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Original Research

CircB3GNTL1 and miR-598 regulation effects on proliferation, apoptosis, and glutaminolysis in gastric cancer cells

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Abstract: This study was performed to research circB3GNTL1 control, miR-598 and its mechanism for cell proliferation, apoptosis and glutamine breakdown. For this purpose, CirB3GNTL1 and miR-598 expressions were detected by qRT-PCR in gastric and cell lines; MTT tests were performed to detect proliferation; flow cytometry was established in flow; glutamine decomposition was evaluated with glutamine, glutamic acid and α-keto-glutaric acid (α-KG) expression; Bcl-2, PCNA, ASCT2 and GLS1 expression levels were calculated; Methods of expression were calculated. The results showed that CircB3GNTL1 expression was up-regulated and miR-598 expression was up-regulated in gastric cancer tissues and cell lines; Knockdown circB3GNTL1 prevented proliferation and glutamine decomposition of the gastric cancer cells and induced apoptosis compared with normal para-cancers and gastric cancer cell lines. Results circB3GNTL1 can target and control miR-598 expression, and miR-598 can reverse the proliferation, apoptosis, and decomposition of glutamine from gastric cancer cells by knockdown circB3GNTL1. It was concluded that CirB3GNTL1 prevents decomposition of glutamines and induces apoptosis by controlling miR-598 in gastric cancer cells.

Key words: CircB3GNTL1; miR-598; Gastric cancer; Proliferation; Apoptosis; Glutaminolysis.

Introduction

Gastric cancer has high morbidity, high mortality, and poor prognosis and survival (1, 2) as one of the common malignancies tumours of the digestive system. It has been recorded that gastric cancer mortality and morbidity are high in Asia while gastric cancer is 5th and 6th worldwide in China (3), respectively. Some studies have found that early gastric cancer is easily affected by lymph node metastasis, due to histology, invasion, the principal role of cancer and other causes, by evaluating clinical data for 383 patients with early gastric cancer (4). Middle and late-stage incidences of gastro-cancer are relatively obscure and most patients. Therefore, further research of gastric cancer pathogens and new therapeutic priorities are of particular importance.

CircRNA is a cyclic RNA molecule with no 5 'to 3'polyA tail. Sanger et al. suggested it for the first time in 1976 (5, 6). Circular RNA plays an anti-cancer and anticancer function in tumour investigations and is involved in the proliferation of the tumour, apoptosis and metastasis (7-9). More and more evidence is being found. CircumRNA expression is closely linked to the incidence and progression of gastric cancer in gastric cancer cells (10). Ding et al. (11) demonstrated an up-regulation of circum-DONSON on gastric cancer linked to TNM stages and bad forecasts. The silence of circum-DONSON expression can inhibit gastric cancer cell growth and metastasis. Zhang et al.'s research results (12) have found that circDLST can function in gastric cancer cells as the sponge of miR-502-5p, and thus influence the spread and metastasis of gastric cancer cells. A new concept for cancer diagnosis and treatment can be given to MiRNA's proliferation and apoptosis of cancer cells (13). This study, therefore, found that circB3GNTL1 influences the propagation, apoptosis and glutamine decomposition of gastric cells by modulation of the miR-598 expression, in order to achieve a new goals for clinical diagnosis and treatment of gastric cancer. CirB3GNTL1 was the potential mechanical mechanism for gastric cancer cells.

Materials and Methods

Cells and main reagents

Shanghai Cell Bank of the Chinese Academy of Sciences was purchased from human gastric, epithelial mucous membrane GES-1 and gastric cancer cell line AGS. Penicillin, streptomycin, glutamine kit and glutamic acid kit are all purchased from Sigma in the USA; LipofectamineTM2000 kit, Trizol kit and the SYBR Green PCR kit are all purchased from the TaKARa Company in Japan. TaKaRa Corporation is the largest company in the world. RNase R was purchased by Epicenter, USA; Bcl-2 antibody, PCNA, ASCT2 antibody and GLS1 antibody, GAPDH and α-KG kits were purchased by the Abcam company in the USA, and were all purchased by Beijing Solebao company; MTT, DMSO solution and Annex V-FITC / PI package. Purchased from Promega Company, USA, Luciferase reporter gene pack.

Organize collection

In this research, we gave 23 samples of gastric can-

cer and adjacent tissues. Three months before the procedure, both patients received chemotherapy. Also, written consent was obtained from each patient with gastric cancer.

Cell culture and grouping

Incubators with RPMI 1640 medium containing foetal bovine serum, penicillin 100 rpm and streptomycin 100 mg / mL were incubated in the GES-1 human gastric mucosal epithelial cell line and gastric cancer cell line AGS and medium were modified on a 1D basis. As the cell density grows up to 70% by around 80%, take the AGS gastric cell line, transfect Si-NC, Si-circB3GNTL1 and Anti-mir-NC into the AGS gastric cell line according to LipofectamineTM 2000 kit operating steps. The group was divided randomly into si-NC, si-circB3GNTL, si-circB3GNTL1, and si-circB3GNTL1 anti-miR-598.

qRT-PCR

The cells were collected in each group. Total RNA has been extracted by the Trizol kit out of gastric cancer tissues and cells, accompanied by reversion of transcription to synthesise complementary DNA. The SYBR Green package PCR Package was introduced in conjunction with the QPCR measures. The internal referral for CircRNA is GAPDH and the internal reference for miRNA is U6. 2- The method of measuring circB3GNTL1 and miR-598 expression in the gene table was used for the same purpose.

RNase R processing

The total RNA was extracted 10 μg RNase R and 40 RNase R and the incubation took 1 h at 37 ° C. The levels of circB3GNTL1, linear B3GNTL1 and qRT-PCR were determined once the expression had been treated with RNase R.

MTT method

Inoculation of cells from each group in 96-well plates, cultivation 48 hours according to 1 to 104 / well, addition to each well with 20 μ l MTT solution, cultivation of the incubator for 4 hours and 150 μ l DMSO to prevent a light reaction. The enzyme labelling instrument (490 nm) defined the optical density.

Flow cytometry

The cells of each group were replaced in the buffer, washed twice with PBS, 10 μl of Annexin V-FITC and 5 μl of PI, added cells for a complete mixture of stain, and covered against light and reacted 30 minutes at room temperature. Flow cytometry was used to detect the apoptosis rate.

Western blotting

Each cell group was lysate with lysate, the supernatant was centrifuged, the concentration of the total protein was detected and mixed with a buffer, the cells were isolated from each other by the Electrophoresis of SDS-PAGE gel and the protein was transferred to the PVDF membrane. Closed culture for 2 hours was detected, first incubation of the antibody, over the nigh.

Determination of glutamine, glutamic acid and α-ketoglutaric acid (α-KG) levels

Inoculated into a six-well plate and cultivated for 48 hours, the cells of each group. Glutamine, glutamate and α-KG levels were then tested using the glutamine kit, glutamate kit and α-KG kit directions.

Double luciferase verification

Circinteractome online data database and wild type (wt-circHIPK3) and luciferase vector mutants (mut-circHIPK3) of circB3GnTL1 were predestined and co-transfected with miR-598 or miR-NC in AGS cells. The presence of binding sites between CircB3GNTL1 and miR-598 was expected. A luciferase reporter gene kit was found 48 hours after transfection.

Statistical method

T-test, single-way ANOVA, using PSSS 22.0 software, analysed all the experimental results. The mean and standard deviation were expressed in all measurement results, and the difference was important statistically.

Results

circB3GNTL1 highly expressed in gastric cancer tissues and cell lines (AGS)

We found that circB3GNTL1 was a circular structure, 536 bp long, (in chrX:139865339-139866824) as shown in Figure 1A when comparing the RNA sequences of circBase circB3GNTL1. Data from GSE141977 showed the upregulation of circB3GNTL1 to normal tissues in gastric cancer tissues (Figure 1B). QRT-PCR findings showed that the expression of circB3GNTL1 was substantially higher than that of paracáncer and human gastric epithelial cell lines GES-1 for gastric cancer and stomach cancer cells (Figure 1C and 1D). qRT-PCR Moreover, RNase R+ Restricted Enzyme Digestion Experiment has also tested the stability of circB3GNTL1. The results showed that circBz3GNTL1 expression had not modified significantly after RNase R+ treatment, whereas linear B3GNTL1 expression decreased considerably, suggesting that circB3GNTL1 was stable (Figure 1E).

Effect of circB3GNTL1 on proliferation and apoptosis of gastric cancer cell line

After si-NC and si-circB3GNTL1 had been transfected into gastric cell line AGS, results of qRT-PCR revealed. In comparison to the si-NC group, expression was considerably decreased in the si-circB3GNTL 1 group (Figure 2A); MTT findings showed a substantial decrease in cell proliferation in the if-circB3GNTL1 group in comparison with Si-NC group (Figure 2B; Flow cytometry showed a considerably higher rate in the si-circlB3GNTL1 group than in a si-NC group (Figure 2C); Western blot result suggested the apoptosis in the si-circB3GNTL1 group was significantly greater.

Effect of circB3GNTL1 on glutamine in gastric cancer cell line

In a gastric cancer cell, we assessed glutamine discomposure by observing glutamine, glutamate, and α-KG expression levels. Glutamine, glutamate and

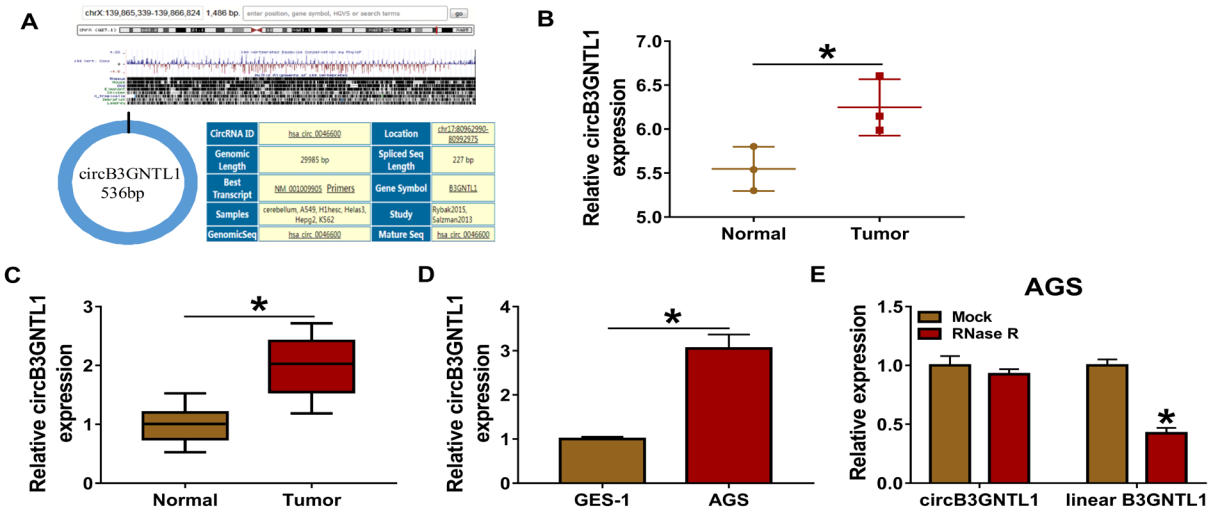

Figure 1. Expression in gastric cancer and cell lines of circB3GNTL1 is strong. A: circB3GNTL1 genomic site; B: microarray data collection GSE141977 circB3GNTL1 in gastric cancer tissue up-regulated; C: circB2GNTL1 in gastric cancer; D: circB3GNTL1 in the gastric cancer line relatively high expression; E: RNase R+ restriction digestive enzyme test to detect the stabilisation of circB3GNTL1.

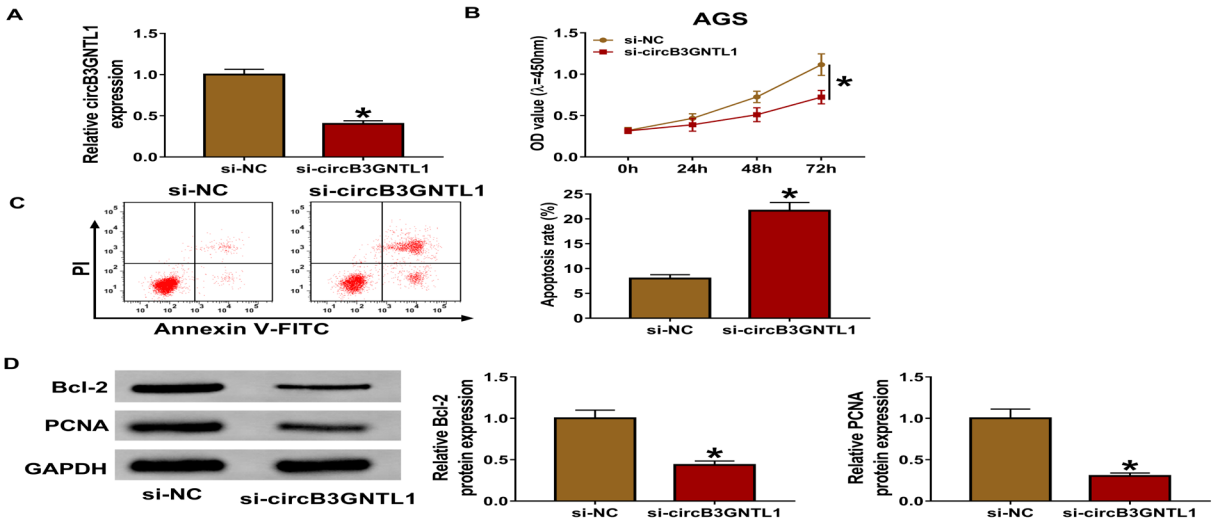

Figure 2. CircB3GNTL1 effect on gastric cancer cell line proliferation and apoptosis. A: knock-down circB3GNTL1 for transfection detection; B: knuckle circB3GNTL1 for gastric cancer cells proliferation detection; C: knock-down circB3GNTL1 for gastric cancer cell apoptosis detection; D: Bcl-2 and PCNA Protein Expression Detection.

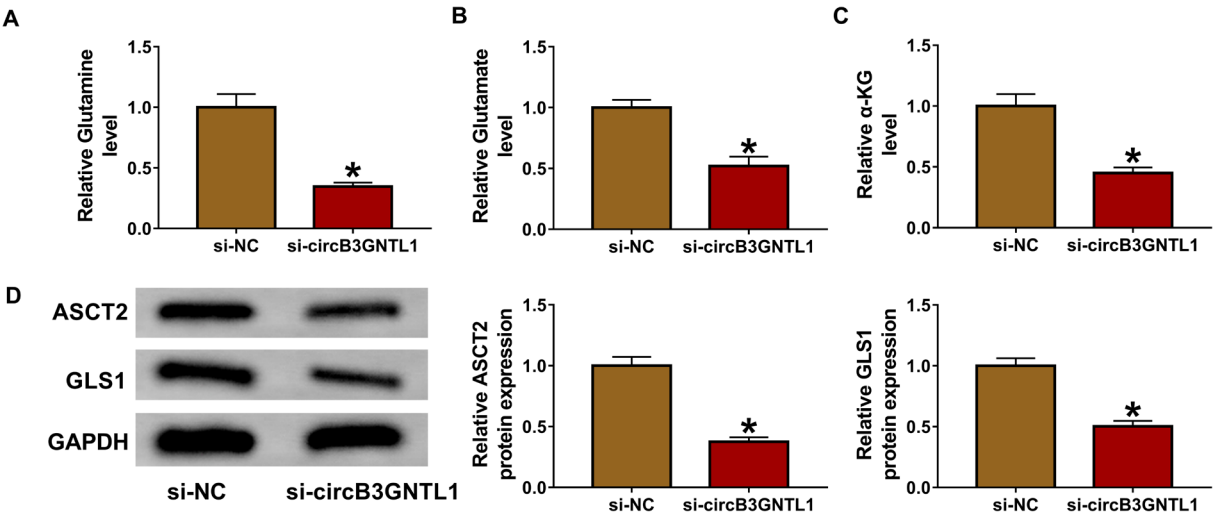

Figure 3. Glutamine effects on the gastric cancer cell line of the circB3GNTL1. B: the impact that circB3GNTL1 is knocked down on glutamine; B: the impact that circB3GNTL1 is knocked down on glutamate; C: the effect of circB3GNTL1 knockdown on α-KG; D: the effect of ASCT2 and GLS1 protein.

α-KG were significantly decreased in expression from si-cercB3GNTL1 compared with a si-NC group (Figure 3A-C). Western blot findings demonstrated that ASCT2 and GLS1 protein expression was significantly lower in the si-circB3GNTL1 Group than in the si-NC groups (Figure 3D).

There is a targeted binding site (AGS) between circB3GNTL1 and miR-598

Online Circinteractome software (Figure 4A) was used for the targeted binding positions of circB3GNTL1 and miR-598. Double luciferase experiments have shown the construction and co-transfection of wt-circHIPK3 and mut-circ HIPK4 reporter vectors in AGS cells with the built Vector, Mir-598 or Mir-NC. Compared to the miR-NC community, the wt-circHIPK3 activity of wt-598 has decreased, whereas there has been no substantial change in luciferase activity of mut-circHIPK3 (Figure 4B). Furthermore, in gastric cancer tissues and cells, we have observed miR-598 expression. The findings from QRT-PCR showed that GES-1, miR-598 expression decreased significantly in gastric cancer tissue and gastric cell line AGS (Figure 4C and 4D). Compared to normal gastric cancer tissue and the gastric mucosal epithelial cell line GES-1, miR-598.

Effects of inhibition of miR-598 partial recovery knock-down circB3GNTL1 on proliferation and apoptosis of gastric cancer cell line

The rate of miR-598 expression and apoptosis, cell proliferation levels, protein Bcl-2, and proteins expression of PCNA were decreased in comparison with Si-cercB3GNTL1 and miR-598 expression and apoptosis rates in the si-circB3GNTL1+anti-miR-598 compare with si-circB3GNTL1+anti-miR-NC classes, whereas in Figure 5 (A-D) the level of miR-598 expression and apoptosis was significantly decreased.

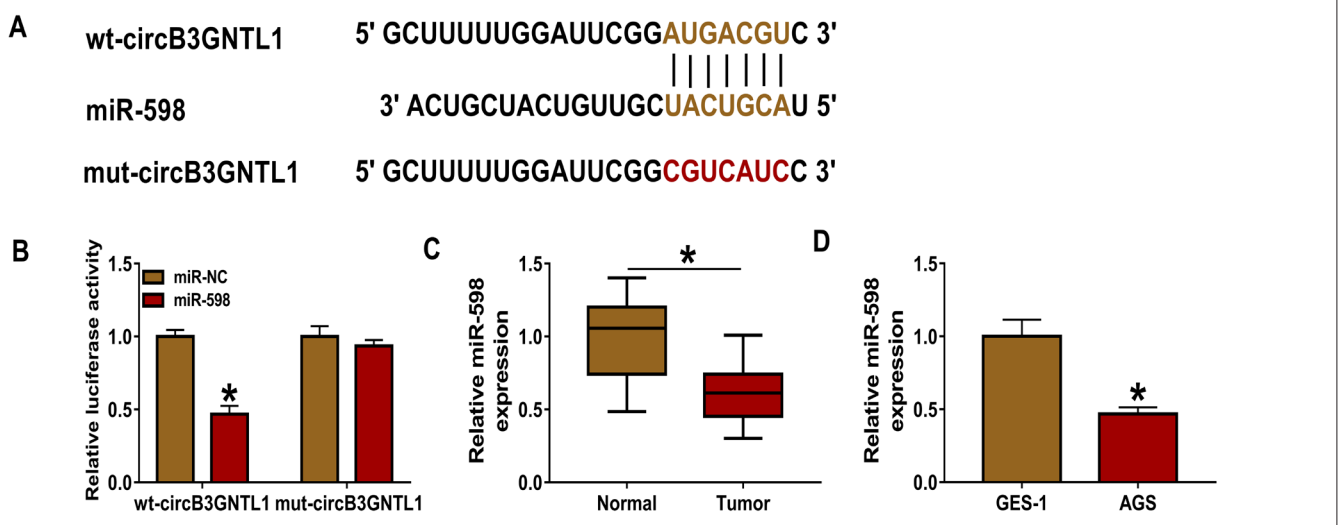

Figure 4. Between circB3GNTL1 and miR-598 there were targeted binding sites. A: targeted circum-B3GNTL1 binding to miR-598; B: twice luciferase to review targeted circB3GNTL1 binding to miR-598; C: relative low miR-598 expression in gastric tissues for cancer; D: Relatively low miR-598 expression in stomach cell lines.

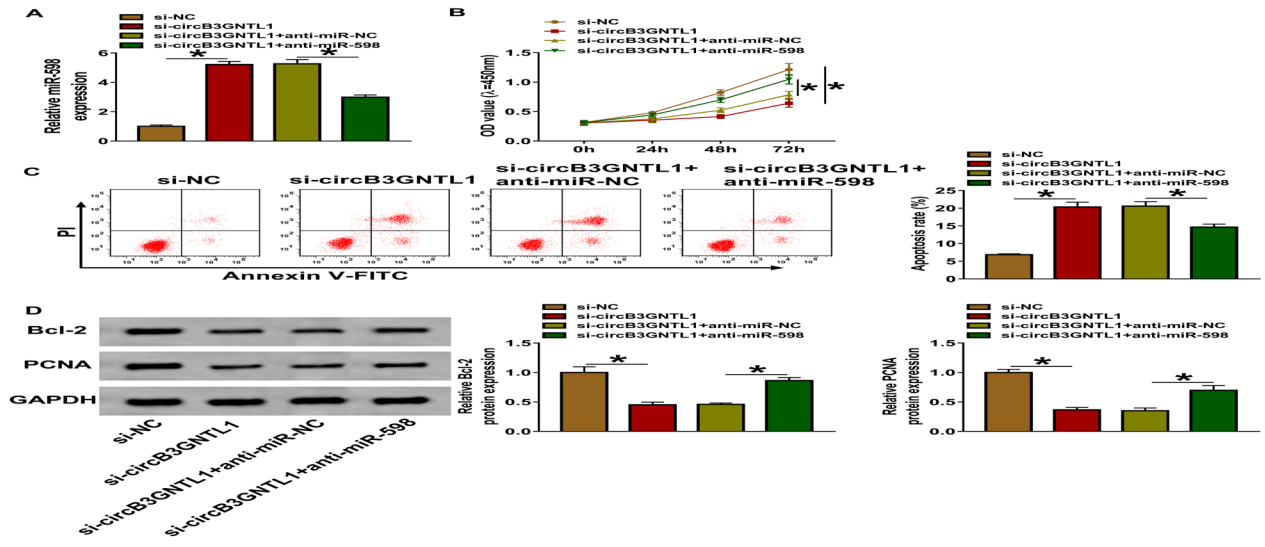

Figure 5. The effect of miR-598 partial recovery knock-down circB3GNTL1 inhibition on gastric cell line proliferation and apoptosis. A: Inhibit miR-598 transfection efficacy; B: inhibit miR-598 circB3GN TL1 part knockdown effect on gastric cancer cells; C: inhibit miR-598 part knockdown circB3GNTL1 effect on the apoptosis of gastric cancer cells; D: Bcl-2 and PCNA expression.

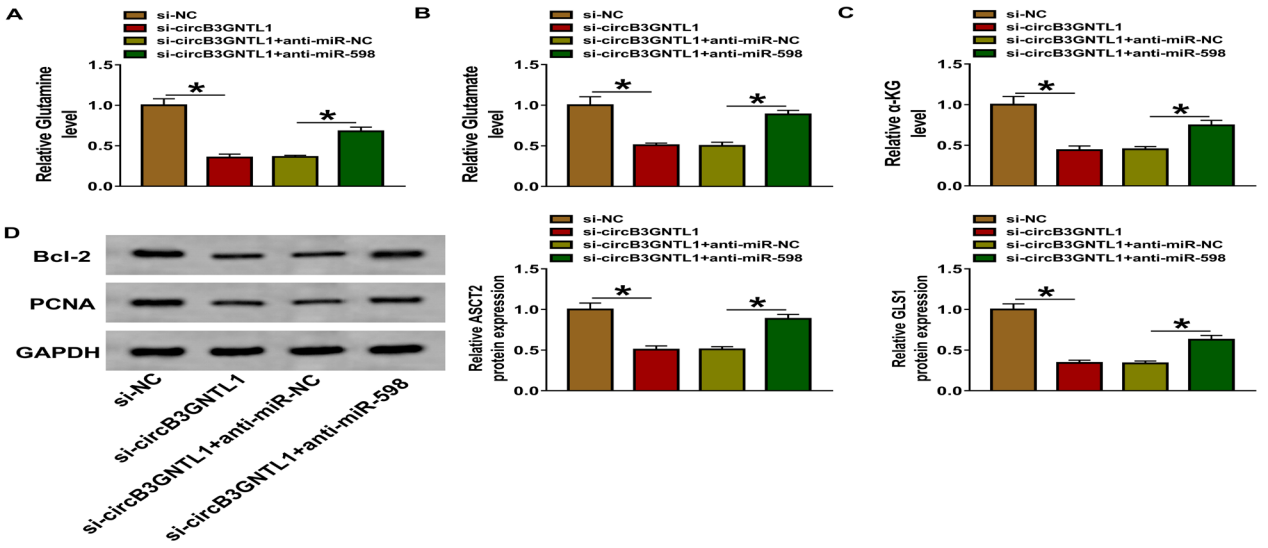

Figure 5. Effect of miR-598 inhibition knock-down circB3GNTL1 partial recuperation on glutamine in the cell line of gastric cancer. A: Inhibit the effects of miR-598 knock-down partial retrieval circB3GNTL1 on Glutamine; B: inhibit the effect of miR-598 partial retrieval circB3GNTL1 knock-down on Glutamate; C: inhale the effect of miR-598 part retrieval circB3GNTL1 on α-KG.

Effect of inhibition of miR-598 partial recovery knock-down circB3GNTL1 on glutamine in gastric cancer cell line

The findings of Figure 6 showed a significantly lower level of expression of si-circB3gNTL1 in glutamine, glutamine, α-KG, ASCT2 and GLS1 proteins, while expression levels for si-circb3GNTL1 + anti-mira 598 protein were significantly higher than for si-circB-3GNTL1 + antifood expression for si-cercB3GnTL1 + antifood expression for si-circB3GNTL1 + anti-mira-NC groups (Figure 6A-D).

Discussion

The prevalence of gastric cancer in recent years has appeared to be younger, easily affected by inheritance, irregular diet, a high level of life stress, high levels of nutritious foods and nutritional supplements and other factors. Currently, much of gastric cancer is medical treatment, but certain adverse drug reactions do occur, such as drug resistance development, poor 5-year survival rate, etc. Currently, gastric cancer treatment is most commonly used. New molecular targeted therapy is therefore urgently needed (14-16).

CircRNA is a sort of non-coding, stable, circularly closed RNA molecule. At the end of its 3 'fin,' the 5' ring structure can be joined together. CircRNA is abnormally expressed in the presence and growth of malignant tumours. Some studies have shown how circRNA0047905, circRNA0138960 and circRNA7690-15 are up-regulated in gastric cancer by the establishment of the co-expression network between circRNA and mRNA. In vitro studies have shown that the proliferation and invasion of gastric carcinoma cells can be encouraged by circRNA0047905, cirrNA0138960 and circRNA7690-15[15-17). The expression of circLARP4 was found to be down-regulated in gastric cancer correlated with the size of the tumour and lymph node metastasis in Zhang et al. (18) by sequencing. Five anomalous miRNA expressions, namely miR-16, miR-15a, miR-15b, miR-590 and miR-424 were found, while miR-424 had a clear association in the stomach cancer. More studies showed that circLARP4 overexpression of miR-424 knockdown can inhibit proliferation and metastasis of gastric carcinoma cells, and a negative association exists between circLARP4 and miR-424. Inhibiting the expression of circHIPK3 has been shown to inhibit the development of gastric cancer cells with glutamine decomposition. By controlling miR-876-5p (19) CircHIPK3 participates in the incidence and growth of stomach cancer. Liu Ming et al. (20) found that circus 009910 expression is up-regulated in stomach cancer cells, while the expression circum 9910 knockdowns of stomach cancer cells will facilitate proliferation, migration and invasion. The findings of this study show that circB3GNTL1 expression was up-regulated in gastric cancer tissue and cells and RNase R restrictive enzyme digestion tests confirmed the stability of circB3GNTL1. CircumB3GNTL1, which has a similar effect as the Li research, can inhibit and cause the proliferation and decomposition of glutamine of gastric cancer cells. The enzyme GLS1 to β-ketoglutaric acid (α-KG) (21). Glutamine is capable to metabolise, whereas the amino acids in the tumours can be transported or metabolised by ASCT2 (22, 23). Consequently, the results of this study showed that the dropping of the circusB3GNTL1 could lead to a decrease of glutamines, glutamates, α-KG, ASCT2 and Gls1 proteins and decomposition of the glutamine by the knockdown of the circleB3GNTL1.

The miR-598 is down-regulated in colorectal cancer, in non-small cell cancer of the lungs and other cancers primarily linked to the stage of TNM and metastases of the lymph node of cancer. In some studies, the expression of mirR-598 has been down-regulated in tissue and cells of gastric cancer, and miR-598 over-expresses can inhibit and promote gastric cancer proliferation and metastasis and apoptosis (23, 24). Zeng et al. (25) found that miR-598 expression in ovarian cancer cells is downregulated and miR-598 inhibition could reverse the circCELSR1 down-regulation on ovarian cancer cell proliferation and metastasis and promote apoptosis. The findings of this study demonstrated that miR-598 was

downregulated with Zeng and Liu-like, stomach cancer tissues and cells. Further experiments demonstrated that targeted binding of circB3GNTL1 to miR-598 and miR-598 inhibition could partially restore circB3GNTL1, by controlling expression, to carcinogenic functions for gastric cancer cells, by inhibiting the inhibition of proliferation of, apoptosis and glutamine decomposition of gastric cancer cells.

To sum up, down-regulation of circB3GNTL1 expression inhibits the proliferation and glutamine decomposition of gastric cancer cells and promotes its apoptosis, and the mechanism may be related to the inhibition of miR-598.

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