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 kind 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.

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 RPM1640 (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 peroxysulfate (APS), Tetramethylthelyenediamine (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: \[ Ct=\frac{-1\log(1+Ex)\log N\log(1+Ex)}{\log(1+Ex)*\log X0+\log N\log(1+Ex)} \]
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. CircRNA 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

Figure1 a

Figure1 b

Figure1 c

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 indicated
Hsa_circ_RNA 0067997 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 prediction shown in Figure 2c.

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 indicated has-circ-0067997 gene silence could increase SGC-7901 and SGC-7901/CDDP cell proliferation as shown in Figure 4a, 4b, 4c.

![Figure 4a](image1)

![Figure 4b](image2)

![Figure 4c](image3)

Figure 4. Has-circ-0067997 gene silence induced increase of cell viability in 24h, 48h, 72h (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 indicated hsa-miR-127-5p 3’UTR mutation could down-regulate the cell proliferation as shown in Figure 5a, 5b, 5c.

![Figure 5a](image4)

![Figure 5b](image5)

![Figure 5c](image6)

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], Circ-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

References


