Pepsinogen C expression–related lncRNA/circRNA/mRNA profile and its co-mediated ceRNA network in gastric cancer

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Abstract

Background

The expression of pepsinogen C (PGC) is considered an ideal negative biomarker of gastric cancer, but its pathological mechanisms remain unclear. This study aims to analyze competing endogenous RNA (ceRNA) networks related to PGC expression at a post-transcriptional level and build an experimental basis for studying the role of PGC in the progression of gastric cancer.

Materials and methods

RNA sequencing technology was used to detect the differential expression profiles of PGC-related long non-coding (lnc)RNAs, circular (circ)RNAs, and mRNAs. The online database, STRING, was used to construct protein−protein interaction (PPI) networks of differentially expressed (DE) mRNAs. A ggcorrplot R package and online database were used to construct DElncRNAs/DEcircRNAs co-mediated PGC expression−related ceRNA networks. *In vivo* and *in vitro* validations were performed using quantitative reverse transcription−PCR.

Results

RNA sequencing found 637 DEmRNAs, 698 DElncRNAs, and 38 DEcircRNAs. The PPI network of PGC expression−related mRNAs consisted of 503 nodes and 1179 edges. CFH, PPARG, and MUC6 directly interacted with PGC. Enrichment analysis suggested that DEmRNAs were mainly enriched in cancer-related pathways. Eleven DElncRNAs, 13 circRNAs, and 35 miRNA−mRNA pairs were used to construct ceRNA networks co-mediated by DElncRNAs and DEcircRNAs that were PGC expression−related. The network directly related to PGC was as follows: SNHG16/hsa_circ_0008197−hsa-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p−PGC. Quantitative reverse transcriptase PCR validation results showed that PGC, PPARG, SNHG16, and hsa_circ_0008197 were differentially expressed in gastric cancer cells and tissues: PGC positively correlated with PPARG (r = 0.276, *P* = 0.009), SNHG16 (r = 0.35, *P* = 0.002), and hsa_circ_0008197 (r = 0.346, *P* = 0.005).

Conclusion

PGC-related DElncRNAs and DEcircRNAs co-mediated complicated ceRNA networks to regulate PGC expression, thus affecting the occurrence and development of gastric cancer at a post-transcriptional level. Of these, the network directly associated with PGC expression was a SNHG16/hsa_circ_0008197−mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p − PGC axis. This study may form a foundation for the subsequent exploration of the possible regulatory mechanisms of PGC in gastric cancer.

1. Introduction

The latest global statistics show that gastric cancer is the fifth most common malignancy and the third most fatal tumor (1). One reason for the high fatality rate lies in its unclear pathogenesis, and the lack of early diagnostic markers and molecular therapeutic targets. Therefore, it is of great importance to explore the potential pathogenesis of gastric cancer to achieve an early diagnosis and to improve the prognosis of each patient.

Pepsinogen C (PGC), a precursor of pepsin C, belongs to the aspartic enzyme family, and is mainly secreted by gastric mucosal principal cells into the gastric cavity (2). PGC expression was found to gradually decrease or even become deficient in the progression of superficial gastritis−atrophic gastritis−gastric cancer, suggesting it might be an ideal negative biomarker of gastric cancer (3). Several scholars have described positive PGC expression also gradually decreased in well differentiated, medium differentiated, and poorly differentiated gastric cancer cells (4). PGC expression was associated with a poor prognosis and might be an independent prognostic factor (4). Functional studies have shown that PGC synthetic fragments in the mouse could stimulate the growth of normal cell lines. PGC expression was increased in acetic acid−induced gastric ulcers, indomethacin-induced gastric lesions, and *Helicobacter pylori* infection, showing that PGC played an important role in gastric mucosal healing (5). In general, PGC might have an important role in multiple gastric diseases, including gastric cancer. However, its pathogenetic mechanism is rarely studied. Current research is limited to PGC as a negative biomarker for gastric cancer, which has a certain clinical application. However, studies on the regulation of PGC expression are lacking.

Competing endogenous RNA (ceRNA) is a class of transcript that might cause mutual regulation by competing for shared microRNA (miRNA) at a post-transcriptional level (6). Multiple molecules function in a similar manner to ceRNAs, such as mRNAs, long non-coding (lnc)RNAs, circular (circ)RNAs, and pseudogenic RNAs (7). The mechanism of ceRNAs is analogous to sponge-absorbing matched miRNAs by miRNA response elements (MREs), thus eliminating the effect of decreased miRNAs on the transcription and translation of target genes (8). The role of the ceRNA network is extensive and likely includes regulating the progression of different tumors, including gastric cancer. This provides an important clue for exploring pathogenetic mechanisms and to construct a ceRNA network mediated via lncRNAs and circRNAs.

MicroRNA can combine with the 3' untranslated region (3'-UTR) of PGC to regulate its expression (9). At present, studies on interactions between PGC and multiple non-coding RNAs have been rare, and its regulatory mechanism at the post-transcriptional level is still unclear in the progression of gastric disease. This study aims to analyze and validate the differential expression profiles of PGC expression–related lncRNAs, circRNAs, and mRNAs, as well as the ceRNA network mediated via lncRNAs and circRNAs. This might be an important foundation for exploring PGC pathogenetic mechanisms in gastric cancer.

2. Materials And Methods

2.1 Establishment of PGC-overexpressing cell line

AGS gastric cancer cells (purchased from National Biomedical Laboratory Cell Resource Bank, Beijing, China) were cultured in RPMI Medium 1640 (Solarbio, Beijing, China) containing 10% embryonic bovine serum (Biological Industries, USA) in a 37°C, 5% CO_2 incubator. Treatment and control groups of cells were used in the study, with three replicates in each group. AGS cells were transfected with PCG-overexpressing and negative control plasmids. According to the protocol of a jetPRIME transfection kit (Polyplus Transfection, USA), 2 µg of plasmid was added to 200 µL transfection buffer and mixed well, vortexed for 10 s, and then briefly centrifuged. Then 4 µL of transfection reagent was added, the sample vortexed for 1 s, and briefly centrifuged. Finally, the premix was incubated at room temperature for 10 min and added to cells in a well of a 6-well plate containing 2 mL of 10% serum/ RPMI Medium 1640. Cells were then incubated for 24 h and then collected, RNA extracted, and RNA sequencing technology (RNA-seq) used to detect PGC expression to verify successful transfection of the PGC plasmid.

2.2 Library construction and high-throughput sequencing

After total RNA was extracted by Trizol and passed a quality inspection, ribosomal RNA (rRNA) was isolated from the two cell groups using an Epicentre Ribo-Zero kit (Illumina, San Diego, CA, USA). The remaining rRNA-depleted RNA (polyA + and polyA-) was purified and recovered. The purified and recovered rRNA-depleted RNA with linear RNA molecules was then digested by 5U RNase R. The purified and recovered circRNA was randomly interrupted into short fragments by Fragmentation Buffer. The fragmented rRNA−depleted RNA was used as a template to synthesize a strand of cDNA with random hexamers. Buffer, deoxyribonucleotide triphosphates, RNaseH, and DNA polymerase I were added for two-stranded cDNA synthesis. AMPure XP beads were used to purify the double-stranded product. T4 DNA polymerase and Klenow DNA polymerase activity were used to repair the sticky end of the DNA to a blunt end. Base A was added to the 3' end as well as a linker, and AMPure XP beads used for fragment selection. A USER enzyme was then used to degrade the second strand of cDNA containing U. Finally, PCR amplification was performed to obtain a final sequencing library. After the library was qualified, it was sequenced using an Illumina Hiseq2000/2500, with a sequencing read length of 2x125 bp (PE125) used.

2.3 Differentially expressed analysis

Fragments per kilobase of exon model per million mapped reads was used to measure the abundance of gene expression. A DESeq R package was utilized for differential analyses with intergroups. |log2FC| ≥ 1 and $P < 0.05$ were considered statistically significant. Heatmaps and ggplot2 R packages were used to visualize the differential expression (DE) profiles of mRNAs, lncRNAs, and circRNAs.

2.4 Construction of protein−protein interaction network and enrichment analysis of DEmRNAs

A protein−protein interaction (PPI) network for PGC expression−related mRNAs was constructed by a STRING online database (https://www.string-db.org/). An interaction score > 0.4 was considered as a cut-off value. DEmRNAs were interpreted using the Gene Ontology (GO) system of classification, including biological processes, cellular components, and molecular functions classifications, and a Kyoto Encyclopedia of Genes and Genomes database. $P < 0.05$ was considered statistically significant.

2.5 Cis regulatory network construction of DElncRNAs

Long non-coding RNAs can modulate adjacent mRNA expression to generate biological roles by a cis-regulatory network. Therefore, the potential target mRNAs of DElncRNAs were predicted. Long non-coding RNA cis-regulated target genes were mainly predicted based on location distribution, with the lncRNA cis-regulated network defined as constructed by DElncRNAs and DEmRNAs within 10 kbp of the chromosome. Pearson correlation analysis was performed on cis lncRNA−mRNA pairs ($1 \geq r \geq -1$, $P < 0.05$). The cis lncRNA regulatory network was visualized by Cytoscape 3.7.2.

2.6 Construction of lncRNA−miRNA−mRNA and circRNA−miRNA−mRNA networks

DElncRNA−DEmRNA and DEcircRNA−DEmRNA pairs were carried out using Pearson correlation analysis by a ggcorrplot R package, respectively. Positive DElncRNA − mRNA pairs and DEcircRNA−circRNA pairs ($r \geq 0.8$, $P < 0.05$) were chosen to construct a ceRNA network mediated by DElncRNAs and DEcircRNAs, respectively. The target miRNAs of DElncRNAs and DEcircRNAs were predicted by StarBase (http://starbase.sysu.edu.cn/starbase2/index.php). The target miRNAs of mRNAs positive with DElncRNAs and DEcircRNAs were predicted by miRTarBase and TarBase of NetworkAnalyst (https://www.networkanalyst.ca/). Finally, lncRNA−miRNA−mRNA and circRNA−miRNA−mRNA were visualized by Cytoscape 3.7.2. The cytoHubba plug-in was used to calculate networks and to choose the top three lncRNAs and circRNAs with a degree score to an in-depth analysis, respectively.

2.7 Construction of co-regulated network mediated by DElncRNAs and DEcircRNAs

The mRNA−miRNA pairs that were shared with lncRNA−miRNA−mRNA and circRNA−miRNA−mRNA were selected to construct a co-medicated ceRNA network by DElncRNAs and DEcircRNAs. Cytoscape 3.7.2 was used to visualize these. The cytoHubba plug-in was also used to calculate the network and select the top three lncRNAs and circRNAs with a degree score to an in-depth analysis, respectively.

2.8 qRT−PCR validation *in vivo* and *in vitro*

In an *in vivo* validation, we employed 46 pairs of gastric cancer and adjacent normal tissues collected from the First Affiliated Hospital of China Medical University. Our research was implemented according to the Declaration of Helsinki and supported by the research ethics committee of the First Affiliated Hospital of China Medical University. Written informed consent was obtained from all patients before samples were collected. For *in vitro* validation, a lentivirus was used to construct PGC stably transfected gastric cancer AGS and HGC-27 cells (purchased from National Biomedical Laboratory Cell Resource Bank, Beijing, China). The required amount of virus was calculated according to multiplicity of infection values for AGS and HGC-27 cells as recommended in each manufacturer's instructions. Opti-MEM (500 μL) was used and polybrene was added to a final concentration of 5 μg/mL. After 72 h, 2 μg/mL of puromycin was added to screen for transfected cells. The infection efficiency was verified by quantitative reverse transcription (qRT)−PCR.

Total RNA was extracted from AGS and HGC-27 cells overexpressing PGC, as well as 46 pairs of gastric cancer and adjacent normal tissues, and the relative expression level of genes was detected by qRT−PCR. Quantitative reverse transcription−PCR experiments were performed using a real-time PCR 480 system and SYBR-green PCR master mix. All curves were a single peak. Measurements were normalized using β-actin. Primer sequences are listed in Supplementary Table 1. SPSSv24.0 (IBM, Chicago, IL, USA) and GraphPad Prism V8.0 (GraphPad software, San Diego, CA, USA) were utilized for data analysis, and $2^{-\Delta Ct}$ was used to calculate relative expression. Differential expression profiles were assessed by Student's *t*-test for normally distributed data while a rank sum test was used for skewed distribution data. Spearman correlation analysis was used to calculate the correlation between the expression of genes. A Chi-square test was utilized to analyze the association of gene expression with clinicopathological parameters. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Differential expression profile of mRNAs, lncRNAs, and circRNAs based on PGC expression

RNA sequencing showed that compared with a negative control, PGC expression was up-regulated by a 111.58-fold change in the overexpressing group, verifying that the PGC plasmid was successfully transfected (Table 1). Differential expression profiles for PGC expression−related mRNAs, lncRNAs, and circRNAs indicated that a total of 637 DEmRNAs (377 up-regulated and 260 down-regulated), 698 DElncRNAs (376 up-regulated and 322 down-regulated), and 38 DEcircRNAs (15 up-regulated and 23 down-regulated) could be identified. The heatmap and volcano map of DERNAs are shown in Fig. 1.

Table 1
PGC expression profiles

Gene_name	FPKM.PGC1	FPKM.PGC2	FPKM.PGC3	FPKM.CON1	FPKM.CON2	FPKM.CON3	FC	P value
PGC	1284.77	670.61	641.18	8.35	6.47	8.46	111.58	< 0.001

CON, control. FPKM, Fragments Per Kilobase of exon model per Million mapped reads.

3.2 PPI network and enrichment function of DEmRNAs

The PPI network of DEmRNAs was constructed by STRING and an interaction score > 0.4 was set as a cut-off value. It was found that the PPI network of DEmRNAs consisted of 503 nodes and 1179 edges (Fig. 2). In addition, this study found that PGC could directly interact with complement factor H (CFH), peroxisome proliferator activated receptor gamma (PPARG), and mucin 6 (MUC6); Table 2, Fig. 2). Gene Ontology enrichment analysis indicated that DEmRNA might be involved in various biological processes, such as signal transduction, the G protein-coupled receptor signaling pathway, regulation of transcription, DNA-templates, and differentiation. These DEmRNAs might take part in cellular components, such as the membrane, plasma membrane, and integral components of the membrane. In addition, the molecular function of DEmRNAs mainly included protein binding, metal ion binding, DNA binding, and hydrolase activity (Fig. 3). Pathway enrichment analysis suggested that PGC expression−related DEmRNAs were mainly involved with cell adhesion molecules, and cAMP, chemokine, and oxytocin signaling pathways (Fig. 4).

Table 2
The potential interaction protein of PGC

Gene 1	Gene 2	gene 1_string_internal_id	gene 2_string_internal_id	gene 1_external_id	gene 2_external_id	Combined score
PGC	CFH	4444085	4442881	9606.ENSP00000362116	9606.ENSP00000356399	0.458
PGC	PPARG	4444085	4436745	9606.ENSP00000362116	9606.ENSP00000287820	0.452
MUC6	PGC	4448430	4444085	9606.ENSP00000406861	9606.ENSP00000362116	0.429

3.3 The cis-regulatory pathway mediated by DElncRNAs

Differential expression genes were selected within 10 kbp upstream and downstream of each DElncRNA host gene as possible cis-regulated genes in order to analyze the regulation of each lncRNA and its neighboring genes. A total of 105 differentially expressed cis-regulatory genes were found upstream and downstream within 10 kbp of 94 DElncRNAs, which constituted 181 cis lncRNA−gene pairs. The Pearson correlation coefficient between each DElncRNA and adjacent genes was calculated. It was found that 8.3% of gene pairs showed a strong positive correlation ($r \geq 0.9$), 25% of gene pairs showed a moderate degree of positive correlation ($0.9 > r \geq 0.5$), 50% of gene pairs had a weaker correlation ($0.5 > r > -0.5$), and 16.7% of gene pairs had a moderately negative correlation ($-0.5 \geq r > -0.9$). No gene pair showed a strong negative correlation (Fig. 5, Supplementary Table 2).

3.4 The ceRNA network mediated by DElncRNAs

Pearson correlation analysis was performed on DElncRNAs and DEmRNAs to select DElncRNA − DEmRNA pairs ($P < 0.05$ and $r \geq 0.8$) in order to construct a ceRNA network. Next, DElncRNA−miRNA and DEmRNA−miRNA pairs were predicted. In addition, PGC-interacting miRNAs verified by our team in previous studies were also included in a subsequent analysis, including hsa-mir-98-5p, hsa-let-7f-5p, hsa-let-7c-5p, hsa-mir-365a-3p, and hsa-520a-5p. It was found that the ceRNA network mediated by DElncRNAs consisted of 19 DElncRNAs, 85 DEmRNAs, and 76 miRNAs (Fig. 6). Further, a cytoHubba plug-in was used to select the top three DElncRNAs with the highest degree scores, including HCG18, SNHG16, and SNHG1. After an in-depth understanding of these DElncRNAs, it was found that only an SNHG16−hsa-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p−PGC regulatory network was directly related to PGC, and that the remaining molecules were indirectly associated (Fig. 6).

3.5 The ceRNA network mediated by DEcircRNAs

Pearson correlation analysis was performed on DEcircRNAs and DEmRNAs to select DEcircRNA−DEmRNA pairs ($P < 0.05$ and $r \geq 0.8$) in order to construct a ceRNA network. Next, DEcircRNA−miRNA pairs and DEmRNA−miRNA pairs were predicted. Twenty-one circRNAs, 32 mRNAs, and 43 miRNAs were used to build a ceRNA network mediated by a DEcircRNA network (Fig. 7). Further, a cytoHubba plug-in was

used to select the top three DEcircRNAs with the highest degree scores, including hsa_circ_0031583, hsa_circ_0008197, and hsa_circ_0036627. After an in-depth understanding of these DEcircRNAs, it was found that only a hsa_circ_0008197−hsa-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p−PGC regulatory network was directly related to PGC, and the remaining molecules were indirectly associated (Fig. 7).

3.6 Co-regulated ceRNA network mediated by DElncRNAs and DEcircRNAs

Based on a ceRNA network mediated by DElncRNAs and DEcircRNAs, DEmRNA−miRNA pairs shared by these were screened. A total of 11 DElncRNAs, 13 DEcircRNAs, and 35 miRNA−mRNA pairs were used to construct a ceRNA network co-mediated by DElncRNAs and DEcircRNAs (Fig. 8). A similar method was used to screen out the top three molecules with the highest degree scores in a ceRNA network co-mediated by DElncRNAs (SNHG14, HCG18, and SNHG16) and DEcircRNAs (hsa_circ_0031583, hsa_circ_0008197, and hsa_circ_0036627). We further analyzed a ceRNA network co-mediated by these DEcircRNA and DElncRNA molecules, and found that an SNHG16/hsa_circ_0008197−hsa-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p PGC network was directly involved in PGC (Fig. 9); other molecules had an indirect relationship.

3.7 qRT-PCR validation *in vitro* and *in vivo*

Compared with a negative control group, PGC-overexpressing AGS and HGC-27 cells showed 1566- and 3177.3-fold changes in upregulation, respectively (Fig. 10A), verifying that PGC-overexpressing cells were successfully constructed. Quantitative reverse transcription-PCR was used to perform *in vitro* validation including PGC-interacting proteins (PPARG, CFH, and MUC6), SNHG16, and hsa_circ_0008197 directly related to PGC. Validation results using AGS and HGC-27 cells showed that compared with the negative control, PPARG, SNHG16, and hsa_circ_0008197 were up-regulated in PGC-overexpressing cells (Fig. 10A). There was no statistically significant difference in CFH and MUC6 levels (Supplementary Figure S1). Statistically significant molecules at a cellular level were identified by *in vivo* validation within 46 pairs of gastric cancer and adjacent normal tissues. Compared with adjacent normal tissues, PGC, PPARG, SNHG16, and hsa_circ_0008197 were down-regulated in gastric cancer (Fig. 10B). Spearman correlation analysis showed that PGC was positively associated with PPARG (r = 0.276, *P* = 0.009), SNHG16 (r = 0.35, *P* = 0.002), and hsa_circ_0008197 (r = 0.346, *P* = 0.005); these results were consistent with our above analysis. The association of PGC, SNHG16, hsa_circ_0008197, and PPARG expression with clinicopathological parameters is detailed in Supplementary Tables 3−6.

4. Discussion

As a marker of organ differentiation and maturation (especially gastric differentiation), PGC has unique physiological and pathological characteristics (10, 11). At present, research on PGC in gastric cancer has mainly focused on its expression level, with its upstream regulatory mechanism yet to be explored.

A ceRNA network mediated by DElncRNA/DEcircRNA plays an important role in the formation and development of tumors and has been used to explore the pathology, diagnosis, and prognosis of different tumors, including gastric cancer (12). In this study, RNA-seq technology was used to detect mRNAs, lncRNAs, and circRNAs based on PGC expression; an enrichment analysis of DEmRNAs was performed and a PPI network was constructed to explore its biological role and potential interacting molecules. A cis lncRNA regulatory network was predicted to analyze its potential role in gastric cancer. The positive correlation between lncRNA−mRNA pairs and circRNA−mRNA pairs was screened and their common target miRNAs were predicted. A ceRNA network was systematically constructed that was co-mediated by PGC expression-related DElncRNAs/DEcircRNAs. The sequencing results were further verified by qRT−PCR using gastric cancer cells and tissues, which formed the basis for further research on the post-transcriptional regulatory mechanisms of PGC in gastric cancer.

RNA sequencing indicated a total of 637 mRNAs, 698 lncRNAs, and 38 circRNAs were differentially expressed, which suggested the association of these RNAs with the expression and function of PGC. Enrichment results showed that DEmRNAs related to PGC expression mainly focused on cell adhesion molecules, and chemokine, oxytocin and cAMP signaling pathways. These pathways were shown to be closely related to the occurrence and development of tumors (13−15). Recently, researchers have claimed that the ceRNA network mediated by non-coding RNA regulated these pathways and thus had an influence on the development of different tumors (16, 17). In other words, PGC regulated and modified the function of these pathways through a ceRNA network mediated by PGC expression−related DElncRNAs and DEcircRNAs, thus leading to a regulatory role in gastric cancer cells at the post-transcriptional level. The PPI network of DEmRNAs was made up of 503 nodes and 1179 edges. A direct interaction relationship exists between PGC−CFH (interaction score = 0.458), PGC−PPARG (interaction score = 0.452), and PGC−MUC6 (interaction score = 0.429). The qRT−PCR validation *in vivo* and

in vitro indicated that only the differential expression of PPARG was statistically significant, which is in the same direction as PGC. Spearman correlation analysis also indicated that PGC was positive with PPARG (r = 0.276, *P* = 0.009). PPARG is a type of nuclear hormone receptor that can regulate various cell functions, including lipogenesis, lipid biosynthesis, energy consumption and storage, and inflammation (18). The study found that PPARG also promoted epithelial cell differentiation and inhibited tumor cell proliferation (19, 20), which is consistent with the conclusion that PGC is a marker of organ differential maturation. Such conclusions suggested that PGC may interact with PPARG to co-regulate the differential maturation of gastric cancer cells, thus affecting tumor progression. But the specific mechanism still needs to be further explored.

To understand the potential roles of PGC expression–related lncRNAs, adjacent target genes were predicted to construct a cis-regulatory network. A total of 105 differentially expressed cis-regulatory genes were found within 10 kbp upstream and downstream of 94 DElncRNAs, which constituted 181 cis lncRNA–gene pairs. The cis-regulation function of non-coding RNA might participate in various biological processes (21) while the cis-regulation function of lncRNA might affect tumor progression (22, 23). Our analysis indicated that DElncRNAs were mainly positive with adjacent protein-coding genes, with a negative correlation being relatively few in number. Therefore, we speculated that PGC expression–related DElncRNAs mainly positively regulated the expression of adjacent protein-coding genes. In addition, a ceRNA network mediated by DElncRNAs was constructed by 19 DElncRNAs, 85 DEmRNAs, and 76 miRNAs, of which HCG18, SNHG16, and SNHG1 showed the highest degree scores. Most research indicated that ceRNA mediated by DElncRNA regulated the progression of gastric cancer, colorectal cancer, and osteosarcoma (24–26). HCG18 was shown to promote gastric cancer progression by up-regulating DNAJB12 via miR-152-3p (27). SNHG1 facilitated the growth and migration of gastric cancer cells via the miR-140/ADAM10 axis (28). This study initially found that these lncRNAs were associated with PGC expression and its mediated ceRNA network might have affected gastric cancer progression.

A ceRNA network mediated via PGC expression–related DEcircRNAs was constituted to explore a potential role. It was found that 21 DEcircRNAs, 32 DEmRNAs, and 43 miRNAs constituted a ceRNA network mediated via DEcircRNAs. Of these, the top three with the highest degree score of DEcircRNAs included hsa_circ_0031583, hsa_circ_0008197, and hsa_circ_0036627. A ceRNA network mediated via DEcircRNAs modulated histological classifications and gastric cancer progression (29) and the stemness properties of colorectal cancer stem cells (30). No report has shown that hsa_circ_0031583, hsa_circ_0008197, and hsa_circ_0036627 affected tumor progression. Using bioinformatics, this study initially revealed that the ceRNA networks mediated by PGC expression–related hsa_circ_0031583, hsa_circ_0036627, and hsa_circ_0036627 may play a major role in the progression of gastric cancer. This provides important clues for subsequent research of mechanisms in gastric cancer.

Based on a ceRNA network mediated by DElncRNAs and DEcircRNAs, a total of 11 DElncRNAs, 13 circRNAs, and 35 miRNA–mRNA pairs were used to construct their co-mediated ceRNA network. As is well known, ceRNA networks mediated via non-coding RNAs play an important role in gastric cancer (31–33). In this study, the co-regulated network included the SNHG16/hsa_circ_0008197–hsa-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p–PGC axis. This was directly related to PGC and was of great significance in exploring its post-transcriptional regulatory mechanisms in gastric cancer. Therefore, qRT–PCR validation for SNHG16 and hsa_circ_0008197 was performed *in vivo* and *in vitro*. It was found that SNHG16 and hsa_circ_0008197 were differentially expressed at the gastric cell and tissue levels, and were positive for PGC, respectively (SNHG16: r = 0.35, *P* = 0.002; hsa_circ_0008197: r = 0.346, *P* = 0.005). This suggested the presence of a ceRNA network directly related to PGC. Small nucleolar RNA host gene 16 (*SNHG16*) was encoded by a 7571-bp region on chromosome 17q25.1 and was considered as a cancer-related lncRNA (34). A previous study showed that SNHG16 sponged miRNA by binding to MREs to modulate the expression of tumor-related target genes, thus regulating the proliferation, apoptosis, migration and invasion of tumor cells (35). A dual luciferase reporter experiment and RNA binding protein immunoprecipitation were used to show that SNHG16 sponged hsa-mir-98-5p to regulate the progression of osteosarcoma (36). SNHG16 promoted the development of bladder cancer via a miR-98/STAT3/Wnt/β-catenin pathway (37). No report exists of SNHG16 sponging hsa-let-7c-5p/hsa-let-7f-5p to modulate tumor progression, but several investigations showed that hsa-let-7c-5p and hsa-let-7f-5p also regulated proliferation, migration, and invasion of tumor cells (38–40). This study initially found and validated the SNHG16–mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p–PGC axis as having an influence on gastric cancer progression. hsa_circ_0008197 is located on chr1:51032749–51061888. Although research on the association of hsa_circ_0008197 with human diseases is lacking, our study initially found and verified that hsa_circ_0008197 and SNHG16 co-mediated a PGC-related ceRNA network to transform the SNHG16/hsa_circ_0008197–mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p–PGC axis. We speculated that this network might cause a change in PGC expression to generate a biological effect via a systemic post-transcriptional regulatory mechanism, thus influencing tumor progression. This yields a novel notion related to PGC pathogenetic mechanisms in gastric cancer.

In conclusion, this study found that the expression of many types of mRNAs, lncRNAs, and circRNAs are related to PGC expression, which, in turn, is involved in cancer-related pathways. DElncRNA related to PGC expression might influence tumor progression by positively regulating the expression of adjacent protein-coding genes. DElncRNAs and DEcircRNAs related to PGC expression co-mediated a complicated ceRNA network to modulate its expression, of which the closest network is SNHG16/hsa_circ_0008197-mir-98-5p/hsa-let-7f-5p/hsa-let-7c-5p-PGC. This study contributes to a foundation for the subsequent exploration of possible regulatory mechanisms related to PGC in gastric cancer.

Declarations

Ethics approval and consent to participate

The study was supported by the research ethics committee of the First Affiliated Hospital of China Medical University ([2021]94). The written informed consents in this study were signed by all patients.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the results of this manuscript are available from the corresponding author upon reasonable request.

Competing interests

All authors disclose no conflicts of interest that might bias their work.

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Authors' contributions

Yuan Yuan and Qian Xu conceived and designed this study. Li-rong Yan, Han-xi Ding, Shi-xuan Shen and Xiao-dong Lu were responsible for the data analysis and performed data interpretation. Li-rong Yan and Han-xi Ding wrote the paper. Qian Xu and Yuan Yuan revised the manuscript.

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Figures
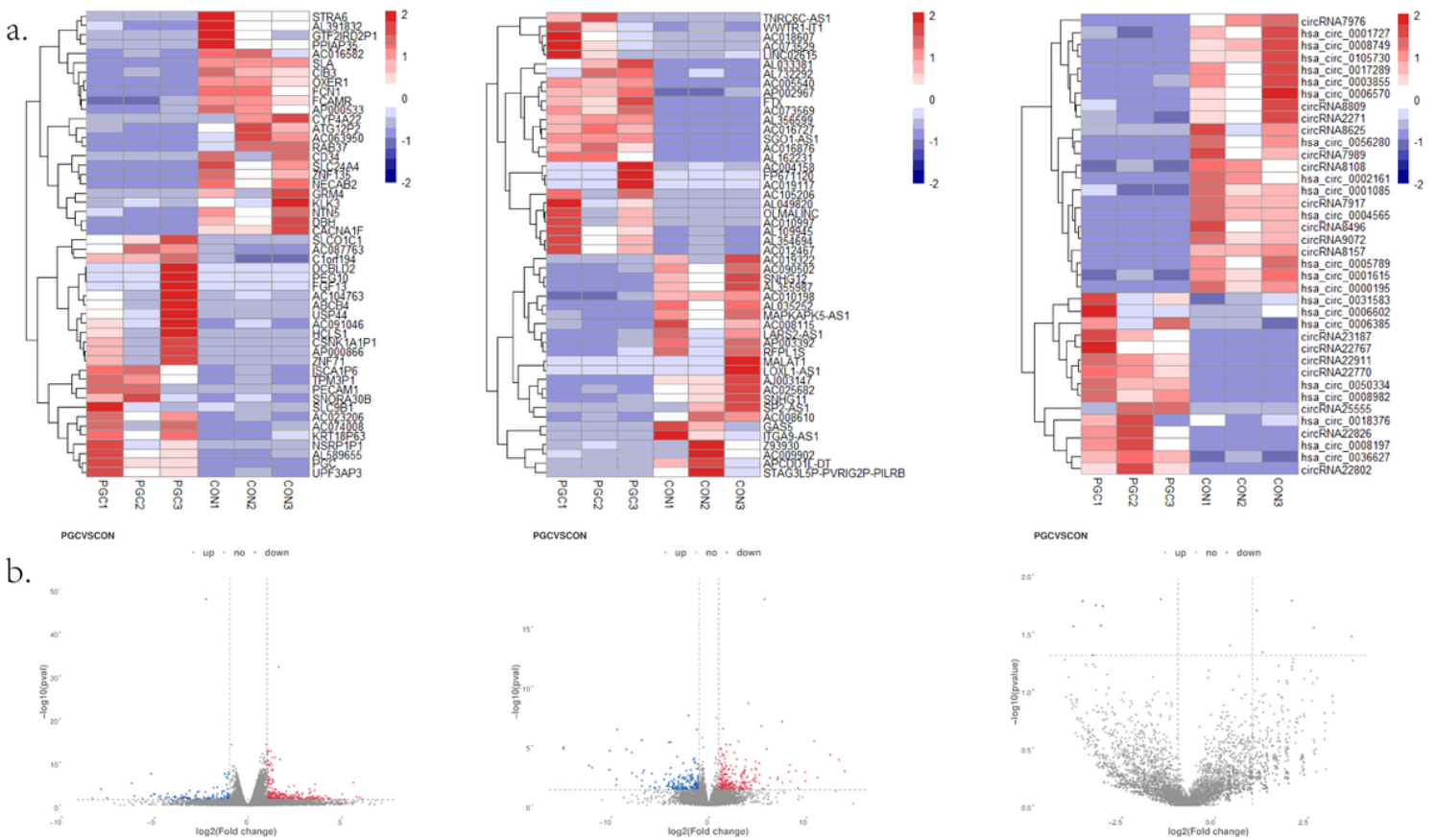
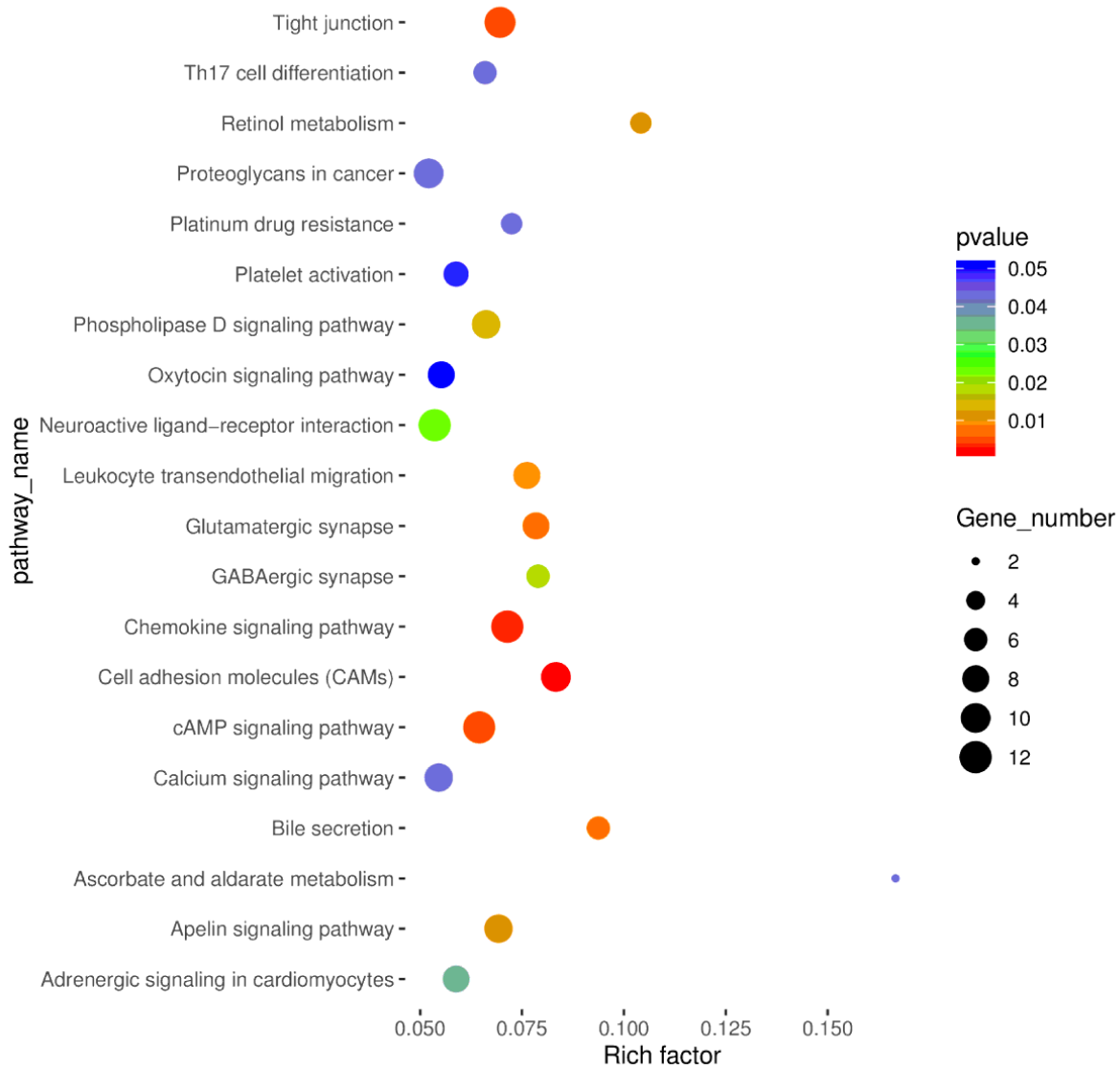
Figure 1

The heatmap (a) and volcano plot (b) of DEmRNAs, DElncRNAs and DEcircRNAs associated with PGC expression. DE, differentially expressed.
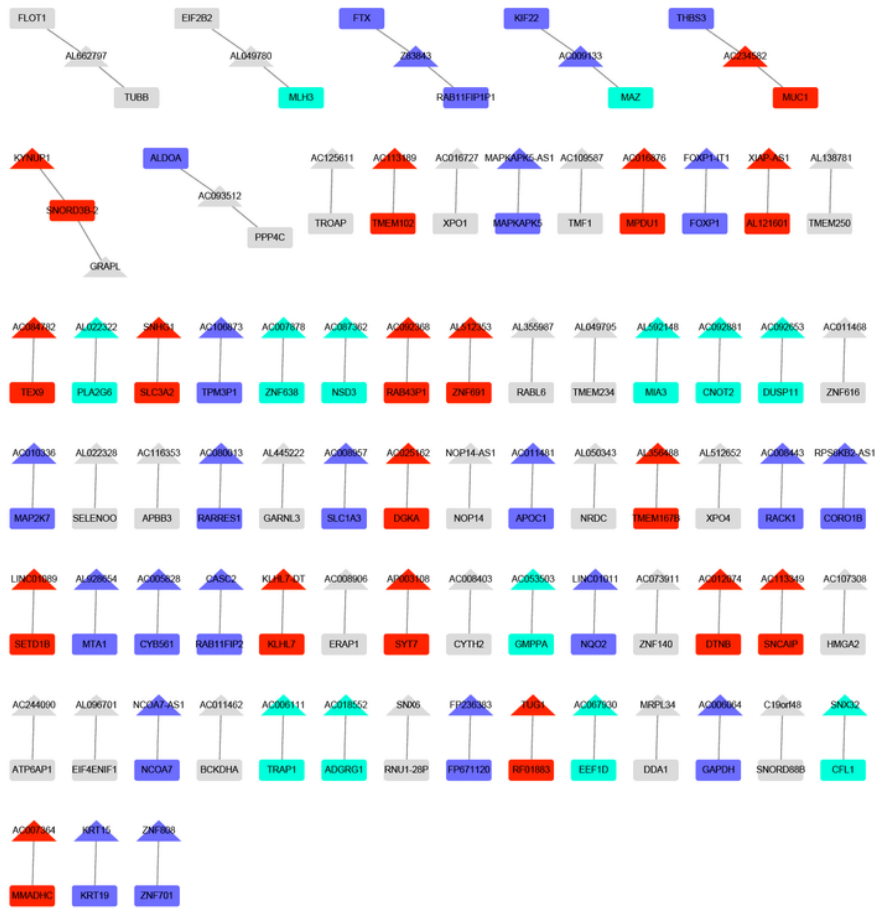
Figure 2

The protein-protein interaction network of DEmRNAs associated with PGC expression. The one circled in red represented protein that probably interact with PGC. DE, differentially expressed.

Figure 3

GO enrichment analysis of DEmRNAs associated with PGC expression. DE, differentially expressed.
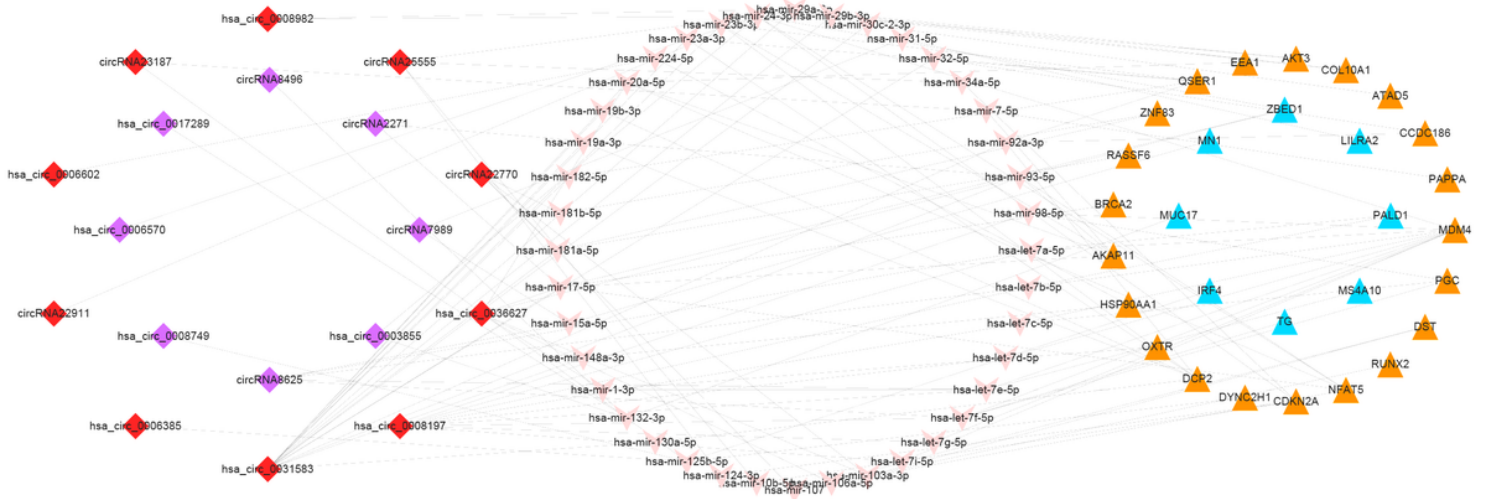
Figure 4

KEGG pathways analysis of DEmRNAs associated with PGC expression. DE, differentially expressed. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Figure 5

The cis-regulatory network of DElncRNAs associated with PGC expression. The rectangle represents DEmRNAs and the equilateral triangle represents DElncRNAs. Blue color represents a medium intensity positive correlation, green color represents a high intensity positive correlation, gray color represents a weak correlation, and orange color represents a medium intensity negative correlation. DE, differentially expressed.
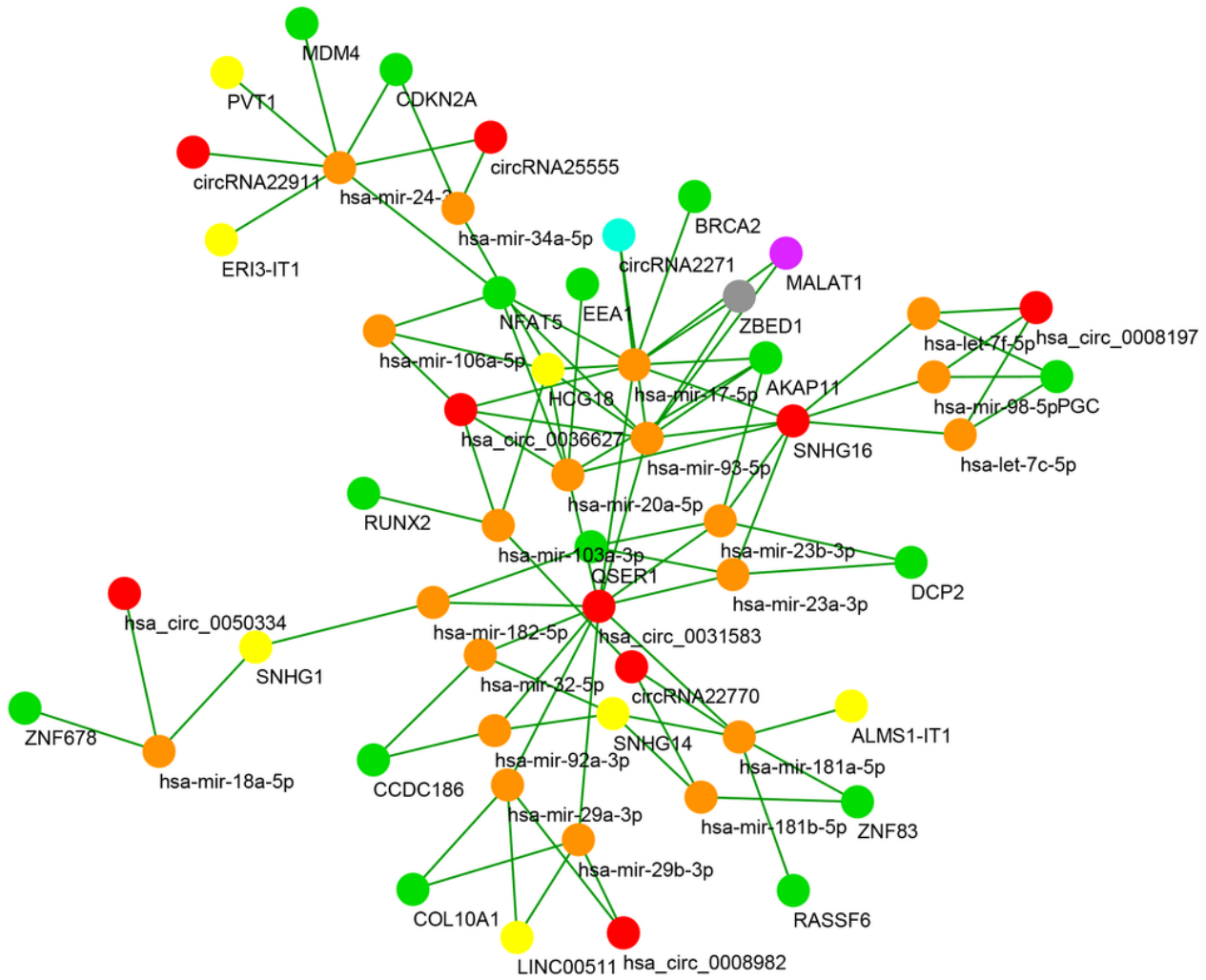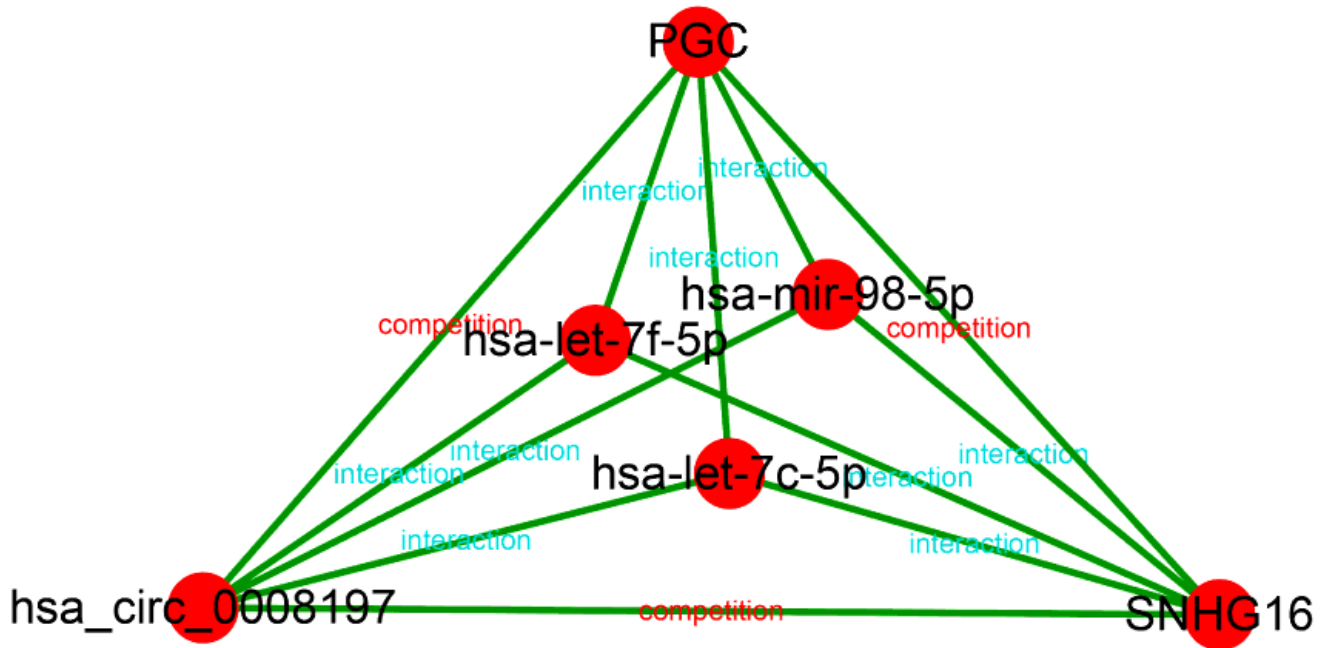
Figure 6

DElncRNAs mediated ceRNA network associated with PGC expression. Red color represents up-regulated lncRNAs, purple color represents down-regulated lncRNAs, Blue color represents up-regulated mRNAs, orange color represents down-regulated mRNAs and green color represents miRNAs. ceRNA, competitive endogenous RNA, DE, differentially expressed.

Figure 7

DEcircRNAs mediated ceRNA network associated with PGC expression. Red color represents up-regulated circRNAs, purple color represents down-regulated circRNAs, Blue color represents down-regulated mRNAs, orange color represents up-regulated mRNAs and pink color represents miRNAs. ceRNA, competitive endogenous RNA, DE, differentially expressed.

Figure 8

DEcircRNAs and DElncRNAs mediated co-regulated network associated with PGC expression. Blue color represents down-regulated circRNAs, purple color represents down-regulated lncRNAs, yellow color represents up-regulated lncRNAs, grey color represents down-regulated mRNAs, green color represents up-regulated mRNA and orange color represents miRNAs. DE, differentially expressed.

Figure 9

PGC directly related DEcircRNAs and DElncRNAs mediated co-regulated network and intermolecular relationships. DE, differentially expressed.
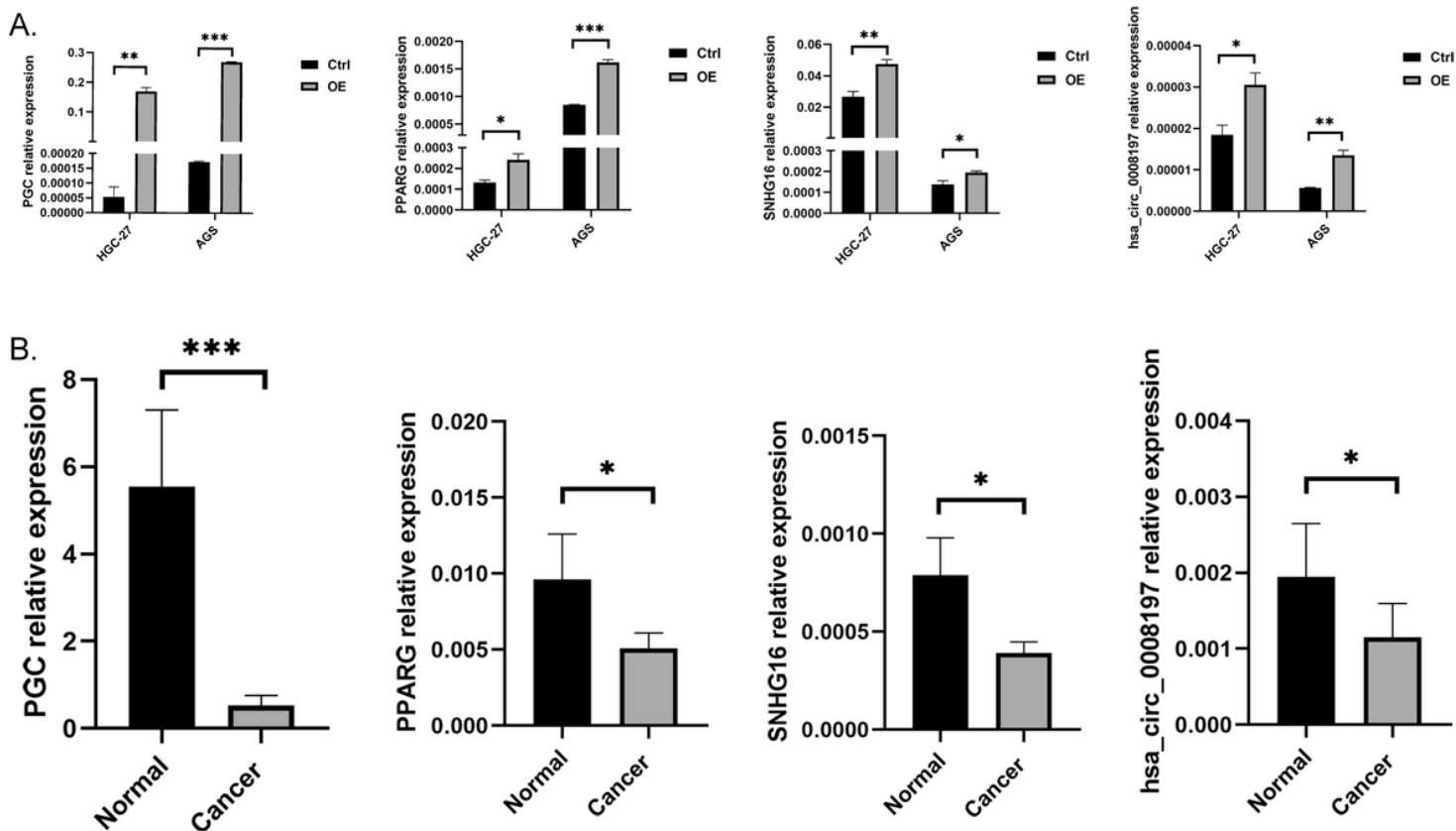
Figure 10

qRT-PCR validation in PGC overexpressed cells and gastric cancer tissues. A. The mRNA relative expression of PGC, PPARG, SNHG16 and hsa_circ_0008197 detected by qRT-PCR in HGC-27 and AGS cells. B. The mRNA relative expression of PGC, PPARG, SNHG16 and hsa_circ_0008197 detected by qRT-PCR in gastric cancer tissues. (Measurement data were expressed as mean ± SEM, *P<0.05, **P<0.01 ***P<0.001). OE, overexpressed. Ctrl, control. qRT-PCR, quantitative real-time polymerase chain reaction.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterials.docx