CNV), and so on (25,26). miRNAs and mRNA expression data tested by experiments in gastric cancer were retrieved from TCGA. The miRNA data met the following criteria: “Disease Type is Adenomas” AND “Primary site is stomach” AND “Project id is TCGA-STAD” AND “Data Category is Transcriptome Profiling” AND “Data Type is miRNA Expression Quantification” AND “Experimental Strategy is miRNA-Seq”, were available. Similarly, the standards set to filter the datasets of mRNAs were as follows: “Disease Type is Adenomas” AND “Primary site is stomach” AND “Project id is TCGA-STAD” AND “Workflow Type is HTseq-Counta” AND “Data Category is Transcriptome Profiling” AND “Data Type is Gene Expression Quantification” AND “Experimental Strategy is miRNA-Seq”.

#### ***Selection of differently expressed miRNAs and genes in gastric cancer from TCGA***

The aberrantly expressed miRNAs and genes were identified using edgeR package, a software used to analyze differential expression in RNA-seq datasets (27,28), and  $|\log_2\text{FoldChange}| > 1$  and P value  $< 0.05$  were defined as screening standards.

## **Results**

### ***Identification of DECs in gastric cancer***

According to the above limitations, four datasets, including GSE78092, GSE83521, GSE89143, and GSE93541, were screened, and the details are shown in *Table 1*. The expression profiles of the four datasets were normalized and log<sub>2</sub>-transformed. Based on these standardized data, we conducted the aberrantly expressed analysis to screen the

**Table 1** Details for datasets from GEO

GEO	Platform	Sample	Normal	Tumor	Submission	Update	Author
GSE78092	GPL21485	Tissue	3	3	Feb 19, 2016	Oct 26, 2017	Huang YS
GSE83521	GPL19978	Tissue	6	6	Jun 20, 2016	Dec 15, 2017	Zhang Y
GSE89143	GPL19978	Tissue	3	3	Oct 25, 2016	Jun 27, 2017	Guo J
GSE93541	GPL19978	Plasma	3	3	Jan 12, 2017	Jan 16, 2017	Guo J

GEO, Gene Expression Omnibus.

differentially expressed circRNAs (DECs) among the four datasets by limma package with  $|\log_2\text{FoldChange}| > 1$  and P value  $< 0.05$ , respectively. Eventually, a total of 196 DECs, consisting of 144 down-regulated circRNAs and 52 up-regulated circRNAs, were identified in GSE78092. In total, 148 DECs were obtained from GSE83521, including 66 down-regulated circRNAs and 82 up-regulated circRNAs. In total, 122 DECs with 94 down-regulated circRNAs and 28 up-regulated circRNAs were selected in GSE89143. Also, we screened 410 DECs consisting of 202 down-regulated circRNAs and 208 up-regulated circRNAs from GSE93541. The aberrant expression of four datasets was expressed in a volcano plot, as shown in *Figure 1*.

#### ***Integrated analysis based on RobustRankAggreg***

The analysis outcome of the single dataset was highly likely to increase the ratio of false-positive results. Taking this into account, we decided to adopt the RobustRankAggreg algorithm to integrate the DECs of four datasets, to improve the authenticity of the results. Through rank analysis, two DECs, which were most likely to be linked to gastric cancer, were screened with an adjusted P value  $< 0.05$ , namely *hsa\_circ\_0007991* and *hsa\_circ\_0067934*. *Figure 2* displays the expression of two DECs among the four datasets and structural patterns of two DECs. The characteristics of two DECs are described in *Table 2*.

#### ***Identification of miRNAs and genes at the downstream of DECs***

As an online web tool that could assist us in obtaining the MREs of circRNAs, the CSCD was utilized to forecast the miRNAs binding to the DECs. As a consequence, a total of 60 miRNAs were discovered at the downstream of *hsa\_circ\_0007991*, and 36 miRNAs were selected at the downstream of *hsa\_circ\_0067934*. Furthermore, we took advantage of three online resources, miRDB, miRTarBase,

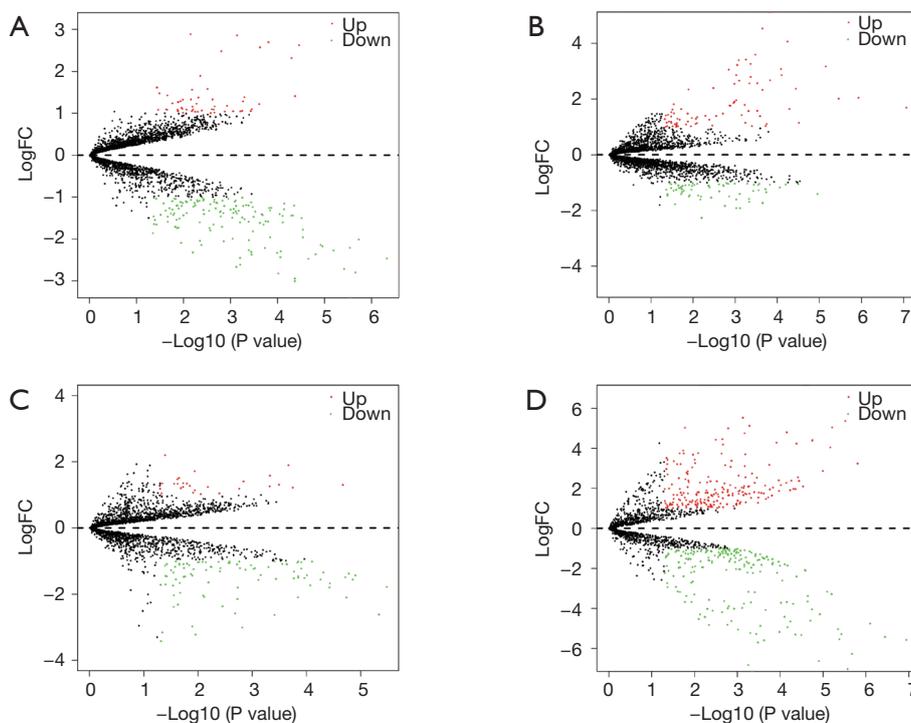
and TargetScan, to predict the miRNA-targeted genes. Genes simultaneously existing in three databases and the relevant miRNAs were included in our study.

#### ***GO enrichment analysis of DECs***

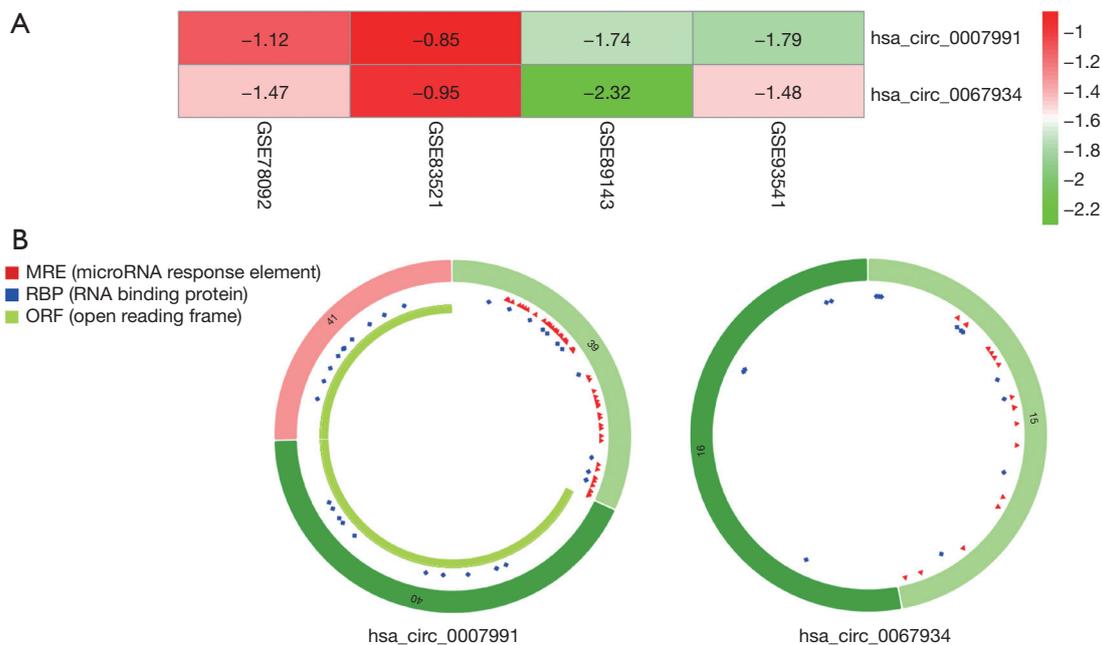
GO enrichment analysis mainly consists of three parts, CC, BP, and MF, and was performed using the DAVID, a powerful online tool. The results are displayed in *Figure 3*. In the cell composition group, the predicted genes at the downstream of *hsa\_circ\_0007991* were primarily enriched in the nucleus, cytoplasm, and focal adhesion. The predicted genes at the downstream of *hsa\_circ\_0067934* were chiefly enriched in nucleoplasm, nucleus, and cytoplasm. In the MF group, the predicted genes at the downstream of *hsa\_circ\_0007991* were primarily concentrated in poly (A) RNA binding, protein binding, and nucleic acid binding. The predicted genes at the downstream of *hsa\_circ\_0067934* were mainly enriched in protein binding, poly (A) RNA binding, and ubiquitin-protein ligase binding. In the BP group, the predicted genes at the downstream of *hsa\_circ\_0007991* were primarily enriched in regulation of transcription, DNA-templated, transcription, and positive regulation of protein insertion into mitochondrial membrane involved in the apoptotic signaling pathway. The predicted genes at the downstream of *hsa\_circ\_0067934* were mainly enriched in Wnt signaling pathway, calcium modulating pathway, negative regulation of transcription from RNA polymerase II promoter. It illustrated that DECs existed in the nucleus and cytoplasm and could bind to protein and RNA, and therein participated in the regulation of transcription and signaling pathway, ultimately leading to the occurrence of diseases. These results are consistent with other reported conclusions.

#### ***Pathway analysis of DECs***

KEGG pathway analysis was conducted with a clusterProfiler



**Figure 1** Differential expression of four datasets from GEO. (A) GSE78092, (B) GSE83521, (C) GSE89143, (D) GSE93541. DECs were screened with  $|\log\text{FoldChange}| > 1$  and P value  $< 0.05$ . DECs, differently expressed circRNAs; FC, fold change; GEO, Gene Expression Omnibus.

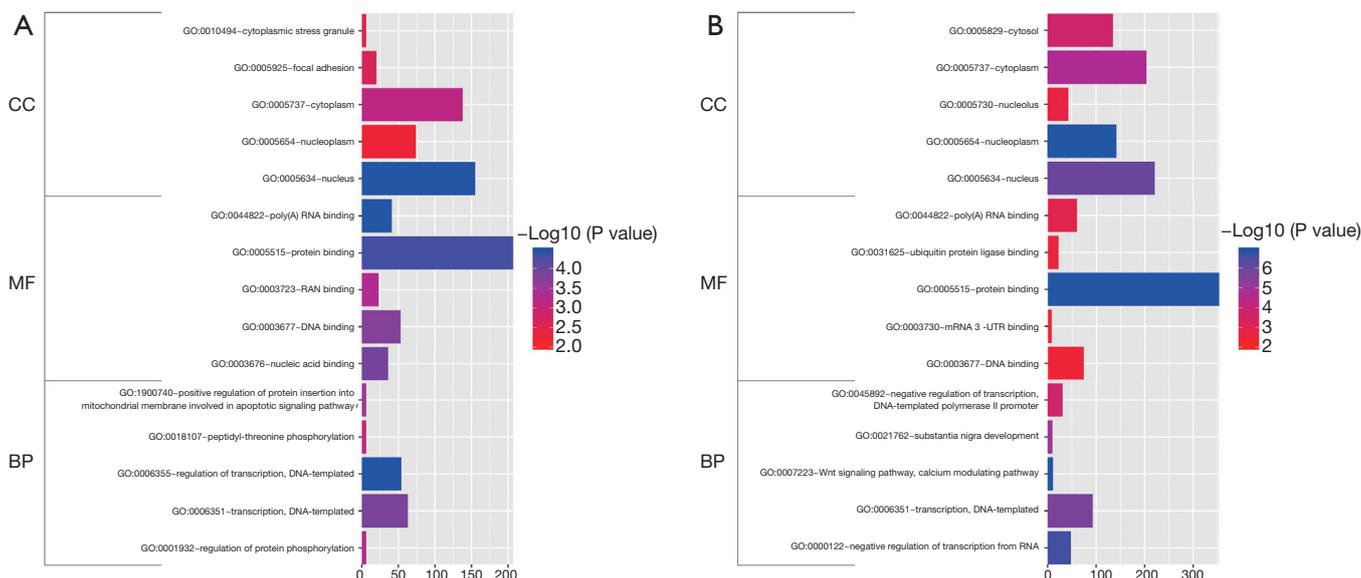


**Figure 2** The expression level and structural information of DECs in gastric cancer. (A) Heatmap of DECs obtained by integrated analysis; adjusted P-value  $< 0.05$  was set as a criterion. (B) Structural information of *hsa\_circ\_0007991* and *hsa\_circ\_0067934* performed by CSCD. DECs, differently expressed circRNAs; CSCD, Cancer-Specific circRNA Database.

**Table 2** The characteristics of two DEC

CircRNA ID	Position	Strand	Best transcript	Gene symbol	CircRNA study
hsa_circ_0007991	chr1:21329205-21415706	-	NM_001198801	EIF4G3	Jeck2013, Maass2017, Rybak2015, alzman2013
hsa_circ_0067934	chr3:170013698-170015181	+	NM_002740	PRKCI	Rybak2015, alzman2013

DECs, differently expressed circRNAs.



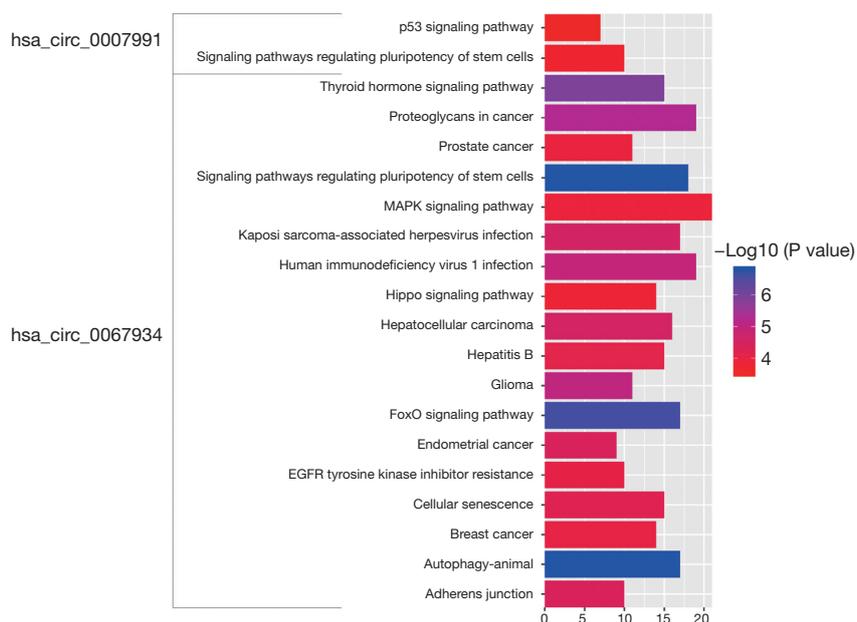
**Figure 3** GO enrichment analyses of genes at the downstream of DEC in gastric cancer. (A) *hsa\_circ\_0007991*-related GO analysis; (B) *hsa\_circ\_0067934*-related GO analysis. The top five terms in three functional groups: molecular function, biological processes, and cell composition, were presented. DEC, differently expressed circRNAs.

package in R software. The results are shown in *Figure 4*. The signaling pathways of genes at the downstream of *hsa\_circ\_0007991* were involved in two pathways: signaling pathways regulating the pluripotency of stem cells and the p53 signaling pathway. The signaling pathways of genes at the downstream of *hsa\_circ\_0067934* were primarily concentrated in signaling pathways regulating pluripotency of stem cells, autophagy-animal, and FoxO signaling pathway. The results proved that *hsa\_circ\_0007991* and *hsa\_circ\_0067934* had a close relationship with cancer-related pathways and were the initiators of gastric cancer.

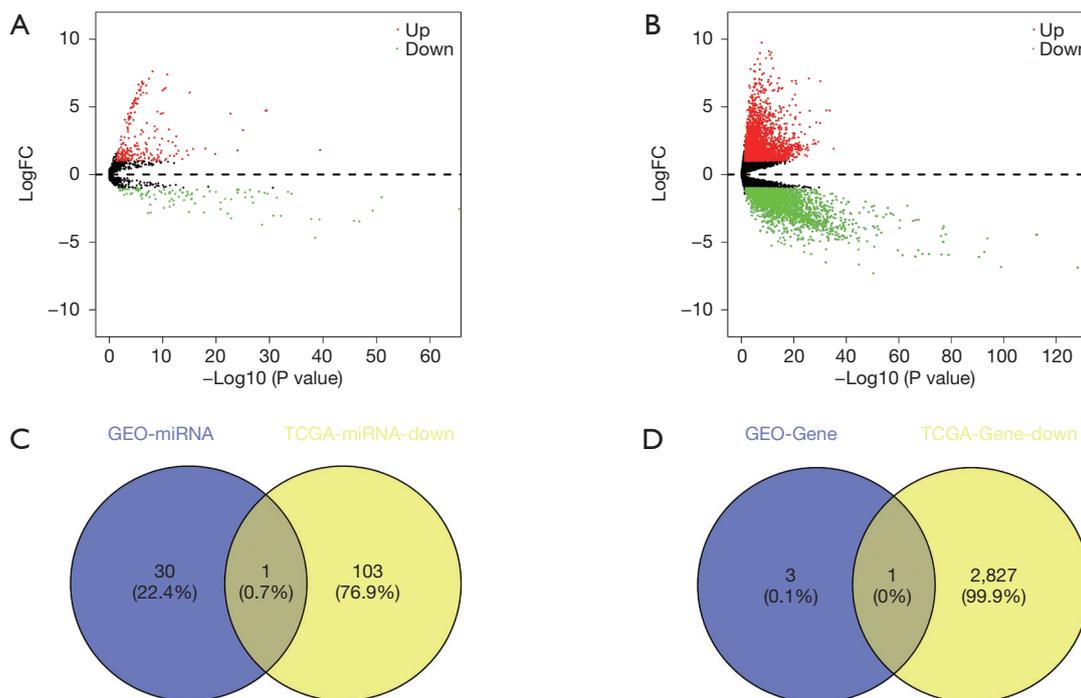
#### Validation of miRNAs and genes at downstream of *hsa\_circ\_0007991* and *hsa\_circ\_0067934* through the data from TCGA

The miRNAs expression profiles of 42 gastric cancer

samples and 410 normal controls were retrieved from TCGA. The expression of genes among 30 gastric cancer samples and 343 normal controls were downloaded from TCGA. All of them were analyzed with edgeR package from the Bioconductor project, independently. Volcano plots are shown in *Figure 5*. As a result, a total of 338 aberrantly expressed miRNAs were determined. Among them, 104 down-regulated miRNAs and 234 up-regulated miRNAs were included. A total of 7042 differentially expressed genes, containing 2828 down-regulated genes and 4214 up-regulated genes, were detected. To examine whether the predicted miRNAs and genes at the downstream of *hsa\_circ\_0007991* and *hsa\_circ\_0067934* obtained through bioinformatics methods were verified in the experiment, we took the intersection between the predicted miRNAs and genes and the aberrantly expressed miRNAs and genes in the expression data from TCGA. No overlapped miRNAs



**Figure 4** KEGG enrichment analyses of genes at the downstream of DEC in gastric cancer. The clusterProfiler package was utilized to screen a significant pathway with P value <0.05. The top 18 pathways related to DEC are shown in the picture. DEC, differently expressed circRNAs.



**Figure 5** Differentially expressed miRNAs and mRNAs in gastric cancer. (A) Differential expression of miRNAs from TCGA; (B) Differential expression genes from TCGA;  $|\log_2(\text{FoldChange})| > 1$  and P value <0.05 were set as criteria; (C) Venn diagram for the miRNAs intersections; (D) Venn diagram for the mRNAs intersections. Because there were no overlapped terms between the group of up-regulated miRNAs or genes from TCGA and the predicted miRNAs or genes at the downstream of *hsa\_circ\_0067934*, the Venn diagram is not shown.

at the downstream of *hsa\_circ\_0007991* were found, and *hsa-mir-4705* at the downstream of *hsa\_circ\_0067934* was identified in both datasets (Figure 5C). Therefore, we took further analysis to study the genes at the downstream of *hsa-mir-4705*. Among four *hsa-mir-4705*-targeted genes (*TMEM68*, *BMPR1B*, *MCFD2*, and *ASH1L*), only *BMPR1B* was verified in the expression data from TCGA (Figure 5D). In summary, we speculate that there may be a *hsa\_circ\_0067934/hsa-mir-4705/BMPRI1B* axis, which plays a vital role in the occurrence and development of gastric cancer.

## Discussion

Gastric cancer is the third most lethal cancer in the world (1,29), and the most common type of gastric cancer is adenocarcinoma, which can be divided into two subtypes, including intestinal and diffuse types, based on the Lauren classification (30). Also, gastric cancer is a complicated disease attributed to the interaction of environmental and host-associated factors. The biological and genetic characteristics may play leading roles in the initiation and development of gastric cancer (4,31). Reports have revealed that the poor outcome of patients with gastric cancer is mainly caused by the lack of diagnosed approach at the early-stage gastric cancer; thus, the gene diagnosis (32) and targeted therapy (33,34) might represent a new dawn for curing gastric cancer. Over the past few decades, the bioinformatics approach has been widely used to detect potential therapeutic targets for gastric cancer with the boom of microarray and high-throughput sequencing technologies. Trastuzumab, the inhibitor of *HER-2*, has been demonstrated to be effective in patients with *HER2*-positive gastric cancer (35,36). However, anti-*EGFR* and anti-*MET/HGF* therapies do not remarkably improve the overall survival (OS) in gastric cancer (37-40). The emerging PD-1inhibitors, pembrolizumab and nivolumab may be useful in suppressing the development of gastric cancer (41,42). There is still much room for exploration in the field of targeted therapy for gastric cancer.

As the highlights of cancers in recent years, circRNAs have been held to have the potential to become diagnostic and prognostic biomarkers due to the highly conserved and stable loop structure and tissue-specificity (11,43,44). An abundance of research has illustrated the importance of circRNAs in the progression of cancers. For instance, Han *et al.* discovered that *circMTO1* is down-regulated in HCC tissues and can repress the development of HCC

by sponging *miR-9* to increase *p21* expression (45). A recent publication in the *Journal of Hepatology* discovered that *cSMARCA5* behaved as a tumor suppressor in HCC, and was able to interact with miR-17-3p and *miR-181b-5p* to promote the expression of an anti-oncogene called *TIMP3* (46). Furthermore, *circ-SHPRH* could code tumor suppressor protein, *SHPRH-146aa*, to regulate the progression of glioblastomas (47). Based on existing research results, we concluded that circRNAs were critical molecules to understand the underlying mechanism of carcinoma progression.

In our study, four expression profiles (GSE78092, GSE83521, GSE89143, and GSE93541) were retrieved from the GEO database and integrated to analyze the aberrantly expressed circRNAs using the RobustRankAggreg algorithm. Through integrated analysis, two circRNAs, namely *hsa\_circ\_0007991* and *hsa\_circ\_0067934*, were screened, and all of them were dysregulated in gastric cancer tissue. According to the theory of MREs, we utilized CSCD, a web tool, to predict the miRNAs interacting with DECs. Sixty miRNAs were forecasted at the downstream of *hsa\_circ\_0007991*, and 36 miRNAs were *hsa\_circ\_0067934*-targeted. Then, three online resources, miRDB, miRTarBase, and TargetScan, were combined to predict the miRNA-targeted genes. Genes simultaneously existed in three databases and relevant miRNAs were included in our study. Forty miRNAs and 371 genes were determined at the downstream of *hsa\_circ\_0007991*, and 31miRNAs and 530 genes were predicted at the downstream of *hsa\_circ\_0067934*.

Furthermore, to understand the functions of *hsa\_circ\_0007991* and *hsa\_circ\_0067934* in gastric cancer, we performed the GO and KEGG analyses. CircRNA *hsa\_circ\_0007991* acted on genes enriched in the nucleus and cytoplasm. These genes could bind to RNA, protein, and nucleic acid, and regulated transcription and protein insertion into mitochondrial membrane involved in the apoptotic signaling pathway, leading to abnormal signal transductions of signaling pathways regulating pluripotency of stem cells and *p53* signaling pathway.

Similarly, the *hsa\_circ\_0067934*-targeted genes were concentrated in the nucleoplasm, nucleus, and cytoplasm, and interacted with RNA, protein, and ubiquitin-protein ligase. Finally, it could have obstructed the signal conduction of the *Wnt* signaling pathway and calcium modulating pathway. This may be a potent mechanism in gastric cancer. These discoveries indicate that *hsa\_circ\_0007991* and *hsa\_circ\_0067934* may be pivotal molecules in the development

mechanism of gastric cancer and could be a potential target for gastric cancer.

To ascertain the expression of predicted miRNAs and genes at the downstream of DECs, we obtained the expression profiles of miRNAs and mRNAs from TCGA database. The differently expressed analyses were performed separately by edgeR package in R software. In total, 104 down-regulated miRNAs and 234 up-regulated miRNAs were identified. A total of 7,042 differentially expressed genes, including 2,828 down-regulated genes and 4,214 up-regulated genes, were detected. Subsequently, we obtained overlapped miRNAs and genes in the predicted results and outputs from TCGA database by the intersection method. The predicted miRNAs at the downstream of *hsa\_circ\_0007991* were not found in expression data from TCGA.

Nevertheless, *hsa-mir-4705*, a *hsa\_circ\_0067934*-targeted miRNA, was overlapped in two datasets, and the expression of it in data from TCGA was down-regulated. Accordingly, we took *hsa-mir-4705* as the focus. *BMPR1B*, one of the four dysregulated genes (*TMEM68*, *BMPR1B*, *MCFD2*, and *ASH1L*) at the downstream of *hsa\_circ\_0067934* was confirmed in DEGs from TCGA database. As mentioned above, we deduced that *hsa\_circ\_0067934* could regulate the expression of *BMPR1B* by interacting with *hsa-mir-4705*, and all of them functioned as a tumor suppressor in gastric cancer. In other words, there may be a *hsa\_circ\_0067934/hsa-mir-4705/BMPR1B* axis to regulate the occurrence, progression, and prognosis of gastric cancer. To study the potential pathway related to the *hsa\_circ\_0067934/hsa-mir-4705/BMPR1B* axis, we researched the predicted pathways that *BMPR1B* at the downstream of *hsa\_circ\_0067934* participated in. Three pathways, including signaling pathways regulating pluripotency of stem cells, the Hippo signaling pathway, and axon guidance, were included. Among them, signaling pathways regulating the pluripotency of stem cells and the Hippo signaling pathway was involved with carcinomas (Figure 6). This implies that the *hsa\_circ\_0067934/hsa-mir-4705/BMPR1B* axis regulated the differentiation of gastric cells and the progression of gastric cancer by impacting signaling pathways regulating pluripotency of stem cells and Hippo signaling pathway.

A previous study had reported that mechanisms mediated by circRNAs are liable for the progression of gastric cancer (48). *hsa\_circ\_002059* (49) and *hsa\_circ\_0014717* (50) were found significantly dysregulated in gastric cancer tissues and could be detected in body fluid. In other

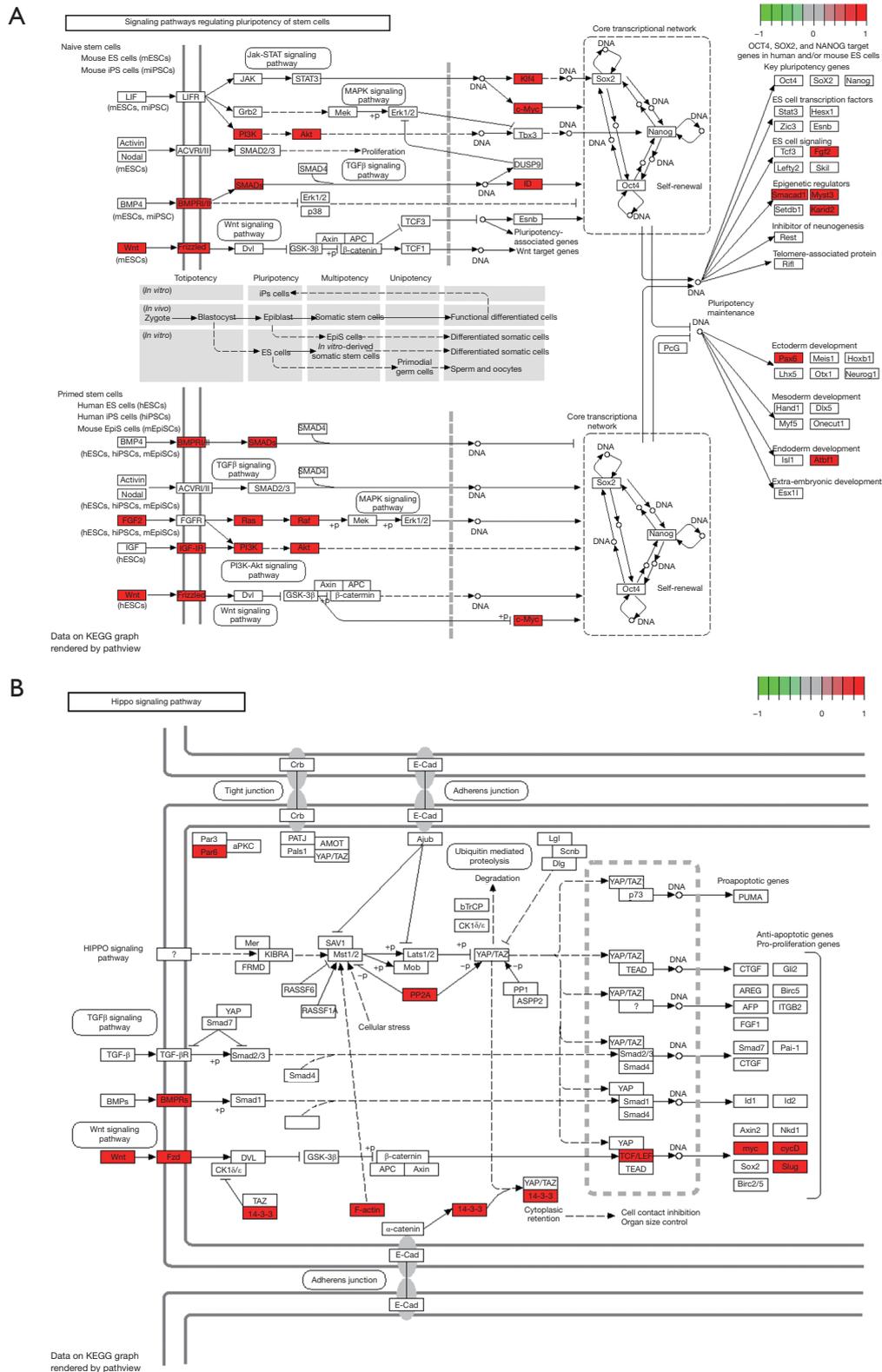
analyses, the low expression of *hsa\_circ\_0001649* (51), *hsa\_circ\_104916* (52), and *hsa\_circ\_100269* (53) were closely related to the development of gastric cancer. As for *hsa\_circ\_0067934* in our study, Xia *et al.* demonstrated that *hsa\_circ\_0067934* is up-regulated in ESCC and promoted the proliferation of ESCC cells. The reason why the result in the study of Xia *et al.* is contrary to our conclusion might be that circRNAs are tissue-specific (54). In the same way, the *hsa\_circ\_0067934* could also be detected in plasma (the samples of gastric cancer were plasma in the GSE93541 dataset) and have a low expression.

Consequently, we inferred that *hsa\_circ\_0067934* might be a novel biomarker and therapeutic target in gastric cancer. The *hsa-mir-4705* was firstly discovered in breast cancer through the next-generation sequencing approach (55). Not much is known about how *hsa-mir-4705* regulates the progression of carcinoma. Our study disclosed that the dysregulated *hsa-mir-4705* suppressed the expression of *BMPR1B* in gastric cancer.

Further experiments are needed to illustrate the role of *hsa-mir-4705* in gastric cancer. *BMPR1B*, as the target of *hsa-mir-4705*, is a transmembrane serine-threonine kinase receptor among the bone morphogenetic protein (BMP) receptor family (56). The responsibility of it is to transduce the BMPs signal by binding to transforming growth factor- $\beta$  (TGF- $\beta$ ) and activin (57). Recent studies indicated that *BMPR1B* acted as an oncogenesis factor, and the declined expression of it was highly connected with the poor prognosis of breast cancer (58,59). These discoveries are consistent with our results. Chapellier *et al.* demonstrated the vital role of *BMPR1B* in the initiation of stem cell transformation (60). The Hippo pathway is conserved, and the pivotal signaling pathway functions as a regulator to modulate cell proliferation, differentiation, and apoptosis, leading to the alteration of tissue growth and homeostasis. The processes are mainly mediated by *YAP* and *TAZ*. Moreover, *BMPR1B* can possibly integrate with the Hippo pathway through crossing talks with TGF- $\beta$  signaling pathways (61). Evidence is available that elucidates the connections between *BMPR1B* and signaling pathways regulating the pluripotency of stem cells and the Hippo pathway.

## Conclusions

In summary, we detected two DECs, *hsa\_circ\_0007991*, and *hsa\_circ\_0067934*, which were involved in the progression mechanisms of gastric cancer and could be novel diagnostic



**Figure 6** Two cancer-related pathway possibly effected by *bsa\_circ\_0067934/bsa-mir-4705/BMPR1B* regulatory axis in gastric cancer. (A) Signaling pathways regulating pluripotency of stem cells; (B) Hippo signaling pathway.

or prognostic markers, as well as therapeutic targets for gastric cancer. Also, a *hsa\_circ\_0067934/hsa-mir-4705/BMPRI1B* regulatory axis was identified in gastric cancer. No matter which molecules among *hsa\_circ\_0067934*, *hsa-mir-4705*, or *BMPRI1B* were abnormally expressed, the signal transduction of signaling pathways regulating pluripotency of stem cells and Hippo pathway would be disturbed and accompany the occurrence and progression of gastric cancer. This may be one of the underlying mechanisms by which gastric cancer develops. Indeed, the absence of experiments is the most significant limitation in our study. We do realize the problem, and further experiments are on the schedule. We will knock down the *hsa\_circ\_0067934* in the normal gastric cell line to examine whether the changes of *hsa-mir-4705* and *BMPRI1B* expression are accordant with the results we predicted in this study. More functional assays will also be conducted to validate the effects of the *hsa\_circ\_0067934/hsa-mir-4705/BMPRI1B* regulatory axis on the biological behavior of gastric cancer cells.

### Acknowledgments

*Funding:* None.

### Footnote

*Conflicts of Interest:* All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2019.10.32>). The authors have no conflicts of interest to declare.

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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**Cite this article as:** He SN, Guan SH, Wu MY, Li W, Xu MD, Tao M. Down-regulated hsa\_circ\_0067934 facilitated the progression of gastric cancer by sponging hsa-mir-4705 to downgrade the expression of BMPRII. *Transl Cancer Res* 2019;8(8):2691-2703. doi: 10.21037/tcr.2019.10.32