Capicua homology protein inhibits the progression of gastric cancer through the PI3K/AKT signaling pathway

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Research

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Abstract

Purpose Capicua homolog protein (CIC) played a broad role in the development of cancer in humans, however, its role in the progression of gastric cancer (GC) specifically has been unclear. This study aimed to explore the expression of CIC and its potential clinical value in patients with GC. Methods The CIC levels in GC tissues and cell lines were examined by quantitative real-time polymerase chain reaction (qRT-PCR). And the in-vitro effects of CIC expression in MGC-803 cells on their proliferation, invasion, and the progression of epithelial-mesenchymal transition were assessed by CCK-8 assays, Matrigel-invasion analysis, qRT-PCR and Western blot assays, separately. In addition, the effects of downregulation of CIC on the activation of PI3K/AKT signaling pathway were measured using Western-blot analysis. Results The results showed CIC levels were lower in GC tissues and GC cell lines, and these lower CIC levels were correlated with tumor differentiation, Helicobacter pylori infection, TNM stage, and patient survival. In addition, CIC overexpression could promote cell proliferation, invasion, and progression of epithelial-mesenchymal transition in MGC-803 cells. Notably, exotic expression of CIC inactivated the phosphoinositide 3-kinase/protein kinase B signaling pathway. Conclusions In conclusion, our finding suggested CIC could serve as a potential diagnostic and prognostic biomarker and a probable therapy target for GC.

Background

Although the morbidity and mortality of gastric cancer (GC) has declined, this disease remains the third most lethal cancer worldwide(1). Because the symptoms of early GC are not readily apparent or easily observed, most patients are already in the advanced stages when diagnosed; therefore, it is critical to investigate a molecular marker for an early diagnosis and treatment of this disease.

Capicua homology protein (CIC) is a member of the SOX-related high-mobility group (HMG) subfamily of proteins that is highly conserved among different species(2). As a transcriptional repressor, CIC contains two highly conserved domains—the HMG-box which mediates DNA binding and nuclear location, and a C-terminal motif C1, which cooperates with the HMG-box for DNA binding(2–5). Recent studies have reported that CIC functions as a transcriptional regulator and is linked to several cancer and non-cancer diseases, including neurodegenerative disease, T cell lymphoblastic leukemia, and hepatocellular carcinoma(6–9). In addition, evidence has implicated that CIC functions as a determinant of the sensitivity to inhibitors of the epidermal growth factor receptor and mitogen-activated protein kinase (MAPK)-extracellular signal-regulated kinase pathway(10), which suggests that CIC plays a broad role in human cancer; however, the function and mechanism of CIC in GC have remained unclear. The epithelial–mesenchymal transition (EMT), a complex cellular process, has been found to promote the migration and invasion of various cancer cells(11–13), including GC(14, 15). Previous studies have proved a variety of molecules, including microRNA(16), long non-coding (Lnc)RNA(17, 18), and messenger (m)RNA(19, 20), contribute to multiple biochemical changes in epithelial cells(21); therefore, it is important to explore the molecules that drive the EMT process to be able to identify a specific drug that could target the metastasis potential of GC. The aim of the present study was to investigate the function of CIC in
metastatic GC and its underlying molecular mechanisms of action in hopes of shedding new light on target therapy for patients with this disease.

Materials And Methods

Patients and tissue samples

This study was approved and supervised by the Research Ethics Committee of the Affiliated Hospital of Nantong University (Nantong, China). All patients provided their signed written informed consent. Paired GC and adjacent normal gastric tissues were obtained in 2017 and 2018 from 20 patients who underwent primary surgical GC resection at the hospital. All tissue samples were immediately frozen in liquid nitrogen until use.

The GC specimens were obtained from 183 GC patients at the Affiliated Hospital of Nantong University (Nantong, China) from 2012 to 2017. All patients had received their first diagnosis of GC but had not received any other treatment, including chemotherapy, before surgery. The clinicopathological data on the 183 GC patients comprised age, sex, degree of differentiation, histological type, lymph node metastasis, distant metastasis, and TNM stage (Table 1).
## Table 1
Clinical characteristics of GC patients

| Parameter                  | n   |
|----------------------------|-----|
| Age(yr)                    | 63(38–86) |
| Gender                     |     |
| Male                       | 128 |
| Female                     | 55  |
| Tumor diameter(cm)         |     |
| ≤ 4                        | 118 |
| >4                         | 65  |
| Tumor Location             |     |
| Up                         | 43  |
| Middle                     | 35  |
| Down                       | 105 |
| Tumor differentiation      |     |
| High                       | 18  |
| Middle                     | 45  |
| Low                        | 120 |
| HP infection               |     |
| Negative                   | 51  |
| Positive                   | 132 |
| T stage                    |     |
| 1a                         | 16  |
| 1b                         | 23  |
| 2                          | 27  |
| 3                          | 102 |
| 4a                         | 12  |
| 4b                         | 3   |
| N stage                    |     |
| Parameter               | n  |
|-------------------------|----|
| 0                       | 65 |
| 1                       | 27 |
| 2                       | 44 |
| 3a                      | 35 |
| 3b                      | 12 |

| TNM stage              |    |
|------------------------|----|
| \( A \)                | 32 |
| \( B \)                | 15 |
| \( A \)                | 28 |
| \( B \)                | 24 |
| \( A \)                | 37 |
| \( B \)                | 29 |
| \( C \)                | 12 |
| \(      \)             |  6 |

| Five-year survival     |    |
|------------------------|----|
| Yes                    | 117|
| No                     | 66 |

**Cell Line Cultures, Plasmids, And Transfection**

Normal gastric epithelial (GES-1) and GC cell lines (MGC-803 and MKN-45) were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, USA) according to manufacturer’s protocol. CIC complementary DNA (cDNA) that was cloned into pcDNA3.1 plasmid was purchased from General Biosystems (An Hui, China), and short heparin RNA for CIC (CIC-shRNA) was purchased from Gene Pharma Co., Ltd (Su Zhou, China).

GC cells were transfected with the CIC-overexpressed plasmid (CIC), CIC-shRNA, or a negative control (pcDNA3.1) at approximately 50% density using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol.
Quantitative Real-time Polymerase Chain Reaction

Total RNA was extracted from the cells or tissues using TRizol reagent (Thermo Fisher Scientific, Carlsbad, CA, USA). Two micrograms of RNA were used with the High Capacity cDNA Reverse Transcription Kit to synthesize cDNA (Thermo Fisher Scientific). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis for the indicated molecules was conducted using SYBR Green Supermix (Thermo Fisher Scientific). The primer sequences are shown in Table 2; β-actin was used as the internal control. The relative expression of each gene was quantified using the $2^{-\Delta\Delta Ct}$ method as reported(22).

| Table 2 | Sequences of primers for qrt-pcr |
|---------|----------------------------------|
|         | Forward (5' to 3') | Reverse (5' to 3') |
| CIC     | ACAGGTACAGAAGCCGAGGA         | GCAGACAAACCTTGAGGGA |
| E-cadherin | GCTGGACCGAGAGAGTTTCC     | CAAAATCCAGGCTTGGGAG |
| N-cadherin | TGGGAAATGGAAACTTGATGC       | AGTTGCTAAACTTCACAGAAG |
| Vimentin | ATTCCTCTTTGCGTCAAGG        | CTTCAGAGAGGAGGCGGA |
| β-actin  | GCTCTCTGCTCCTCTGTTCC       | CGACCAATCCGTGTACTCC |

Western Blot Assay

Whole-cell extracts from the cultured cells transfected with the indicated molecule or tissues were prepared using radioimmunoprecipitation assay lysis buffer (Beyotime, Shanghai, China). Thirty micrograms of protein were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred into polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA), which were then incubated with rabbit anti-CIC (cat. no. ab123822, dilution, 1:1000), mouse anti-β-actin (cat. no. ab8226, dilution, 1:5000), rabbit anti-pan-protein kinase B (AKT) (cat. no. ab8805, dilution, 1:1000), rabbit anti-pan-AKT (phospho T308) (cat. no. ab38449, dilution, 1:1000), mouse anti-vimentin (cat. no. ab8978, dilution, 1:1000), rabbit anti-E cadherin (cat. no. ab40722, dilution, 1:1000), and rabbit anti-N cadherin (cat. no. ab18203, dilution, 1:1000) (all purchased from Abcam, Cambridge, MA, USA). PI3 kinase p85 antibody (cat. no. 4292, dilution, 1:1000) and phospho-PI3 kinase p85 (Tyr458)/p55 (Tyr199) antibody (cat. no. 4228, dilution, 1:1000) were purchased from Cell Signaling Technology (Boston, MA, USA) and incubated at 4°C overnights. After washing four times with Tween 20, the membranes were immunoblotted with goat anti-rabbit immunoglobulin (Ig)G or goat anti-mouse IgG secondary antibody, purchased from Jackson Immuno Research (Lancaster, PA, USA), at room temperature (RT) for 2 h. The expression levels of the indicated molecules were detected using chemiluminescent immunoassay, β-actin was used as the internal control.
Cell invasion assay

The 24-well Transwell plates were precoated with Matrigel (BD Biosciences Pharmingen, San Jose, CA, USA) at 37°C for 30 min (BD Biosciences, San Jose, CA, USA). The cells transfected with the indicated molecules were cultured overnight, harvested, and resuspended with 2% FBS. Approximately $1 \times 10^5$ cells were added into the inner chamber, and the outer chamber contained RPMI-1640 medium with 20% FBS. After incubating at 37°C for 24 h in 5% carbon dioxide, the cells on the upper surface of the inner chamber were washed with cold PBS and fixed with cold methanol at RT for 20 min. The cells were then rinsed with crystal violet at RT for 30 min. Those cells on the upper space of inner chamber were removed using cotton swabs, and those adhered to the lower surface of the inner chamber were counted using the Olympus BX-43 microscope (Tokyo, Japan).

Cell proliferation assay

Briefly, approximately 5,000 cells transfected with the indicated molecules and suspended in 100 μL RPMI-1640 medium were cultured in 96-well plates. After incubating separately for 0, 24, 48, 72, 96 h, 10 μL cell counting kit-8 (CCK-8) solutions was added to each well and let stand for 4 h. The absorbance of each well at 450 nm was detected using the Multiskan FC automated microplate reader (Thermo Fisher Scientific).

Statistical analyses

Statistical analyses were conducted using SPSS 19.0 (SPS Inc., Chicago, IL, USA). Patient survival rates were calculated using the Kaplan-Meier survival analysis method. Statistically significant differences were found using one-way analysis of variance. The results are expressed as the mean ± SD. P < 0.05 was considered statistically significant.

Results

CIC is downregulated in human GC tissues and GC cell lines

To reveal the function of CIC in GC tissues and GC cell lines, CIC expression was detected using qRT-PCR and Western blotting. The results indicated that CIC expression in GC tissues was lower than that in adjacent normal tissues in both mRNA and proteins (Fig. 1). In addition, the MKN-45 and MGC-803GC cell lines showed decreased levels of CIC compared with that in GES-1 cells (Fig. 1). Furthermore, low expression of CIC was associated with poor differentiation, lymph node metastasis, and an advanced clinical stage of GC, but not with age, sex, tumor diameter, or tumor location (Tables 3, 4). In addition, the survival time of patients with low CIC expression was shorter than that in those with high CIC expression (Fig. 2).
| Groups                      | Patients,n | % of total | CIC expression level | \( \chi^2 \) | P value |
|-----------------------------|------------|------------|----------------------|--------------|---------|
|                             |            |            | Low (n, %)           | High (n, %)  |
| Age (yr)                    | 0.260      | 0.643      |                      |              |
| \( \leq 50 \)               | 20         | 10.93      | 8(40.00%)            | 12(60.00%)   |         |
| \( >50 \)                   | 163        | 89.07      | 75(46.01%)           | 88(53.99%)   |         |
| Gender                      | 0.094      | 0.872      |                      |              |
| Male                        | 128        | 69.95      | 59(46.09%)           | 69(53.91%)   |         |
| Female                      | 55         | 30.05      | 24(43.64%)           | 31(56.36%)   |         |
| Tumor diameter (cm)         | 1.966      | 0.167      |                      |              |
| \( \leq 4 \)                | 118        | 64.48      | 49(41.53%)           | 69(58.47%)   |         |
| \( >4 \)                    | 65         | 35.52      | 34(52.31%)           | 31(47.69%)   |         |
| Tumor location              | 2.158      | 0.340      |                      |              |
| Up                          | 43         | 23.50      | 21(48.84%)           | 22(51.16%)   |         |
| Middle                      | 35         | 19.13      | 19(54.29%)           | 16(45.71%)   |         |
| Down                        | 105        | 57.38      | 43(40.95%)           | 62(59.05%)   |         |
| Tumor differentiation       | 7.037      | 0.030      |                      |              |
| High                        | 18         | 9.84       | 3(16.67%)            | 15(83.34%)   |         |
| Middle                      | 45         | 24.59      | 20(44.44%)           | 25(55.56%)   |         |
| Low                         | 120        | 65.57      | 60(50.00%)           | 60(50.00%)   |         |
| HP infection                | 9.145      | 0.003      |                      |              |
| Negative                    | 51         | 27.87      | 14(27.45%)           | 37(72.55%)   |         |
| Positive                    | 132        | 72.13      | 69(52.27%)           | 63(47.73%)   |         |
| T stage                     | 36.299     | < 0.001    |                      |              |
| 1a                           | 16         | 8.74       | 2(12.50%)            | 14(87.50%)   |         |
| 1b                           | 23         | 12.57      | 4(17.39%)            | 19(82.61%)   |         |
| Groups | Patients, n | % of total | CIC expression level | χ² | P value |
|--------|------------|------------|----------------------|----|---------|
|        |            |            | Low (n, %)          | High (n, %) |        |        |
| 2      | 27         | 14.75      | 5(18.52%)           | 22(81.48%) |        |        |
| 3      | 102        | 55.74      | 62(60.78%)          | 40(39.22%) |        |        |
| 4a     | 12         | 6.56       | 9(75.00%)           | 3(25.00%)  |        |        |
| 4b     | 3          | 1.64       | 1(33.33%)           | 2(66.67%)  |        |        |
| N stage|            |            |                     | 75.725     | <0.001 |        |
| 0      | 65         | 35.52      | 8(12.31%)           | 57(87.69%) |        |        |
| 1      | 27         | 14.75      | 5(18.52%)           | 22(81.48%) |        |        |
| 2      | 44         | 24.04      | 30(68.18%)          | 14(31.82%) |        |        |
| 3a     | 35         | 19.13      | 30(85.71%)          | 5(14.29%)  |        |        |
| 3b     | 12         | 6.56       | 10(83.33%)          | 2(16.67%)  |        |        |
| TNM stage|          |            |                     | 105.512    | <0.001 |        |
| A      | 32         | 17.49      | 5(15.63%)           | 27(84.38%) |        |        |
| B      | 15         | 8.20       | 3(20.00%)           | 12(80.00%) |        |        |
| A      | 28         | 15.30      | 1(3.57%)            | 27(96.43%) |        |        |
| B      | 24         | 13.11      | 2(8.33%)            | 22(91.67%) |        |        |
| A      | 37         | 20.22      | 34(91.89%)          | 3(8.11%)   |        |        |
| B      | 29         | 15.85      | 25(86.21%)          | 4(13.79%)  |        |        |
| C      | 12         | 6.56       | 9(75.00%)           | 3(25.00%)  |        |        |
| C     | 6          | 3.28       | 4(66.67%)           | 2(33.33%)  |        |        |
| Five-year survival|            |            |                     | 46.556     | <0.001 |        |
| Yes   | 117        | 63.93      | 31(26.50%)          | 86(73.50%) |        |        |
| No    | 66         | 36.07      | 52(78.79%)          | 14(21.21%) |        |        |
Table 4
Univariate and multivariable analysis of prognostic factors in GC patients for 5-year survival

| Variable                  | Univariate analysis |         |         |         |                      | Multivariable analysis |         |         |
|---------------------------|---------------------|---------|---------|---------|----------------------|------------------------|---------|---------|
|                           | HR                  | P>|z|    | 95% CI  | HR                  | P>|z|    | 95% CI  |
| Age (yr)                  |                     |         |         |         |                      |                        |         |         |
| ≤ 50 vs >50               | 0.526               | 0.132   | 0.228–1.214 |        |                      |                        |         |         |
| Gender                    |                     |         |         |         |                      |                        |         |         |
| Male vs Female            | 1.245               | 0.456   | 0.700–2.211 |        |                      |                        |         |         |
| Tumor diameter (cm)       |                     |         |         |         |                      |                        |         |         |
| ≤ 4 vs >4                 | 1.639               | 0.058   | 0.984–2.729 |        |                      |                        |         |         |
| Tumor location            |                     |         |         |         |                      |                        |         |         |
| Up vs Middle vs Down      | 1.096               | 0.554   | 0.809–1.485 |        |                      |                        |         |         |
| Tumor differentiation     |                     |         |         |         |                      |                        |         |         |
| High vs Middle vs Low     | 1.381               | 0.232   | 0.814–2.343 |        |                      |                        |         |         |
| HP infection              |                     |         |         |         |                      |                        |         |         |
| Negative vs Positive      | 1.518               | 0.280   | 0.712–3.236 |        |                      |                        |         |         |
| T stage                   |                     |         |         |         |                      |                        |         |         |
| 1 vs 2 vs 3 vs 4          | 2.430               | 0.007   | 1.282–4.608 | 2.161   | 0.016 | 1.154–4.047         | |
| N stage                   |                     |         |         |         |                      |                        |         |         |
| 0 vs 1 vs 2 vs 3          | 2.106               | 0.001   | 1.333–3.327 | 2.056   | 0.001 | 1.319–3.203         | |
| TNM stage                 |                     |         |         |         |                      |                        |         |         |
| Variable       | Univariate analysis | Multivariable analysis |
|----------------|---------------------|------------------------|
| Low vs High    | 0.391               | 0.522                  |
|                | 0.016               | 0.040                  |
|                | 0.182–0.840         | 0.281–0.970            |
|                | 0.081               | 0.281–0.970            |

**Ectopic expression of CIC inhibits the proliferation, invasion, and migration of GC cells in vitro**

Based on the expression levels of CIC in cells, MKN-45 and MGC-803 cells could be chosen for further study, while the subsequent studies revealed that MGC-803 cells were partially contaminated with HeLa cells(23), so we chose MKN-45 cells for further study. As shown in Fig. 3A, CIC levels were successfully reduced in MKN-45 cells when transfected with CIC-shRNA but increased when transfected with CIC-overexpressed plasmid.

To investigate the function of CIC on GC progression, the CCK-8 assay revealed that overexpressed CIC significantly inhibited cell growth (Figs. 4A, B) after 48 h, and that ectopic expression of CIC in MKN-45 cells inhibited the number of invasive cells compared with that in the control cells (Fig. 4C). These data indicated that CIC was also involved in the invasion of GC expect for proliferation.

**CIC inhibited the progression of EMT and activation of the phosphoinositide 3-kinase/protein kinase B signaling pathway**

Because EMT is associated with GC metastasis(19, 24), the function of CIC was observed on the progression of EMT in MKN-45 cells. As shown in Fig. 3, the mRNA and protein levels of N-cadherin decreased; whereas, E-cadherin increased in the pcDNA-CIC group compared with those in the controls.

When CIC was downregulated, the mRNA and protein levels of N-cadherin increased and that of E-cadherin decreased compared with those in the controls. These results indicated that CIC suppressed EMT progression in the GC cell lines; however, the mechanism remained unclear.

It has been well documented that the phosphoinositide 3-kinase/protein kinase B signaling pathway (PI3K/AKT) signaling pathway plays an important role in the proliferation, invasion, and EMT metastasis of GC (25, 26); therefore, we hypothesized whether CIC affects PI3K/AKT signaling. As shown in Fig. 5, CIC significantly decreased phosphorylation levels of p-PI3K and p-AKT (T308) but not PI3K and AKT in MKN-45 cells, which suggested that CIC might also play a role in the inactivation of the PI3K/AKT pathway in GC cells.
Discussion

CIC has been proved to be a tumor suppressor and participate in the progression of many cancers, including leukemia(6) and hepatocellular carcinoma(7). Previous studies have revealed that low expression of CIC is associated with tumor differentiation and lymph node metastasis, and to our knowledge, CIC is reported to be involved in several important processes, including organ growth(27), stem-cell proliferation(28), and cell proliferation (29); however, CIC’s actual biological functions and its underlying mechanisms have not yet been well elucidated. We first addressed this issue using MGC-803 and MKN-45 cells, both of which contained lower levels of CIC compared with that in GES-1 cells.

Our results indicated that cells transfected with CIC overexpressed plasmids led to the efficient increased levels of CIC in both mRNA and protein in vitro. In addition, low levels of CIC were associated with poor differentiation, lymph node metastasis, and advanced clinical stages of GC, but not with age, sex, tumor diameter, or tumor location, which suggested that CIC may be a dependent probable prognostic marker in the disease. Moreover, CIC upregulation significantly reduced the invasive and proliferative capacity of MKN-45 cells, which suggested that CIC may be associated with GC cell metastasis. Because EMT is a critical process during cancer progression, many molecules have been found to be involved in EMT regulation in cancers. In this study, ectopic CIC resulted in elevated levels of E-cadherin and vimentin and reduced levels of N-cadherin in GC cells, which was the first time that CIC was demonstrated to act as an inhibitor of EMT modulation and participate in GC progression.

Previous data have shown that CIC is involved in the activation of several signaling pathways, including MAPK (30), Toll/IL-1 (31), and epidermal growth factor(32); however, its involvement in the activation of the PI3K/AKT signaling pathway in GC has not been reported. In the current study, we observed that CIC overexpression triggered inactivation of AKT and PI3K in GC cells, which suggested that activation of PI3K/AKT is regulated by CIC in these cells. It is well known that the activated PI3K/AKT signaling pathway directly modulates the growth, migration, and invasion of several types of cancer cells, including GC cells(33–35); therefore, it is reasonable to hypothesize that decreased cell proliferation and invasion rates observed in CIC-overexpressed plasmid–transfected GC cells were partly the result of decreased PI3K/AKT activity. While there were some limitations to our study. First, the role of CIC in normal gastric tissues must be further investigated, which would help to better illustrate the expression of CIC in GC. Second, although our findings suggested that CIC inhibits the proliferation and invasion of GC cells in vitro, there is no evidence on the roles of CIC in vivo; therefore, we would conduct in vivo experiments to further explore the functions of CIC in GC cells in future.

Conclusions

In conclusion, our current study implicated that CIC, as a new prospect as a diagnostic marker, remarkably inhibits cell proliferation, invasion, migration, and EMT in vitro. In addition, CIC overexpression suppressed the activation of the PI3K/AKT signaling pathway, which might provide valuable information for developing targeted therapeutic mechanisms against GC.
List Of Abbreviations

CIC, Capicua homolog protein; GC, gastric cancer; HMG, SOX-related high-mobility group; MAPK, mitogen-activated protein kinase; qRT-PCR, quantitative real-time polymerase chain reaction; EMT, epithelial–mesenchymal transition; FBS, fetal bovine serum; CCK-8, cell counting kit-8; PI3K/AKT, phosphoinositide 3-kinase/protein kinase B;

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Affiliated Hospital of Nantong University. And all patients provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/ or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Author's contributions

LG and CL. H performed the experimental study and data collection. ZH. G and CR. W analyzed and interpreted the data. LG and JH. H wrote and reviewed the manuscript. JH. H revised the manuscript and provided material support. JH. H conceived and supervised the whole project. All the authors have read and approved the final version of this manuscript.

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Figures
Expression of CIC is decreased in GC tissues and cell lines. Expression of CIC was significantly decreased in GC tissues (T1-T4) compared with adjacent healthy tissues (N1-N4) both in mRNA and protein levels (A, C, D). β-actin was used as a loading control to normalize the CIC protein levels in each sample (n=20). Also CIC was downregulated in MKN-45 and MGC-803 cells than that in GES-1 cells (B, E, F). **P <0.01, vs adjacent healthy tissues or GES-1 cells.
Kaplan-Meier survival curves. Patients in the low-expression CIC group had significantly shorter overall survival rates (P<0.001).

Figure 2

Kaplan-Meier Survival estimates

Log Rank, Chi-Square=44.174
P<0.001
Figure 3

Ectopic CIC inhibits EMT progression. Representative images of western blotting analysis (A) and qRT-PCR (B) for EMT marker (Vimentin, E-cadherin, N-cadherin) and CIC in MKN-45 cells transfected with indicated molecules. (C) Density assays for EMT marker (Vimentin, E-cadherin, N-cadherin) and CIC, β-actin was used as internal controls. *P <0.05 and **P <0.01, vs Mock.
Figure 4

Ectopic expression of CIC inhibits cell proliferation and invasion in GC cells. (A-B) CCK-8 assays showed ectopic expression of CIC inhibited the proliferation of MKN-45 cells after transfection for 48 h. (C) Representative images of invasive cells transfected with indicated molecules. **P < .01, vs Mock.
**Figure 5**

Ectopic CIC inhibited activation of PI3K/AKT signaling pathway. Representative images of western blotting analysis (A) and density statistic assays (B) of phosphorylated PI3K/AKT and total PI3K/AKT. *P <0.05 and **P <0.01, vs Mock.