Gene expression profile analysis of ENO1 knockdown in gastric cancer cell line MGC-803

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Abstract. Gastric cancer (GC) is the third leading cause of cancer-associated mortality. In a previous study, we identified that α-enolase (ENO1) promoted cell migration in GC, but the underlying molecular mechanisms remain to be fully elucidated. In the present study, small interfering RNAs were identified to interfere with ENO1 expression. The cDNA expression profiling was performed using an Affymetrix mRNA array platform to identify genes that may be associated with ENO1 in human GC cell line MGC-803. The differentially expressed genes (DEGs) were identified using the reverse transcription-quantitative polymerase chain reaction, followed by a series of bioinformatic analyses. As a result, there were 448 DEGs, among which 183 (40.85%) were downregulated. The most significant functional terms for the DEGs were the nuclear lumen for cell components (P=2.83x10⁻⁴), transcription for biological processes (P=3.7x10⁻³) and transcription factor activity for molecular functions (P=1.16x10⁻³). In total, six significant pathways were enriched, including the most common cancer-associated forkhead box O signaling pathway (P=0.0077), microRNAs in cancer (P=0.0183) and the cAMP signaling pathway (P=0.0415). Furthermore, a network analysis identified three hub genes (HUWE1, PPP1CB and HSPA4), which were all involved in tumor metastasis. Taken together, the DEGs, significant pathways and hub genes identified in the present study shed some light on the molecular mechanisms of ENO1 involved in the pathogenesis of GC.

Introduction

Gastric cancer (GC) is the third leading cause of cancer-related mortality (1-3), with ~1,033,701 novel diagnoses and 782,685 mortalities worldwide in 2018 (3). Previous studies have indicated that GC may be driven by a number of different genetic abnormalities, such as mutations in cadherin 1 (4) and catenin α1 (5). Chromosomal aberrations including Erb-b2 receptor tyrosine kinase 2 (6), adenomatous polyposis coli, tumor protein p53 and NME/NM23 nucleoside diphosphate kinase 1 (7) have also been frequently identified in GC. Certain SNPs, such as interleukin 17A (rs2275913) (8), mucin 1 (rs4072037) (9) and prostate stem cell antigen (rs2976392) (10), have indicated genetic predispositions towards an increased risk of GC. However, the aforementioned results are not sufficient to clarify the complex pathogenesis of GC. Therefore, further research into the molecular aspects involved in carcinogenesis is required, which will offer new insights into GC treatment.

The α-enolase (ENO1) gene encodes a glycolysis-associated enzyme, which contains 434 amino acids and has a molecular mass of ~57 kDa (11). Previous studies have revealed ENO1 to be abnormally expressed in a number of cancer types and serves pivotal roles in tumorigenesis (12-16). For example, in endometrial cancer, ENO1 silencing significantly decreased malignant biological behavior; furthermore, the expression level of ENO1 could affect the prognosis of patients (14). In breast cancer, ENO1 promoted vascular endothelial cell proliferation, inhibited apoptosis and accelerated blood vessel formation (15). In non-small cell lung cancer, stably upregulated ENO1 could activate the focal adhesion kinase/phosphoinositide 3-kinase (PI3K)/protein kinase B pathway and its downstream signals, and then activate...
glycolysis, the cell cycle and epithelial-mesenchymal transi-
tion-associated genes (13,16). In colorectal cancer tissues, the
expression level of ENO1 was significantly increased, which
was associated with tumorigenesis and metastasis in patients
with colorectal cancer (17). In addition, an in vitro study
suggested that overexpression of ENO1 promoted prolifera-
tion, migration and invasion of the colorectal cancer cell line
HCT116 (17). However, research regarding the role of ENO1
in GC is insufficient, and further studies are required. To date,
only a few studies have indicated that ENO1 can promote
chemoresistance in GC, and that increased protein levels of
ENO1 lead to a poor prognosis for the patient (18). Previous
studies indicated that overexpression of ENO1 can enhance
proliferation and migration in GC cell line AGS (19), and that
ENO1 can be upregulated by a well-known GC-associated
protein, CagA, in AGS cells (20). Combined with the afore-
mentioned results, we hypothesize that ENO1 serves a role in
the pathogenesis of GC. Microarray is a powerful tool that can
present the whole gene expression profile (21) and, as such,
a microarray analysis was performed on ENO1-silencing GC
cells with the aim of gaining further understanding into the
molecular mechanism(s) of ENO1 in the progression of GC.

Materials and methods

Cell culture and treatment. The human GC cell line MGC-803
(Sun Yat-sen University Cell Library, Guangdong, China)
was cultured as described previously (22). The small frag-
ment small interfering RNA (siRNA) against ENO1 and
the scrambled (control) siRNA were synthesized by Beijing
Oligobio (Beijing, China). The siRNA-ENO1 sequences
were as follows: Forward, 5'-GCAUGAGGAGAGGCUU
ATT-3' and reverse, 5'-UAACUCUGCUUCAUGCTT-3'.
The siRNA transfection experiment was conducted using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific
Inc., Waltham, MA, USA), according to the manufacturer's
protocol. The cells were plated onto 6-well plates at a density
of 8.0x10⁴ cells. Following reaching ~50% confluence, cells
were transfected. The cells were assigned to two groups:
NC group, transfected with 50 nM scrambled siRNA; and
ENO1-knockdown group, transfected with 50 nM siRNA
against ENO1. Each group had three parallel samples.
Cells were transfected with Lipofectamine 2000 (Thermo
Fisher Scientific, Inc., Waltham, MA, USA) in serum-
and antibiotic-free Opti-MEM (Thermo Fisher Scientific, Inc.),
according to the manufacturer's instructions. After 24 h, the
cells were treated with TRIzol® (Thermo Fisher Scientific,
Inc.) and the total RNA was extracted.

Microarray analysis. Following extraction of the total
RNA from the NC group and ENO1-knockdown group, the
quality was determined using NanoDrop™ 2,000 (Thermo
Fisher Scientific, Inc.), and the 2100 Bioanalyzer (Agilent
Technologies, Santa Clara, CA, USA). The amplified RNA
(aRNA) was prepared using an Affymetrix GeneChip™ 3’IVT
Express kit (Thermo Fisher Scientific, Inc., Waltham, MA,
USA), according to the manufacturer's protocol. The aRNA
was purified, fragmented and hybridized with the chip probes.
Following hybridization, the chip was stained and the final
scanned images and raw data were obtained by the Shanghai
GeneChem Co., Ltd. (Shanghai, China). The raw data were
processed using the two-way semi-linear model, and the genes
with fold change (FC) >1.5 and P<0.05 were regarded as
significantly DEGs.

Reverse transcription-quantitative polymerase chain reaction
(RT-qPCR) analysis. To determine the interference efficiency
of siRNA-ENO1 and to validate the gene chip results, ENO1
and five random genes were selected for RT-qPCR analysis and
were as follows: AVL9 cell migration-associated (AVL9), glia
maturation factor β (GFMB), G-protein-coupled receptor 180
(GPR180), microfibrillar-associated protein 3 (MFAP3) and
septin 8 (SEPT8). The total RNA was extracted from the cells
and the quality was assessed using the aforementioned method.
RNA was reverse-transcribed into cDNA using a reverse
transcription kit (cat. no. 05091284001; Roche Diagnostics,
Basel, Switzerland), according to the manufacturer's protocol.
qPCR was carried out in a volume of 10.0 µl, including
5.0 µl SYBR® Select Master mix (Roche Diagnostics), 3.4 µl
DNase/RNase-free water (Beijing Solarbio Science and
Technology Co., Ltd., Beijing, China), 1.0 µl cDNA, 0.30 µl
forward primer and 0.30 µl reverse primer. β-actin was selected
as the internal reference gene. The Piko Real detection system
(Thermo Fisher Scientific, Inc.) was used for the amplification
according to the manufacturer's protocol. The primers were
synthesized by Generay Biotech Co., Ltd. (Shanghai, China)
and the sequences were as follows: ENO1 forward, 5’-GGG
AATCCCCACTGTTAGGT-3’ and reverse, 5’-CGGAGCTCT
AGGGCCCTCATA-3’; β-actin forward, 5’-GGGAAATCGTG
GTGACATTAAGG-3’ and reverse, 5’-CGGAGAAGAGG
CGGGAAGAGTG-3’; ALV9 forward, 5’-TTTCTTTGTTGG
GGCAAGT-3’ and reverse, 5’-ACATCCGTTGTTGTCGG
GATTTC-3’; GMFB forward, 5’-CAGCGTTGTGCTCTTTTCT
TGC-3’ and reverse, 5’-GTCTTTGTTTTGTATGTT
TGC-3’; MFAP3 forward, 5’-AATGACATAGATGCCAC
TTG-3’ and reverse, 5’-GTGCTCCTTCTCCACCTCCTA-3’;
SEPT8 forward, 5’-GGGATAATGTTCCACCTGCTGT
CTC-3’ and reverse, 5’-TTTGCGCTCTACTTCAGCCG-3’. For all
RT-qPCR experiments, the samples were amplified in triplic-
te, each consisting of three replicates. The relative levels of
target gene mRNA were calculated and normalized relative to
β-actin using the 2⁻ΔΔCt method (23).

Functional enrichment analysis. The functional enrichment
analyses of the DEGs were performed using DAVID 6.7
(https://david.ncifcrf.gov) (24). Briefly, all the differentially
expressed genes (DEGs) were uploaded in the ‘Functional
Annotation’ section of DAVID 6.7, and set E=0.01. The result
would indicate the DEGs mapping to different Gene Ontology
(GO) terms. The GO annotation (www.geneontology.org)
includes three parts: Biological processes (BP), cellular com-
ponents (CC) and molecular functions (MF), which provide a
descriptive framework and functional annotation of DEGs.
The pathway enrichment analysis was performed using Kyoto
Encyclopedia of Genes and Genomes (KEGG; http://www.
genome.jp/kegg) (25,26). P<0.05 was considered to indicate
statistically significant functional terms and pathways.

Protein-protein interaction (PPI) network construction and
module selection. A PPI network was constructed based
on Biological General Repository for Interaction Datasets (BioGRID) in WebGestalt (http://www.webgestalt.org/option.php). The DEGs were mapped to BioGRID and PPI pairs were acquired. Interactions with a confidence score >0.4 were retained in the network and were visualized using Cytoscape (version 3.5.1; http://cytoscape.org). In the PPI network, a node represents a protein product of a DEG and the degree represents the number of proteins linked to this node. The nodes with a high degree (>10) were considered to be important and named ‘hub genes’ in the present study. The PPI modules were screened using the ClusterONE plugin (version 1.0; http://www.paccanarolab.org/clusterone) in Cytoscape (27). Results were considered statistically significant when P<0.0005.

Statistical analysis. All the data were analyzed using SPSS software (version 15.0; SPSS, Inc., Chicago, IL, USA). The measurement data were expressed as the mean ± standard deviation. Comparison between two groups was performed using an independent sample t-test. P<0.05 was considered to indicate a statistically significant result.

Results

Successful knockdown of the ENO1 gene in MGC-803 cells. The mRNA expression levels of ENO1 were downregulated 12.08-fold (array data; Fig. 1) and 11.43±0.39-fold (RT-qPCR data; Fig. 2) in the ENO1-knockdown group compared with in the NC group. The results indicated that the siRNA fragments targeting the ENO1 gene were successful and that silencing was efficient.

Gene expression profile analysis and hierarchical clustering. The microarray included two groups with six samples, and the heat map results are presented in Fig. 1. As a result, there were 448 DEGs with a FC value >1.5 and P<0.05, among which, 183 (40.85%) were downregulated and 265 (59.15%) were upregulated. The top ten DEGs with high FC were identified to be downregulated in the present study has previously been reported to serve an important role in the development of breast cancer (29). In addition, FGF2, which also demonstrated significantly decreased expression, is a well-known oncogene (30,31).

Verification of the array data using RT-qPCR analysis. Five DEGs (AVL9, GMFB, GPR180, MFAP3 and SEPT8) were selected for qPCR analysis. The results (Fig. 2) indicated that the mRNA levels of AVL9, GMFB, GPR180, MFAP3 and SEPT8 were downregulated 2.78-, 2.68-, 2.72-, 2.67- and 2.23-fold, respectively. In the RT-qPCR experiment, these genes were downregulated 3.05±0.07-, 2.33±0.12-, 3.15±0.06-, 2.77±0.08- and 2.89±0.12-fold, respectively. The array data were in concordance with the RT-qPCR results.

Functional annotation analysis of the DEGs. The gene annotation analysis was performed using DAVID and the detailed results (Table 1) identify the number of significant functional classifications for BP, CC and MF as 26, 7 and 10, respectively. The DEGs were mainly enriched in transcription, blood vessel morphogenesis and cell cycle for BP. CC enrichment was detected for genes associated with the nuclear lumen, organelle lumen and nucleoplasm, and MF enrichment was identified for genes associated with transcription factor activity, transcription regulator activity and cytoskeletal protein binding.

KEGG pathway enrichment analysis. The DEGs responding to ENO1 silencing were enriched in six significant pathways: Systemic lupus erythematosus [Homo sapiens (hsa05322; P<0.001), viral carcinogenesis (hsa05203; P=0.00141), alcoholism (hsa05034; P=0.003), forkhead box O (FoxO) signaling pathway (hsa04068; P=0.0077), miRNAs in cancer (hsa05206; P=0.0183) and cAMP signaling pathway (hsa04024; P=0.0415) (Table II).

PPI network analysis. The PPIs among the 448 DEGs were predicted using WebGestalt with information from BioGRID. The constructed network consisted of 209 proteins (nodes) and 293 interactions (edges) (Fig. 3). In addition, there were seven genes that had high degrees with edges ≥10 in the PPI network. These seven genes were HECT, UBA and WVE domain-containing 1, E3 ubiquitin protein ligase (HUWE1; degree, 16), protein phosphatase 1 catalytic subunit β (PPP1CB; degree, 16), heat shock protein family A (Hsp70) member 4 (HSPA4; degree, 16), signal transducer and activator of transcription 3 (STAT3; degree, 13), anillin actin-binding protein (degree, 12), Sre homology 3 domain-containing kinase-binding protein 1 (degree, 10) and casein kinase 2α (degree, 10), respectively. Among these, HUWE1, PPP1CB and HSPA4 were the top three nodes with 16 edges.

Module analysis and protein domain analysis. ClusterONE was applied for module analysis to further predict potential protein complexes. For the network constructed above, there were three significant modules (P<0.0005; Fig. 4), as follows: Module A (nodes, 24; density, 0.101; quality, 0.549; P=0.00000791); Module B (Nodes, 18; density, 0.183; quality, 0.683; P=0.000136) and Module C (nodes, 17; density, 0.132; quality, 0.581; P=0.00045) (Table III). For the protein domain analysis, one significant domain was found for Module B: Myosin head, motor domain (IPR001609) (P=0.034). No significantly enriched protein domains were identified in Modules A and C.

Discussion

In the present study, a total of 448 DEGs responded to ENO1 knockdown in the human GC cell line MGC-803. Certain DEGs that demonstrated significantly decreased expression in the present study, including TPM4, have been reported to be associated with clinical progression in patients with colon cancer (28). Another gene, SGK3, which was identified to be downregulated in the present study has previously been reported to serve an important role in the development of breast cancer (29). In addition, FGF2, which also demonstrated significantly decreased expression, is a well-known oncogene (30,31).

The DEGs were enriched in six significant pathways: Systemic lupus erythematosus, viral carcinogenesis, alcoholism, FoxO signaling pathway, miRNAs in cancer and...
Table I. GO analysis of the DEGs regulated by ENO1 silencing.

| Identifier | Functional term | Count | P-value         |
|------------|-----------------|-------|----------------|
| **Biological process** | | | |
| GO:0006350 | Transcription | 86    | 6.43x10^{-7}   |
| GO:0006355 | Regulation of transcription, DNA-dependent | 73    | 5.37x10^{-6}   |
| GO:0045449 | Regulation of transcription | 96    | 1.08x10^{-5}   |
| GO:0051252 | Regulation of RNA metabolic process | 73    | 1.18x10^{-5}   |
| GO:0010629 | Negative regulation of gene expression | 28    | 1.00x10^{-4}   |
| GO:0016481 | Negative regulation of transcription | 26    | 1.40x10^{-4}   |
| GO:0006357 | Regulation of transcription from RNA polymerase II promoter | 35    | 1.81x10^{-4}   |
| GO:0048514 | Blood vessel morphogenesis | 16    | 1.98x10^{-4}   |
| GO:0010605 | Negative regulation of macromolecule metabolic process | 35    | 2.17x10^{-4}   |
| GO:0045934 | Negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process | 27    | 3.13x10^{-4}   |
| GO:0010558 | Negative regulation of macromolecule biosynthetic process | 28    | 3.74x10^{-4}   |
| GO:0051172 | Negative regulation of nitrogen compound metabolic process | 27    | 3.82x10^{-4}   |
| GO:0045892 | Negative regulation of transcription, DNA-dependent | 21    | 4.39x10^{-4}   |
| GO:0051253 | Negative regulation of RNA metabolic process | 21    | 5.34x10^{-4}   |
| GO:0031327 | Negative regulation of cellular biosynthetic process | 28    | 5.50x10^{-4}   |
| GO:0009890 | Negative regulation of biosynthetic process | 28    | 7.64x10^{-4}   |
| GO:0001568 | Blood vessel development | 16    | 9.55x10^{-4}   |
| GO:0001525 | Angiogenesis | 12    | 9.60x10^{-4}   |
| GO:0007049 | Cell cycle | 34    | 1.189962x10^{-3} |
| GO:0001944 | Vasculature development | 16    | 1.215671x10^{-3} |
| GO:0031099 | Regeneration | 8    | 1.374438x10^{-3} |
| GO:0051726 | Regulation of cell cycle | 18    | 3.03174x10^{-3} |
| GO:0031100 | Organ regeneration | 5    | 3.326962x10^{-3} |
| GO:0007507 | Heart development | 13    | 6.280962x10^{-3} |
| GO:0030593 | Neutrophil chemotaxis | 4    | 8.795164x10^{-3} |
| GO:0007167 | Enzyme-linked receptor protein signaling pathway | 17    | 9.52888x10^{-3} |
| **Cell component** | | | |
| GO:0031981 | Nuclear lumen | 52    | 2.58x10^{-4}   |
| GO:0070013 | Intracellular organelle lumen | 60    | 3.75x10^{-4}   |
| GO:0043233 | Organelle lumen | 60    | 6.74x10^{-4}   |
| GO:0031974 | Membrane-enclosed lumen | 60    | 1.097428x10^{-3} |
| GO:0005654 | Nucleoplasm | 34    | 1.312471x10^{-3} |
| GO:0005783 | Endoplasmic reticulum | 34    | 4.951755x10^{-3} |
| GO:0044451 | Nucleoplasm part | 22    | 8.735324x10^{-3} |
| **Molecular function** | | | |
| GO:0003700 | Transcription factor activity | 43    | 2.18x10^{-4}   |
| GO:0030528 | Transcription regulator activity | 58    | 5.22x10^{-4}   |
| GO:0008092 | Cytoskeletal protein binding | 26    | 6.25x10^{-4}   |
| GO:0016564 | Transcription repressor activity | 18    | 1.995073x10^{-3} |
| GO:0043014 | α-tubulin binding | 4    | 2.056678x10^{-3} |
| GO:0003677 | DNA binding | 77    | 3.813995x10^{-3} |
| GO:0046914 | Transition metal ion binding | 88    | 6.180415x10^{-3} |
| GO:0003779 | Actin binding | 17    | 6.453361x10^{-3} |
| GO:0008270 | Zinc ion binding | 75    | 6.826083x10^{-3} |
| GO:0004672 | Protein kinase activity | 26    | 7.297114x10^{-3} |

GO, Gene Ontology.
cAMP signaling pathway (Table II). Among these, some were reported to be associated with cancer development, such as the cAMP signaling pathway and FoxO signaling pathway. cAMP signaling regulates protein levels by controlling gene transcription via transcriptional activators that are involved in cancer cell migration, proliferation, apoptosis, and cytoskeleton remodeling in bladder cancer (32), breast cancer (33) and lung cancer (34). Furthermore, an exchange protein directly activated by cAMP (EPAC1) has been regarded as a prognostic marker and may be a potential therapy target for GC (35), which suggests the important role that the cAMP signaling pathway serves in the pathological processes of GC. The FoxO signaling pathway has also been reported to be associated with breast cancer (36), bladder cancer (37), prostate cancer (38) and lung cancer (39). Previous studies have demonstrated that there may be crosstalk between the cAMP signaling pathway and the FoxO signaling pathway. On one hand, activation of the cAMP signaling pathway increased FoxO1 phosphorylation (40). On

| Identifier | Name                          | Count | Gene                                                                 | P-value          |
|------------|--------------------------------|-------|----------------------------------------------------------------------|-----------------|
| hsa05322   | Systemic lupus erythematosus   | 6     | HIST1H2BD, HIST1H2BG, HIST1H2BF, HIST1H2BE, HIST1H2BI, HIST1H2BC     | <0.001          |
| hsa05203   | Viral carcinogenesis           | 10    | CDKN1A, HIST1H2BD, CCND1, STAT3, TRAF3, HIST1H2BG, HIST1H2BF, HIST1H2BE, HIST1H2BI, HIST1H2BC | 1.41x10⁻³       |
| hsa05034   | Alcoholism                     | 6     | HIST1H2BD, HIST1H2BG, HIST1H2BF, HIST1H2BE, HIST1H2BE, HIST1H2BI, HIST1H2BC | 3.11x10⁻³       |
| hsa04068   | FoxO signaling pathway         | 6     | CDKN1A, SGK3, CCND1, STAT3, TGFB1, SETD7                             | 7.7x10⁻³        |
| hsa05206   | MicroRNAs in cancer            | 8     | CDKN1A, CYP1B1, DICER1, PDCD4, MCL1, PLAU, CCND1, STAT3              | 1.83x10⁻²       |
| hsa04024   | cAMP signaling pathway         | 5     | ADCY9, HTR1D, PPP1CB, RAC1, CREB3L2                                  | 4.15x10⁻²       |

hsa, Homo sapiens; FoxO, Forkhead box O; cAMP, cyclic adenosine monophosphate.
the other hand, FoxOs supported the metabolic requirements of normal and tumor cells via the PI3K signaling pathway, which was reported to interact with the cAMP signaling pathway in a number of physiological processes (41). In addition, systemic lupus erythematosus was the most significant pathway response to ENO1 inhibition, and this result was similar to that in our previous study on TPI silencing (22). As previously discussed, although no definitive evidence has suggested the involvement of systemic lupus erythematosus in the pathogenesis of GC, there may be an association between the two since certain sporadic patients were affected by GC and systemic lupus erythematosus simultaneously (22,42,43). The molecular mechanisms underlying the involvement of systemic lupus erythematosus in the pathogenesis of GC are not currently fully understood and further research is required.

The constructed PPI network based on BioGRID included 209 nodes and 293 edges. There were seven DEGs with a degree ≥10, among which, the first three were PPP1CB, HUWE1 and HSPA4, which were regarded as hub genes and may interact with ENO1 in GC progression. These
three genes have been reported to be associated with cancer development. PPP1CB, encoding protein phosphatase 1 catalytic subunit β isoform, has been reported in prostate cancer, chronic lymphocytic leukemia (44) and even used as a potential biomarker for distinguishing malignant melanoma from other melanocytic lesions (45). HUWE1, as a ubiquitin ligase, has been regarded as a tumor suppressor and served key roles in tumorigenesis (46). For example, compared with normal thyroid tissue, HUWE1 was downregulated in human thyroid cancer tissues, and overexpression of HUWE1 in thyroid cancer cells enhanced chemotherapeutic sensitivity and inhibition of cell proliferation, cell migration and invasion (47). The third hub gene HSPA4 encodes a heat shock protein (HSPA12A, HSP90B1, HSPA4, HSPA5 and HSPA6) in tumor tissues is associated with poor outcomes from hepatitis B virus-associated early-stage hepatocellular carcinoma (48). Furthermore, HSPA4 has been reported to regulate cell migration and delay gastric ulcer healing (49). To the best of our knowledge, the present study is the first to identify the possible association between PPP1CB, HUWE1, HSPA4 and ENO1. Further studies are required to confirm these connections and their functions in the pathogenesis in GC tumorigenesis.

In summary, the results of the present study provide a comprehensive bioinformatic analysis of the genes associated with ENO1. The important signaling pathways (such as cAMP signaling pathway and FoxO signaling pathway) and key genes

### Table III. Detailed information of three modules screened from the PPI network of all DEGs.

| Module | Nodes | Density | Quality | P-value          | Members                                                                                                                                 |
|--------|-------|---------|---------|-----------------|----------------------------------------------------------------------------------------------------------------------------------------|
| A      | 24    | 0.101   | 0.549   | 7.905x10⁻⁶     | HSPE1, PGM2L1, HSPA4, ARFIP1, TMED7, TMEM30A, HUWE1, GANAB, GBAS, TROVE2, ATL3, SEC24D, FAMIL14A1, ZW10, HDLBP, FAP, PA2G4, DNAJA2, WIP12, MCL1, PRSS23, THRβ, PSMD10, HIST1H2BC |
| B      | 18    | 0.183   | 0.683   | 1.356x10⁻⁴     | SORBS2, ARHGAP21, ANLN, PPP1CB, TPM4, SSFA2, MYO1C, LARP4, SSH1, ACTR2, EFHD2, STBD1, PPP1R3C, MYO6, CPM, RIF1, AP0BEC3B, TMEM33 |
| C      | 17    | 0.132   | 0.581   | 4.502x10⁻⁴     | RGS19, MAPK6, CUX1, FOXX2, NFATC2, TLK2, MAP1B, GATAD2A, RBPI, MTA1, HNRNPA0, NFIA, KDSR, CA12, MAX, GADD45A, FNBP1 |

Figure 4. Modules identified from the network based on BioGRID. Three modules were identified with P<5x10⁻⁴. Module A (nodes, 24; density, 0.101; quality, 0.549; P=7.91x10⁻⁶); Module B (nodes, 18; density, 0.183; quality, 0.683; P=1.36x10⁻⁴) and Module C (nodes, 17; density, 0.132; quality, 0.581; P=4.50x10⁻⁴).
(such as PPP1CB, HUWE1 and HSPA4) may help to narrow down the role of ENO1 in the pathogenesis and treatment of GC.

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Availability of data and materials

The datasets generated and/or analyzed during the study are available from the corresponding author upon request.

Authors’ contributions

ZH and PO designed the experiments, performed the bioinformatic analysis and wrote the paper; BL and HP identified the correct siRNA fragment and performed the array; JD carried out the statistical analysis; RH performed the RT-qPCR experiment; SZ cultured the cells.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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