Revista Argentina de Clínica Psicológica
2021, Vol. XXX, N°2, 259-265
DOI: 10.24205/03276716.2020.40224

Association between Has-circ-0067997 with PI3K/AKT Signaling Pathway in Gastric Carcinoma Oncological Characteristics

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Abstract

Objective: To investigate the relationship between has-circ-0067997 and PI3K/AKT signaling pathway effecting gastric carcinoma cell line SGC-7901 oncological characteristics.

Methods: Circ-RNA chips used for mining different gene expression, mining different gene, High-throughput sequencing combined with computational systems mining relevant signaling pathway and biological process, Pubmed online database retrieved for miRNA connection with circ-RNA, Crisper/Cas9 structured circ-RNA-knock down cell line, CCK-8 used for cell proliferation analysis, Western blotting and Real-Time PCR used for tested the levels of PI3k/AKT protein and gene expressed.

Results: Circ-RNA chips showed has-circ-0067997 high expressed in chemical drug resisted patients, targeted miRNA High-throughput sequencing indicted has-circ-0067997 related to PI3k/AKT signaling pathway, Circ-RNA interaction online tool mined has-circ-0067997 maybe connect with hsa-miR-127-5p, the biological process and signaling pathway refers to pathway hsa05200: pathways in cancer. The cells' proliferation increased combined with has-circ-0067997 and hsa-miR-127-5p gene silence. WB and PCR examined PI3k/AKT protein and gene down-regulated combined with has-circ-0067997 and hsa-miR-127-5p gene silence.

Conclusion: has-circ-0067997 connects with hsa-miR-127-5p through PI3K/AKT signaling pathway promotes gastric carcinoma cell line's oncological characteristics

Keywords: Has-circ-0067997, PI3K/AKT Signaling Pathway, Gastric Carcinoma, Oncological Characteristics

Introduction

The incidence of gastric cancer occupies the top fifth in all kinds of malignant disease [1], the poor progress and the rapid increasing occurrence of gastric cancer become a common challenge of world public health [2], Circ-RNA is a king of deduce closed circular and non-coding RNA [3]. Recently reports investigate crucial role in regulating tumor and tissue's canceration, participate tumor formation and deterioration [4-6], circ-RNA derives from reverse splicing or exon skipping of pre-mRNAs and mutated miRNA 3'UTR [7], miRNA 3'UTR mutation could induce promoting or inhibiting tumor activities [8], our advanced study indicted has-circ-0067997 up-regulated in chemical resisted patients, this study we will investigate the relationship between circ-0067997 and molecular signaling pathway and predict the connection of circ-0067997 and micro-RNA, explored the impaction on gastric cancer cell lineSGC-7901.

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Materials and Methods

Cell line assessment and progression

STAD cell line SGC-7901 and SGC-7901/CDDP assessed from the Global Bioresource Center (ATCC, EY-X0724, USA), incubated SGC-7901/ SGC-7901/CDDP with a total medium contained RPMI1640 (Gibco, USA), final concentration 10% fetal bovine serum (FBS, Gibco, USA), 1% penicillin solution (Beyotime, China) and streptomycin solution (Beyotime, China) for 24h after cell-thawing, 5%-10% trypsin digestion fluid (Beyotime, China) used for sub-culturing, as

different concentrations of cisplatin () covered on the STAD cells.

CCK-8 Kit analysis

STAD cells seeded into 96wells culture plates, covered as a final concentration cisplatin as 0、1、2、4、8、16ug/ml for 24h, 48h, 72h, CCK-8 cell proliferation Kits () examined STAD cells cell availability. Gen5.3 software analyzed absorbance value.

Western-blotting

Tris-HCL(PH=8.8), Tris-HCL(PH=6.8), 30%acrylamide, 1% ammonium peroxydisulfate (APS), Tetramethylethylenediamine (TEMED,) used for SDS-PAGE, 100v electrophoresis for 1h, 110v transmembraned for 1h 40min, NC membrane used for transferring the protein, 5%BSA() blocking the protein for 1h, incubated the primary anti-body overnight(the concentration of antibody shown in supplementary Table1), 5% PBS-T washed the membrane three-five times, HRP-secondary anti-body incubated for1h, ECL Western Blotting Substrate Kit (ThermoFisher, 32109, Wuhan, CHINA) used for detected the level of protein expressed.

Real-Time PCR

TROzil lysate (Beyotime, China) smashed STAD cells, according to organic extraction principle Isopropanol and Tri-chloromethane purified RNA, FastKing One Step RT-PCR Kit (Tiangen, KR123, Beijing, CHINA) used for reverse transcription and detection of gene levels, the protocol contained: reverse transcription, denaturation, primer annealing, polymerization (primer sequence shown in supplementary Tables). Primary cq value calculated: Ct=-1/lg(1+Ex)*lgX0+lgN/lg(1+Ex) Crisper/Cas9 structure has-circ-0067997 silence and hsa-miR-127-5p 3'UTR mutation STAD cell lines.

Computing data from online database

Pubmed database screened circ-RNA different expression for GSE83521, GSE78092, GSE89143. GEO2R online tool computed for details of datasets. CircrRNA interaction tools predicted for targets miRNA(https://circinteractome.nia.nih.gov/index), MetaScape online analyzed tools predicted relevant mRNA(gene)'s Biological process (BP) and signaling pathway, micro-bioinformatics tools (http://www.bioinformatics.com.cn) designed the binding sites of miRNA and circRNA.

Results

GSE datasets analyzed has-circ-0067997 expression in gastric cancer:

We screened Pubmed GSE83521, GSE78092, GSE89143 datasets, and different gene expression shown in Figure 1a, 1b, 1c. The interaction of datasets shown in Figure 1d.

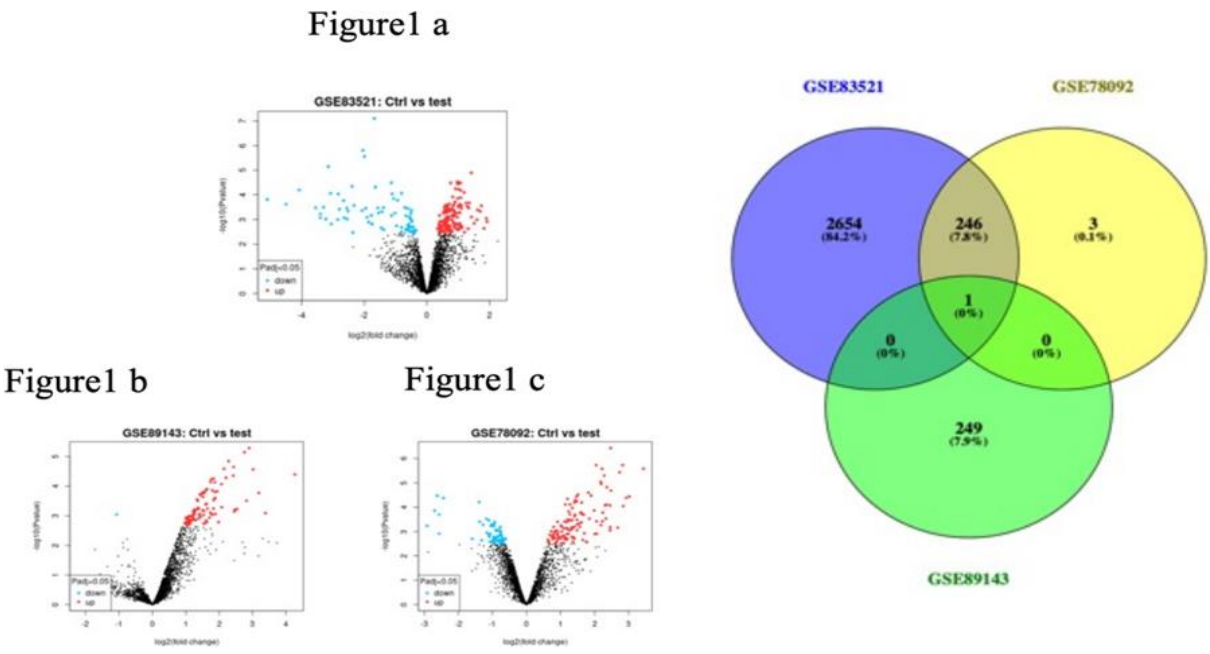


Figure 1. **GSE datasets analyzed has-circ-0067997 expression in gastric cancer.**

(GSE83521, GSE78092, GSE89143 datasets different hsa_circ_RNA expression and combination of each datasets, Log10(P)>1, *P<0.05*)

The performance indicted

hsa_circ_RNA0067997 expressed significantly in cancer tissues.

Hsa_circ_RNA 0067997 targets miRNA and gene prediction and Biological process enrichment:

Hsa_circ_RNA 0067997 shown in Figure 2a. According to the previous reports, miRNA-127-5p promotes tumorigenesis and progression, we predicted the chromosomes binding sites shown in Figure 2b. The relevant gene's biological process

Figure2 a

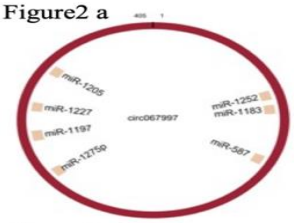
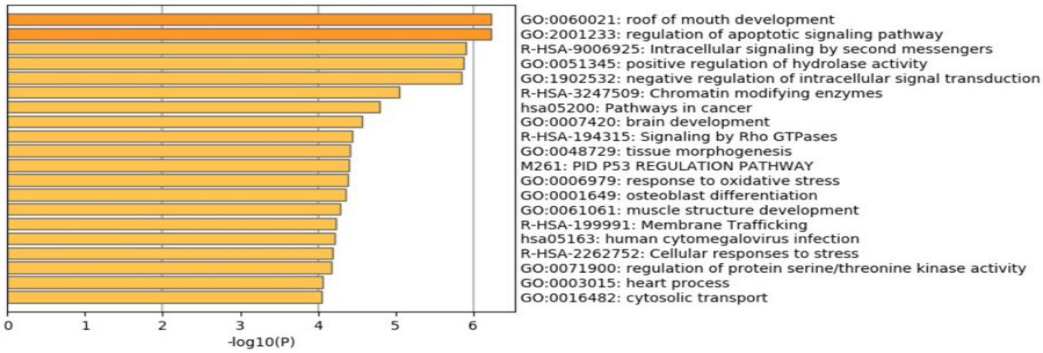


Figure2 b

hsa_circ-0067997 (5'...3')	UGCUCCGAGCUGUU-AGCUUCAC
hsa-miR-127-5p (5'...3')	UAGUCUCGGGAGACUCGAAGU

Figure2 c



The targets and binding sites miRNA of prediction shown in Figure 2c.

Figure 2. **Hsa_circ_RNA 0067997 targets miRNA and gene prediction and Biological process enrichment.**

(Targets of hsa_circ_RNA 0067997 and miRNA shown in Figure 2a, b, biological process of miRNA targets gene enrichment on KEGG and GO, Log10(P)>1)

Mainly biological process focused on pathways in cancer-hsa05200 shown in Figure 3, we designed hsa05200 as PTEN-PI3K-AKT signaling, the performance indicted hsa_circ_RNA 0067997 maybe refer to PI3K-AKT signaling induce gastric cancer progression.

KEGG pathway refers to hsa_circ_RNA 0067997 targeted genes:

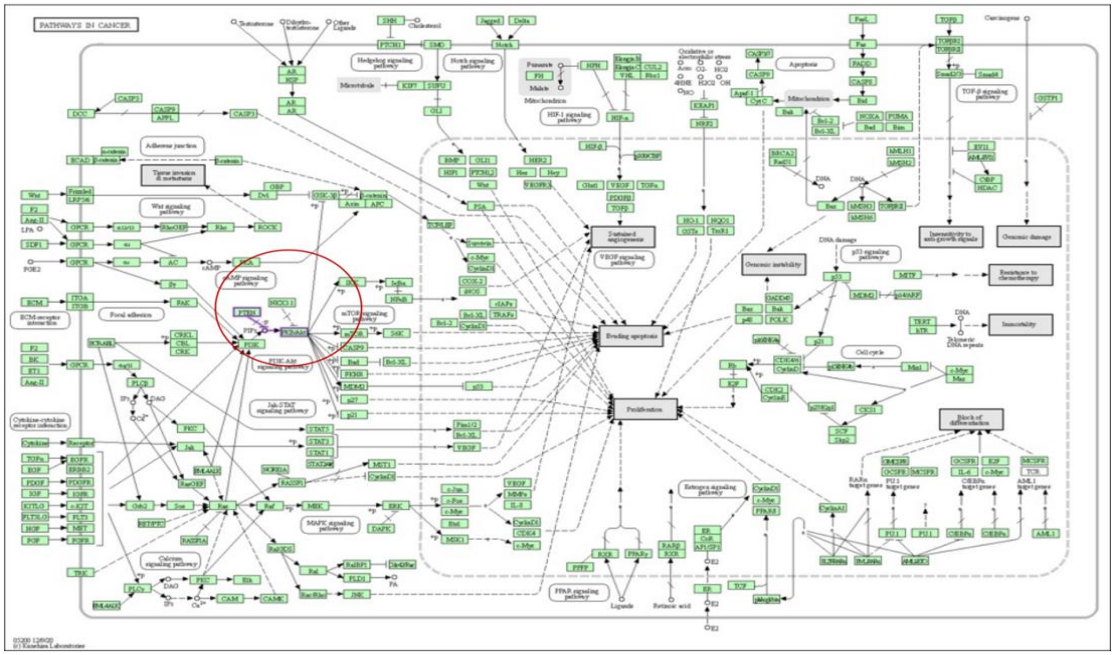


Figure 3. **KEGG pathway refers to hsa_circ_RNA 0067997 targeted genes.**

(The details of pathway hsa05200: pathways in cancer shown in Figure 3)

Has-circ-0067997 gene silence inhibits STAD cell proliferation:

To investigate the proliferation of SGC-7901 and SGC-7901/CDDP induced by has-circ-0067997, CCK-8 analysis examined the 24h, 48h, 72h cell proliferation, the exploration indicted has-circ-0067997 gene silence could increase SGC-7901 and SGC-7901/CDDP cell proliferation as shown in Figure 4a, 4b, 4c.

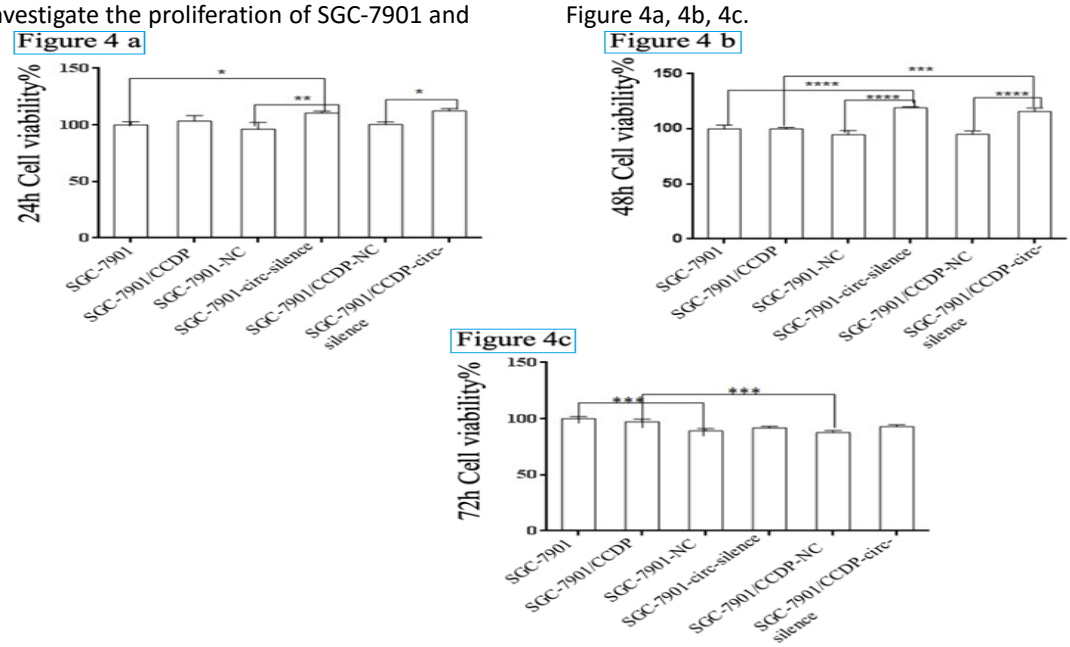


Figure 4. **Has-circ-0067997 gene silence induced increase of cell viability in 24h, 48h, 7(SGC-7901 and SGC-7901/CDDP cells' proliferation percentage was shown in Figure 4a, 4b, 4c presented data represent the expression of Mean± SE, * p <0.05)**

Hsa-miR-127-5p 3'UTR mutation regulation STAD oncological characteristics:

TargetScan database showed has-circ-0067997 could connect hsa-miR-127-5p 3'UTR sequence as shown in supplementary Figures, to investigate the function of hsa-miR-127-5p 3'UTR in regulating of STAD cells, CCK-8 examination indicted hsa-miR-127-5p 3'UTR mutation could down-regulate the cell proliferation as shown in Figure 5a, 5b, 5c.

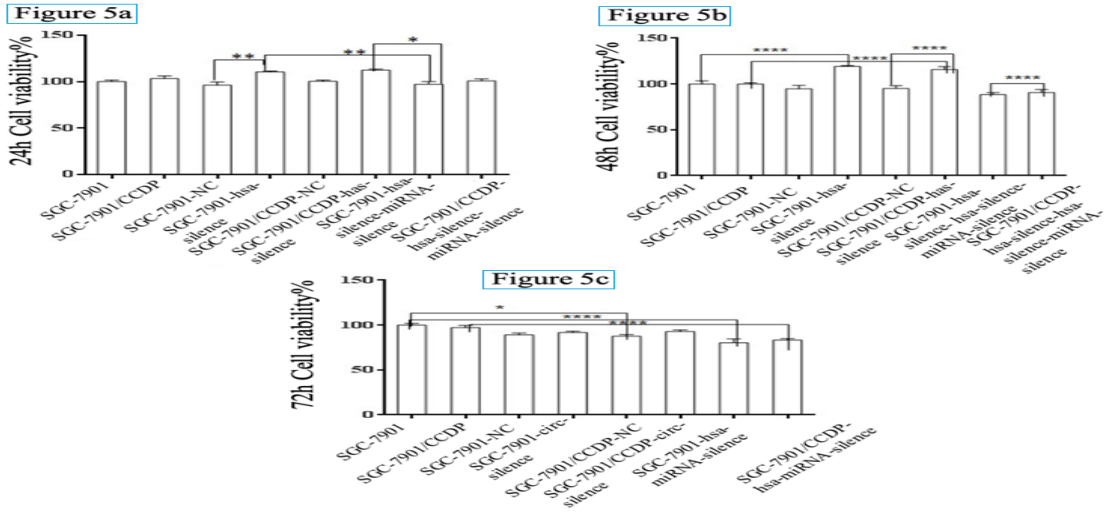


Figure 5. **hsa-miR-127-5p silence induced increase of cell viability in 24h, 48h, 72h.**

(SGC-7901 and SGC-7901/CDDP cells' with miR-127-5p silence proliferation percentage was shown in Figure 5a, 5b, 5c The presented data represent the expression of Mean± SE, * p <0.05)

Has-circ-0067997 silence and hsa-miR-127-5p silence inhibits PI3K/AKT signaling pathway activities:

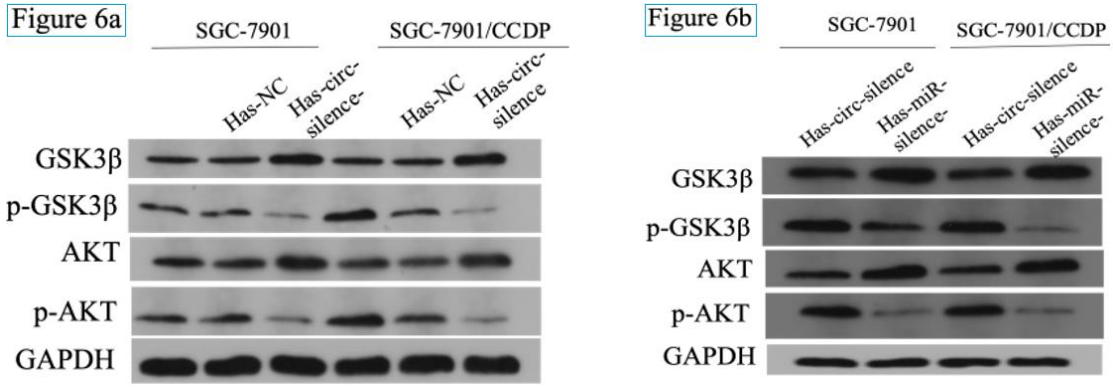


Figure 6. **The regulation of has-circ-0067997 and miR-127-5p silence in PI3K/AKT signaling pathway**

(A representative blot depicting the bands for GSK3β and AKT in has-circ-0067997 silence SGC-7901 and SGC-7901/CDDP cells; representative blot depicting the bands for GSK3β and AKT in miR-127-5p 3'UTR mutation SGC-7901 and SGC-7901/CDDP cells.)

According to our advanced exploration enriched hsa-circ-0067997 related to PI3K/AKT signaling pathway, to investigate the regulation of the relationship, WB analysis showed has-circ-0067997 silence could inhibit PI3K/AKT activities as shown in Figure 6, while has-circ-0067997 overexpressed could not restore the hsa-miR-127-5p silence induced down-regulation of PI3K/AKT activities as shown in Figure 6. Real-Time PCR examination showed the same phenomenon as shown in Figure 7a, 7b.

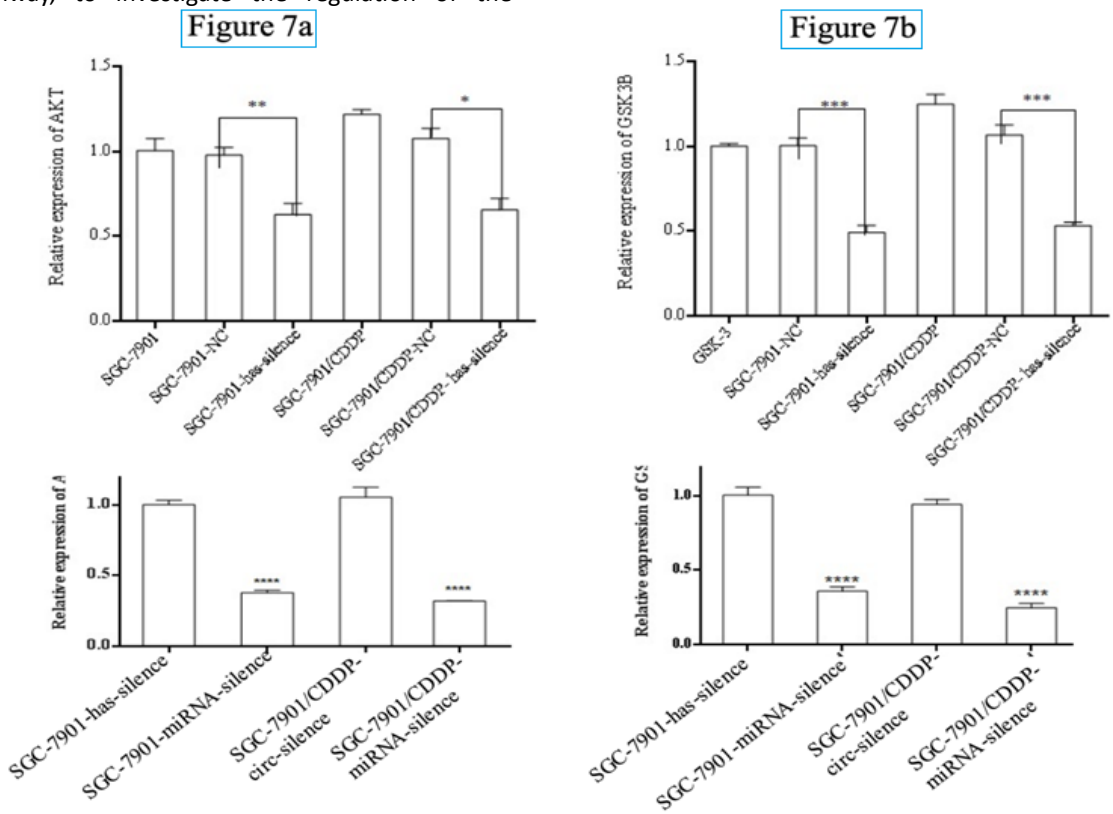


Figure 7. **Relevant gene expression of PI3K/AKT signaling pathway induce by has-circ-0067997 and miR-127-5p silence**

(Representative images of GSK3β and AKT expression in has-circ-0067997 silence and miR-127-5p silence SGC-7901 and SGC-7901/CDDP cells. The presented data represent the expression of

Mean± SE, * p <0.05)

Discussion

Gastric adenocarcinoma continues a common cause of death over the world with poor progress and highest incidences [9], the causes of morbidity identified as smoking, Helicobacter pylori infection and less commonly by autoimmune gastritis, high salty-diets [10-12] and microenvironment, immunocyte, molecular signaling pathway impacts on Gastric adenocarcinoma tumorigenesis, progress [13, 14], Cric-RNA is one of the noncoding-RNA, participating in regulating the protein transcription in the eukaryotic cells, unique circular structure of Circ-RNA refers to degradation by exonuclease and provides stability for linear parental genes [15, 16], a vast number of researches indicted Circ-RNA could impact on the cancer diseases in biogenesis, function, and clinical significance [17-20], according to our research, we found has-circ-0067997 overexpression in the gastric cancer patients High-throughput sequencing enriched has-circ-0067997 targets to miR-127-5p which related to PI3K/AKT signaling pathway, this study we established hsa-circ-0067997-silence and overexpressed SGC-7901 and SGC-7901/CDDP cell lines (examination of gene silence and overexpressed shown in supplementary Figures), the proliferation analysis indicted has-circ-0067997-silence could inhibit the function of SGC-7901 and SGC-7901/CDDP cell proliferated, as the literature reported circ-RNA may function in microRNA binding, protein interaction [21], we used TargetScan (http://www.targetscan.org/mamm_31/) for prediction microRNA sequence, we established hsa-miR-127-5p silence in the hsa-circ-0067997 overexpressed SGC-7901 and SGC-7901/CDDP cell lines, the examination explored the ability of proliferation could not be restored, so we got a primary conclusion: has-circ-0067997 combined with hsa-miR-127-5p impact on the gastric cancer cell oncological characteristics, finally we examined the molecular signaling pathway PI3K/AKT activities induced by has-circ-0067997 and hsa-miR-127-5p, WB analysis explored the levels of AKT, GSK3β increased combined with hsa-circ-0067997 silenced, he levels of p-AKT, p-GSK3β decreased combined with has-circ-0067997 silenced, whatever hsa-circ-0067997 overexpressed, hsa-miR-127-5p 3'UTR mutation induced the same examination, a vast number of researches reported AKT regulates vital downstream effector molecules and effectors via phosphorylation cascade reaction to control cell growth, proliferation, survival, genome stability [22-24], as all above experiments we got a conclusion: has-circ-0067997 combined with hsa-miR-127-5p regulated the gastric cancer cell's proliferation through PI3K/AKT signaling pathway.

Conclusion

It has-circ-0067997 combined with hsa-miR-127-5p regulated the gastric cancer cell's oncological characteristics through PI3K/AKT signaling pathway

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