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ORIGINAL RESEARCH

Study on Clinical Significance of LncRNA EGOT Expression in Colon Cancer and Its Effect on Autophagy of Colon Cancer Cells

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Background: Colon cancer (CC) is a common digestive tract tumor, and the increase of new and dead patients every year still puzzles clinical workers. LncRNA eosinophil granule ontogeny transcript (*EGOT*), as a newly discovered long-chain noncoding RNA (lncRNA), is differentially expressed in other tumors, but there are fewer studies of it in colon cancer.

Methods: The relative expression and diagnostic value of *EGOT* in CC were detected and analyzed by starBase online website and qRT-PCR. The patients were followed-up for five years, and Cox regression was used to analyze the independent prognostic factors of CC. The effects of *EGOT* overexpression (pcDNA-RGOT) on CC cell function were detected by CCK-8, transwell and flow cytometry. WB was applied to detect autophagy. The influence of knocking out *EGOT* (sh-*EGOT*) on tumor growth was observed by tumor allogeneic inhibition. The microRNA (miR) and mRNA in the downstream of *EGOT* were predicted and the ceRNA network map was drawn.

Results: The online database and qRT-PCR detection showed that *EGOT* was highly expression in patients with CC and had good diagnostic value. The five-year survival rate of patients with high expression of *EGOT* decreased. *EGOT* and TNM staging were independent prognostic factors of patients with CC. Functional analysis revealed that the growth and invasion abilities of cells increased, and the apoptosis rate decreased after overexpression. Upregulation of *EGOT* inhibited autophagy of CC cells and promoted cell growth. However, the tumor in nude mice was significantly lessened after knockout of *EGOT*. Bioinformatic analysis showed that microRNA-33a-5p and microRNA-33b-5p had targeted binding sites with *EGOT*.

Conclusion: *EGOT* is highly expressed in CC and has high diagnostic value. In addition, inhibition of *EGOT* can promote autophagy of CC cells and inhibit cell growth and metastasis, which is expected to be a potential therapeutic index.

Keywords: LncRNA *EGOT*, colon cancer, diagnosis, autophagy

Introduction

Colon cancer (CC) is still one of the general gastrointestinal tumors and one of the primary causes of carcinoma-related deaths.[1] According to the report,[2] it is estimated that more than 10 million new patients will be increased each year, and the number of dead patients will exceed 700 million. With the change of people's eating habits, the sick population tends to be younger in China.[3] The prognosis depends on the stage of the disease at the time of diagnosis, with a five-year survival rate of 90% in early diagnosing and less than 10% in distal metastasis.[4] However, patients with early diagnosed CC are not common in clinic, mainly

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because the patient's condition cannot be accurately detected by routine physical examination.[5] Serum tumor markers *CA199* and CEA have certain value in the diagnosis of CC, but their specificity is low.[6] Therefore, it is urgent to develop rapid and highly sensitive CC screening markers to improve this situation.

In recent years, noncoding RNA (ncRNA) has gradually become a hot topic in various disciplines.[7,8] According to its length, ncRNA can be divided into long-chain noncoding RNA (lncRNA) and microcoding RNA (microRNA).[9] The research of microRNA in tumors has been reported greatly, but the study of lncRNA in tumors is still in the exploratory stage. The lncRNA is an RNA molecule with a length of more than 200 nucleotides. Early studies have shown that lncRNA cannot directly encode proteins, but they play a regulatory role in biological processes.[10] A large number of studies have shown that lncRNA is closely bound up with the development and progression of CC,[11,12] and it also plays a crucial role in the diagnosis and prognosis of CC. For example, studies by Zhou et al[13] have shown that *LncRNA XIRP2-AS1* predicted good prognosis of CC. Another study has shown that lncRNA *CALIC* upregulated *AXL* to promote the metastasis of CC.[14] LncRNA eosinophil granule ontogeny transcript (*EGOT*) is a newly discovered lncRNA, which is located on human 3p26.1 chromosome.[15] Early studies have shown that *EGOT* is highly expressed in hepatocellular carcinoma and breast cancer.[16,17] Some studies have shown that *EGOT* can regulate autophagy of renal tubular cells induced by hypoxia.[18] However, there is little research on whether *EGOT* affects the development and progression of CC.

In this research, we have found that *EGOT* is expressed to a high degree in cases of CC through TCGA database analysis, which suggests that *EGOT* may play a certain role in the progression of CC. Therefore, this research aimed to explore the clinical value and related mechanism of *EGOT* in CC, to provide potential therapeutic targets and diagnostic indicators for clinical practice.

Materials and Methods

GEPIA2 Online Analysis

We logged into starBase[19] (http://starbase.sysu.edu.cn) to choose the pan-Cancer→Gene Differential Expression, analyze the expression of *EGOT* in CC and draw a box diagram.

Clinical Data

From February 2012 to February 2014, 86 patients with CC who were treated in North China University of Science and Technology Affiliated Hospital were selected as the study group (SG). The serum of patients was collected before operation. The cancer tissues and adjacent tissues were collected during the operation. Then they were transported to the laboratory for detection by liquid nitrogen. In addition, the serum of 50 normal physical examinees who received medical examination in our hospital in the meantime were obtained and they were included in the control group (CG). There were no statistical differences in baseline data between the SG and CG. In this research, all patients were diagnosed with CC for the first time, and they had not received antitumour therapy before. All patients and physical examinees provided informed consent. This research was ratified by the Medical Ethics Committee of North China University of Science and Technology Affiliated Hospital, and conformed to the Declaration of Helsinki.[20]

Cell Culture

CC cells SW480, SW620, SW1116, LoVo, CaCo2 and normal colon mucosa cell line NCM460 were from American Type Culture Collection (ATCC; Manassas, VA, USA). The purchased cells were cultivated in DMEM containing (Gibco, CA, USA) 10% FBS (Thermo Fisher Scientific, USA), and the cells were cultured in 5% CO_2 incubator at 37°C.

Cell Transfection

To overexpress *EGOT*, the full-length *EGOT* sequence was introduced into cells by pcDNA3.1 plasmid (GenePharma, Shanghai, China). The constructed vector was named pcDNA-*EGOT*, and the blank pcDNA3.1 plasmid (pcDNA3.1-NC) was used as control. The cell transfection was conducted using lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) according to the makers's protocol. After transfecting for 48 h, the cells were collected for follow-up detection.

In addition, an *EGOT* knockdown (sh-*EGOT*) recombinant lentivirus and a control recombinant lentivirus (sh-NC) (Sangon Biotech Company, China) were constructed. The concentrated virus was used to infect 5×10 5 cells with 4–6 μg/mL in a six-well plate. Then infected cells were cultured with 1 μg/mL puromycin for two weeks.

Stable knockdown cell strains were identified via qRT-PCR.

qRT-PCR

Total RNA was extracted from the collected tumor tissues, serum and cells by Total agent. Then, the total RNA was reverse transcribed by the First Strand cDNA kit to obtain cDNA (Thermo Fisher Scientific, USA). The 7500 Fast Real-time PCR system (ABI, USA) and SYBR® PrimeScript™ RT-PCR kit (Dalian, China) were applied for real-time PCR amplification. The amplification system and reaction conditions were configured according to the kit instructions. 2-ΔΔCT was used to quantitative gene expression.[21] *GAPDH* expression was used for normalization. *EGOT* upstream primer was 5′-GGTAGGTGGACT GCGTTGTT-3′, and downstream primer was 5′-ATG GGGGAGCTGAGCACTTA-3′. GAPDH upstream primer was 5′-AGACTCGCTGATGATCCATGC-3′, and downstream primer was 5′-AGGTGACCACAGTGTTCTG-3′.

Detection of Cell Proliferation

In this study, the cell counting kit-8 (CCK-8, Dojindo Molecular Technologies, Japan) was applied to detect cell growth. The transfected cells were resuspended to 5×10^3 cells/well, and then put in 96-well plate for culture. Then the CCK-8 solution (10 μL) was put in each well at 0, 24, 48, and 72 h respectively, and incubated for one hour. Absorbance analysis (A) was performed at 450 nm with a enzyme-labeling instrument (Bio-Rad, USA). The experiment was repeated three times.

Detection of Cell Invasion

Cells were collected and made into 1×10 5 cells/mL cell suspension. The medium (600 μL) comprising 10% FBS was put in the lower chamber, and the cell suspension (200 μL) was put in the upper chamber (precoated Matrigel) and cultivated for 24 h. Then the fluid from the upper and lower chambers was discarded. The cells in the lower chamber were fastened with 4% paraformaldehyde for 30 min. Thereafter, the paraformaldehyde was eliminated, and cells that did not pass through the film were wiped clean with cotton swabs. The lower chamber was dyed with 0.1% crystal violet for 10 min, while the lower chamber was rinsed with PBS three times. Next, the migrated cells were analyzed and counted under a microscope (Olympus, Japan).

Apoptosis Test

Apoptosis was analyzed by flow cytometry. The cells in logarithmic growth phase were inoculated in a 96-well plate at a density of 1×10 4 cells/well, cultivated for 24 h, rinsed twice with PBS, fastened with 70% ethanol and conserved at 4°C for one night. They were washed once with PBS to regulate the cellular density to 1×10 6 mg/mL. The final concentration was adjusted to 0.05 mg/mL by adding propidium iodinate staining solution, and the cells were dyed at 4°C for 30 min. Then, the apoptosis was analyzed via flow cytometry (Miltenyi Biotec, USA). Each group was provided with three repeating wells.

WB Test

The transfected cells and tumor tissues of nude mice were collected and lysed in RIPA lysis buffer (Beyotime Biotechnology, Shanghai, China) to obtain total proteins. Then, BCA™ protein detection kit (Pierce, Appleton, WI, USA) was used to detect the concentration of protein extract. Then, it was isolated by 10% SDS-PAGE, and then moved to polyvinylidene fluoride (PVDF) film. The membrane was cultivated with 5% nonfat dried milk at ambient temperature for one hour, and Bcl-2, Bax, pro-caspase-3, cleaved-caspase-3, LC3-Ⅰ, LC3-Ⅱ, Beclin-1, p62 and primary antibody were added to incubate overnight at 4°C. Then, the appropriate secondary antibody labeled with HRP (1:500, Abcam) was added at room temperature and incubated for one hour. After rinsing, chemiluminescence testing was performed. Anti-β-actin antibody (1:1000, Affinity, USA) was applied as intraprotein control.

Immunofluorescence Staining

Immunofluorescence staining was used to detect the changes of autophagy-related proteins in cells. After washing with PBS three times (five minutes each time), the cells were fixed for 10 min, and then soaked with Triton X-100 (0.1%) PBS solution for 10 min. Then they were sealed at room temperature for one hour and incubated with anti-LC3A/B antibody at 4°C overnight. The next day, the cells were washed thoroughly with PBS and cultured at room temperature for three hours with Cy3-labeled goat anti-rabbit IgG (Beyotime, China). The chromatin was stained with DAPI for 10 min, and the cells were evaluated under

a fluorescence microscope (Olympus IX71, Olympus, Japan). ImageJ was used to quantify the fluorescence intensity of Alexa Fluor 488.

Bioinformatics Analysis

The potential microRNA of *EGOT* was predicted through online prediction websites of microRNADB, microRNA code, starBase, LncBase. For the predicted common microRNA, TargetScan, starBase, microRNADB and microRNADIP were applied to predict the potential common targeted genes of common microRNA. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were applied to analyze the targeted gene with R language "clusterProfiler" package, and the bubble diagram was drawn.

In vivo Study

The study was approved by the Medical Ethics Committee of North China University of Science and Technology Affiliated Hospital, and the animal experiment was conducted according to the Laboratory animal-guideline for ethical review of animal welfare issued by China in 2018.[22] Six four-week-old male BALB/c athymic nude mice (Viton Lihua, Beijing, China) were used for xenotransplantation experiment. SW620 cells infected with sh-*EGOT* and sh-NC (2×10^7 /mL) were digested by trypsin, then centrifuged, washed with PBS three times, and then resuspended in PBS. The cell suspensions were inoculated to the right side (sh-*EGOT*) of nude mice in sh-*EGOT* group (n=3) and sh-NC group (n=3). The change of tumor size in nude mice was measured every seven days

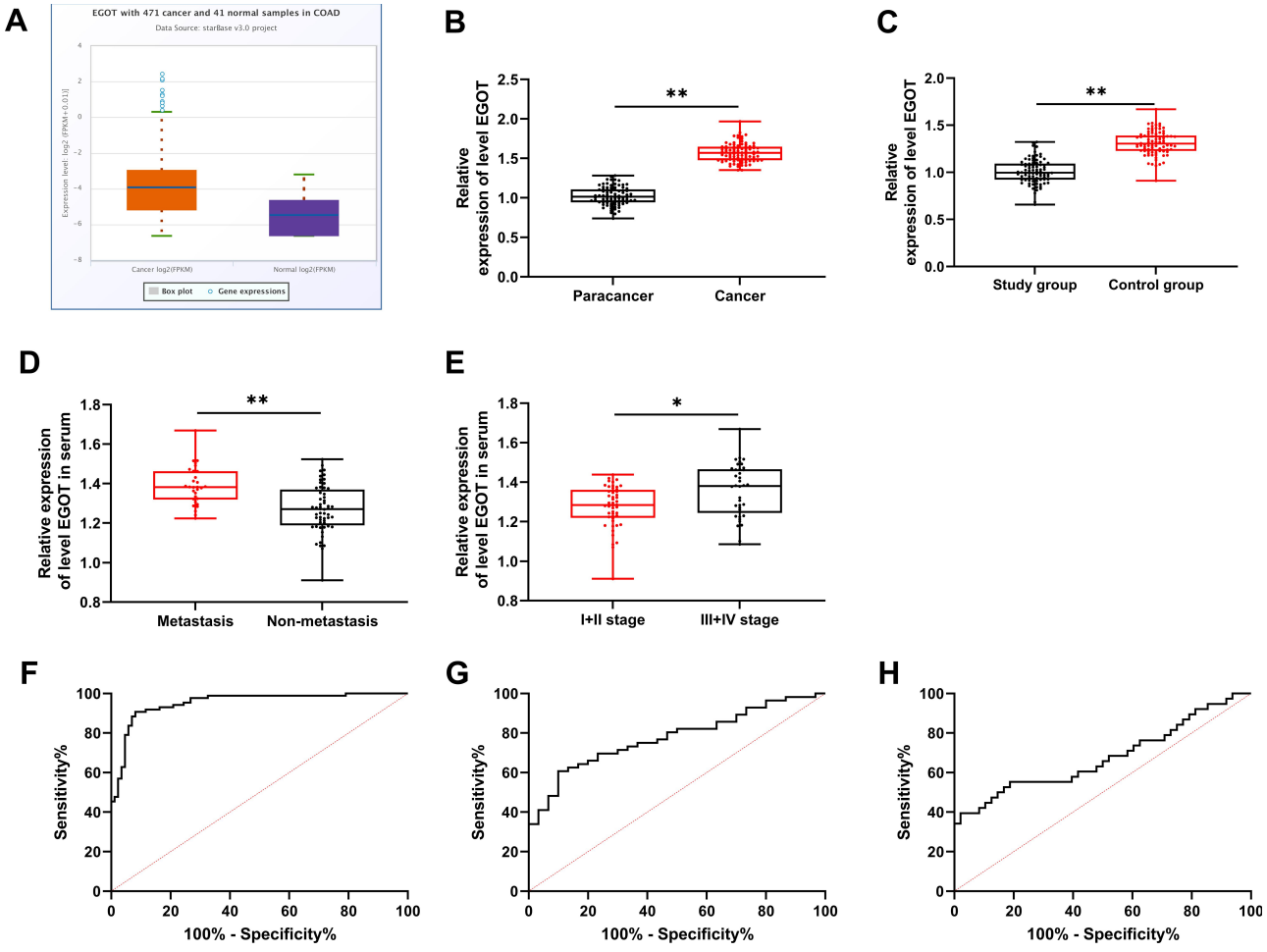

Figure 1 Expression and diagnostic value of *EGOT* in CC patients. (**A**) The starBase online website was used to analyze the *EGOT* relative expression of CC in TCGA database. (**B, C**) qRT-PCR was used to detect the relative expression of *EGOT* in tumor tissue and serum of CC patients. (**D, E**) qRT-PCR was used to detect the relative expression of *EGOT* in serum of CC patients with metastasis and nonmetastasis, low stage and high stage of TNM. (**F**) ROC curve was used to analyze the area under the curve of *EGOT* in diagnosing CC patients and normal population (0.952), and the best specificity and sensitivity were 91.8% and 90.6% when the Youden index was 82.5. (**G**) ROC curve was used to analyze the area under the curve of *EGOT* in diagnosing lymph node metastasis of CC patients (0.777), and the best specificity and sensitivity were 90.0% and 60.7% when the Youden index was 50.7. (**H**) ROC curve was used to analyze the area under the curve of *EGOT* in diagnosing low stage and high stage of TNM of CC patients (0.678), and the best specificity and sensitivity were 97.9% and 37.4% when the Youden index was 37.3. *P<0.01, **P<0.001.

Table 1 Relationship Between *EGOT* and Clinical Data of CC Patients

Factors	*EGOT*		*P*-value
	High Expression (n=43)	Low Expression (n=43)	
Gender			0.822
Male (n=55)	28	27	
Female (n=31)	15	16	
Age			0.388
≥60 years old (n=44)	20	24	
<60 years old (n=42)	23	19	
Tumor size			0.193
≥3cm (n=38)	22	16	
<3cm (n=48)	21	27	
Lymphatic metastasis			0.002
Metastasis (n=30)	22	8	
Nonmetastasis (n=56)	21	35	
TNM staging			0.009
I+II (n=48)	18	30	
III+IV (n=38)	25	13	
KRAS status			0.366
Wild type (n=73)	35	38	
Mutant (n=13)	8	5	
Microsatellite status			0.268
MSS (n=70)	33	37	
MSI (n=16)	10	6	
Cancer location			0.664
Right (n=38)	18	20	
Left (n=48)	25	23	

Abbreviation: CC, colon cancer.

by caliper (volume calculation formula: volume =(0.5× length× width 2)). After 28 days, the mice were killed and the tumor tissues of nude mice were collected.

Statistical Analysis

GraphPad prism 8 (GraphPad Software Inc., San Diego, California, USA) and SPSS 20.0 (IBM Corporation, Armonk, NY, USA) were used for statistic analysis of all data. Independent-samples *t*-test was applied for comparison between groups. The counting data were represented by percentage (%) and compared by chi-squared test. One-way ANOVA was used for comparison among groups (represented by F), and LSD *t*-test was applied for posterior comparison. The expression profiles at different time points were verified by repeated measurement analysis of variance (F), and the post-test was completed by Bonferroni. Pearson's test was applied to analyze the connection between genes. The relationship between *EGOT* and five-year survival of patients with CC was analyzed by Kaplan–Meier test. The independent prognostic factors of patients with CC were analyzed by Cox regression. The diagnostic value *EGOT* in CC was analyzed by receiver operating curve (ROC). $P<0.05$ was considered significant.

Results

EGOT Was Highly Expressed in CC and Had Diagnostic Value

We first analyzed the relative expression of *EGOT* in CC in TCGA database according to starBase online. The results revealed that the *EGOT* of CC patients' tumor tissues in the database was significantly increased. Then, in order to verify the expression of *EGOT* in patients with CC, we detected the expression of *EGOT* in tumor tissues and serum of patients with CC by qRT-PCR (Figure 1A). The findings revealed that the *EGOT* in tumor tissues and serum of patients with CC was significantly increased (Figure 1B and C), which was consistent with the database results. This showed that *EGOT* might be involved in the development of CC. In order to further determine the relationship between *EGOT* and patients with CC, the patients were separated into high expression group (n=43) and low expression group (n=43) in accordance with the median value of *EGOT*. By observing the clinical baseline data of patients in both groups (Table 1), it was concluded that the patients in *EGOT* high expression group had high TNM stage (III+IV), and the probability of lymphatic metastasis increased obviously. Furthermore, we found that *EGOT* had better diagnostic value in diagnosing patients with CC (Figure 1D and E), and distinguishing CC low stage (I+IV) and patients with lymph node metastasis (Figure 1F–H). Therefore, *EGOT* was expected to be a potential biological index for diagnosis of CC.

The Five-year Survival Rate of Patients with High EGOT Expression Decreased

In the above research, we revealed the diagnosis value of *EGOT* in CC, and we also analyzed the five-year survival of *EGOT* in patients with CC. Through analysis, we concluded that the five-year survival rate of patients decreased obviously in *EGOT* high expression group (Figure 2A). In

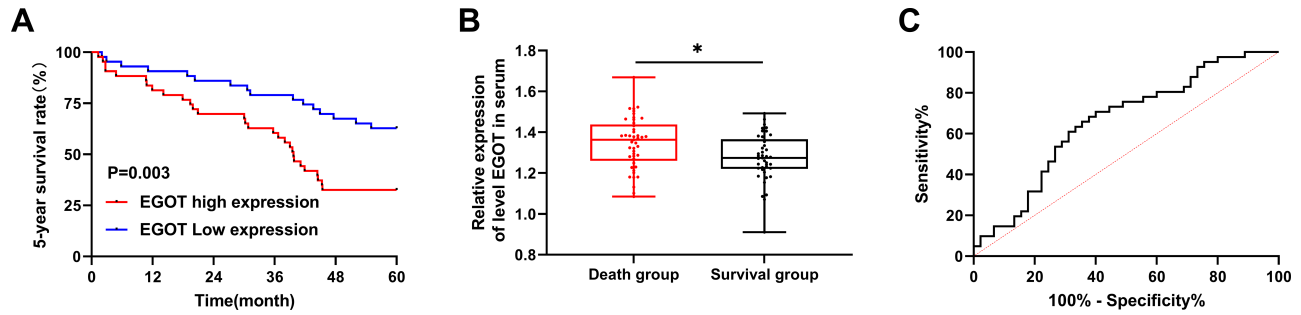

Figure 2 Survival analysis of *EGOT* and CC patients. (**A**) K-M survival analysis was used to analyze the five-year survival of patients in the *EGOT* high and low expression groups. (**B**) qRT-PCR was used to detect the relative expression of *EGOT* in serum of dead patients and surviving patients. (**C**) ROC curve was used to analyze the area under the curve of *EGOT* in predicting survival and death of CC patients (0.659), and the best specificity and sensitivity were 60.0% and 70.7% when the Youden index was 30.7. **P*<0.01.

addition, Cox regression analysis showed that *EGOT* and TNM stages were independent prognostic factors of patients with CC (Table 2). Finally, we divided the patients into survival group and death group according to their survival situation. Further comparison showed that the expression of *EGOT* in the death group was obviously higher than that in survival group, and ROC curve analysis showed that *EGOT* had a certain value in predicting death of patients with CC (Figure 2B and C).

Upregulation of EGOT Inhibited Autophagy of CC Cells and Promoted Cell Growth

In the above research, we have revealed the clinical significance of *EGOT* in CC, but it is not clear whether *EGOT* is bound up with the development and progression of CC. Therefore, we first detected the *EGOT* relative expression in CC cells. qRT-PCR detection showed that the *EGOT* in CC cell strains was significantly increased. Then, we established pcDNA-*EGOT*, which was transfected into CC cells. The findings revealed that the relative expression level of *EGOT* in CC cells was significantly increased (Figure 3A and B). The biological function of the cell was further analyzed. CCK-8 experiment showed that the proliferation activity of CC cells transfected with pcDNA-*EGOT* was significantly enhanced compared with that of pcDNA-NC (Figure 3C). Transwell experiment showed that after pcDNA-*EGOT* transfection, the number of cells passing through the membrane was significantly higher than that of CC cells transfected with pcDNA-NC (Figure 3D). In addition, flow cytometry experiment showed that the apoptosis rate of CC cells transfected with pcDNA-*EGOT* was lower than that of cells transfected with pcDNA-NC (Figure 3E). Moreover, WB detection showed that the expression of cle-Caspase-3 and Bax protein decreased significantly and the expression of Bcl-2 protein increased (Figure 3F). This indicated that *EGOT* might be involved in the development of CC. Early studies have shown that *EGOT* can protect cell growth and development by regulating autophagy. However, it is not clear whether *EGOT* plays the same role in CC. Therefore, we detected autophagy-related proteins in CC cells transfected with pcDNA-*EGOT* by WB experiment. The result showed that the relative expressions of LC3II/I and Beclin1

Table 2 Cox Regression Analysis

Factors	Univariate Cox			Multivariate Cox		
	Sig.	Exp (B)	95%CI	Sig.	Exp (B)	95%CI
Gender	0.849	1.061	0.576–1.954			
Age	0.430	0.789	0.439–1.419			
Tumor size	0.781	1.087	0.604–1.958			
Lymphatic metastasis	0.539	0.821	0.436–1.543			
TNM staging	0.002	0.391	0.215–0.708	0.008	0.443	0.242–0.811
EGOT	0.004	2.455	1.328–4.537	0.016	2.146	1.150–4.005
KRAS status	0.321	0.691	0.332–1.435			
Microsatellite status	0.219	0.643	0.318–1.3			
Cancer location	0.484	1.232	0.687–2.212			

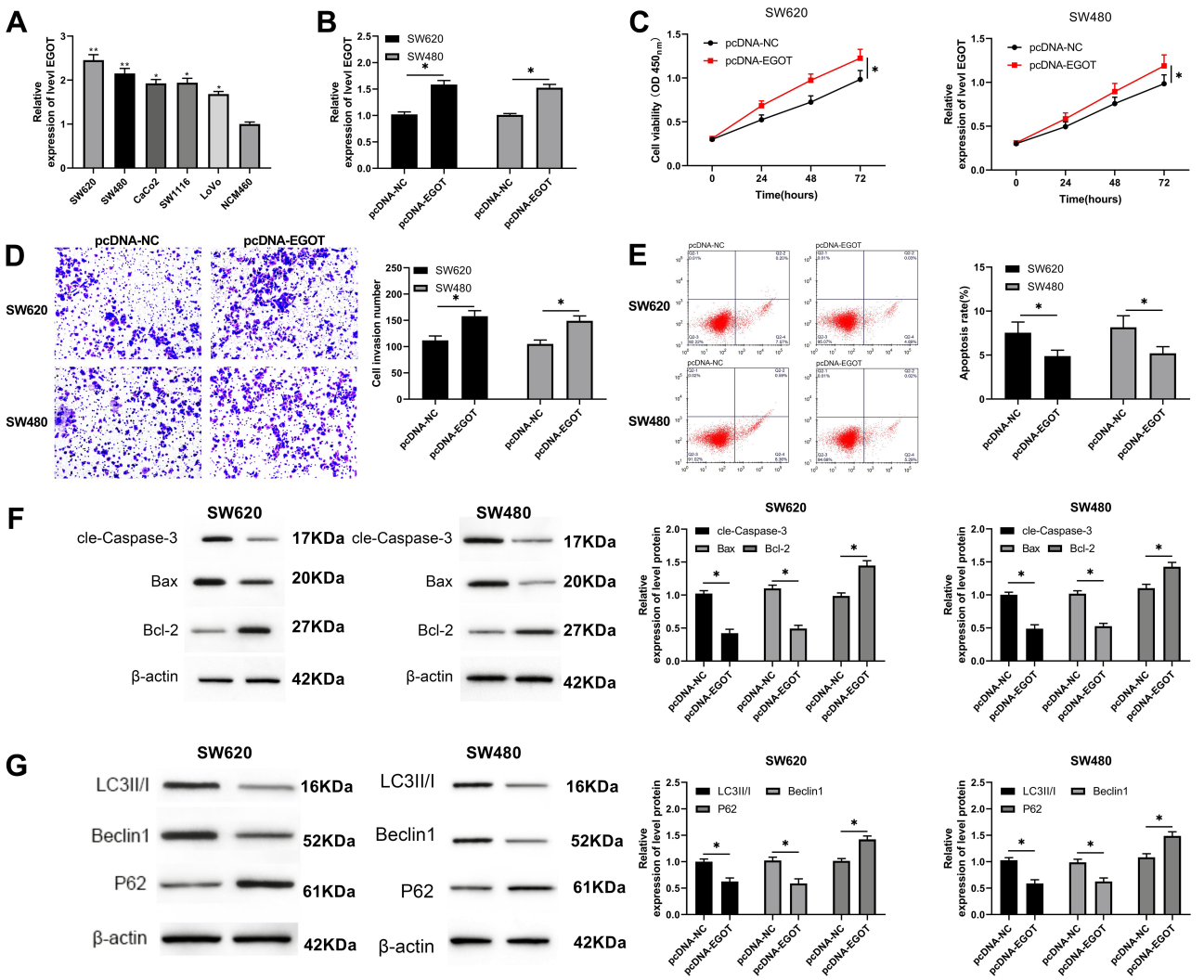

Figure 3 *EGOT* inhibited autophagy and promoted proliferation and invasion of CC cells. (**A**) qRT-PCR was used to detect the relative expression of *EGOT* in CC cells. (**B**) qRT-PCR was used to detect the relative expression of *EGOT* in CC cells transfected with pcDNA-*EGOT*. (**C**) CCK-8 test was used to detect the change of proliferation ability in CC cells transfected with pcDNA-*EGOT*. (**D**) Transwell test was used to detect the change of invasion number in CC cells transfected with pcDNA-*EGOT*. (**E**) Flow cytometry was used to detect the apoptosis of CC cells transfected with pcDNA-*EGOT*. (**F, G**) WB test was used to detect the changes of relative expression of apoptosis-related proteins and autophagy-related proteins in CC cells transfected with pcDNA-*EGOT*. *$P<0.05$.

protein were obviously decreased, while the expression of P62 protein was obviously increased (Figure 3G), In addition, the immunofluorescence staining also showed that the ratio of LC3II/LC3I increased significantly after transfection of pcDNA-*EGOT* (Figure 4), suggesting that *EGOT* might facilitate the growth and metastasis of CC cells by adjusting autophagy.

Knocking Down EGOT Could Suppress the Growth of Tumor Volume in Nude Mice

In the above study, we revealed the biological function of *EGOT* in vitro of CC. To further ascertain the value of *EGOT* in CC, we established a nude mouse model of allogeneic tumor transplantation. Then the sh-*EGOT*, which was stably expressed after knocking down, was injected into nude mice for observation. Compared with injection of sh-NC, the tumor volume of nude mice was significantly reduced after injecting CC cells stably transfected with sh-*EGOT* (Figure 5A and B). By detecting the tumor quality of nude mice, it was also found that the sh-*EGOT* group was lower than the sh-NC group, suggesting that *EGOT* could suppress tumor growth in nude mice in vivo. In addition, the WB test showed that the expression of cle-Caspase-3, Bax, LC3II/I and Beclin1 in the tumor tissues of nude mice in the sh-*EGOT* group was

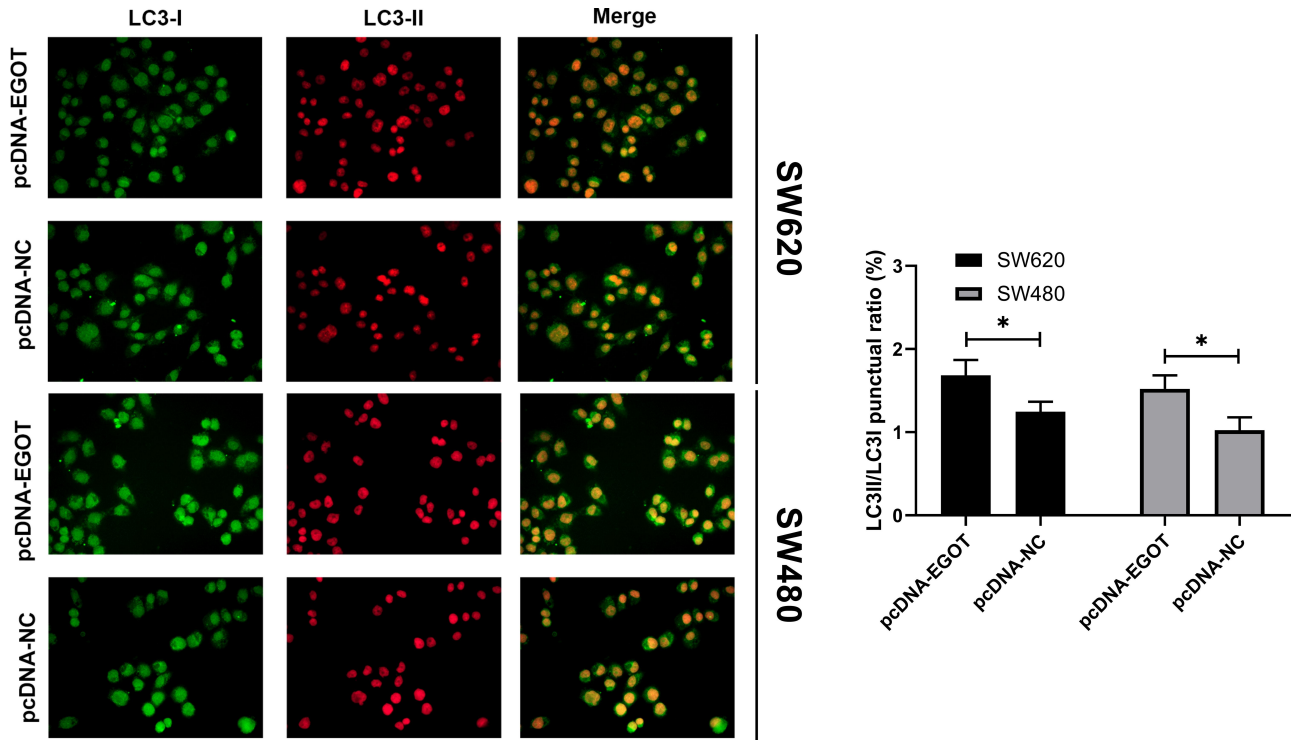

Figure 4 Cell immunofluorescence staining to detect changes in the ratio of LC3II/LC3I in cells after transfection *P<0.05. Magnification x200.

significantly increased, while the expressions of Bcl-2 and P62 protein were declined (Figure 5C). This indicated that *EGOT* could inhibit the growth and metastasis of CC cells by promoting autophagy. It was expected to become a potential therapeutic target of CC.

Bioinformatics Analysis of EGOT

Early studies have shown that lncRNA can act as a sponge of microRNA to regulate its downstream target genes and participate in the development and progression of tumors. In this research, in order to ascertain the potential function of *EGOT*, we predicted the potential microRNA of *EGOT*, and found that microRNA-33a-5p and microRNA-33b-5p had targeted binding sites with *EGOT* (Figure 6A). Then we predicted the target genes downstream of microRNA-33a-5p and microRNA-33b-5p and drew the ceRNA network map (Figure 6B). In addition, nine potential GO functions and 10 potential signal pathways were found by GO enrichment and KEGG analysis (Figure 6C and D), which is the potential direction for us to explore *EGOT* in the future.

Discussion

Although some progress has been made in the treatment of CC, clinicians are still faced with the challenge of how to improve the early diagnosis of diseases by developing methods that can detect malignant tumors.[23] In this research, we found that *EGOT* was expressed to a high degree in cases of CC, and it had high clinical value in diagnosing patients with CC, lymphatic metastasis and TNM staging. In addition, we also found that *EGOT* could inhibit the growth and metastasis of CC cells by regulating autophagy, which was expected to be a potential target and diagnostic index for treatment of CC.

LncRNA is a long-chain noncoding RNA. At present, many studies have shown that lncRNA has high clinical value in the diagnosis of tumor.[24–26] For example, the research of Liu et al[27] have shown that LncRNA H19 can be used as a cancer biomarker with high value in the detection and diagnosis of human cancer. *EGOT*, as a newly discovered lncRNA, is found to be highly expressed in glioma and other diseases in early studies.[28] Furthermore, it is found that *EGOT* can promote the apoptosis of CC through microRNA-33b-5p/CROT axis.[29] However, the predictive value of *EGOT* in clinical diagnosis of CC is still unclear. In this research, we first analyzed the expression of *EGOT* in CC. qRT-PCR analysis revealed that the relative expression of *EGOT* was high in serum, tumor tissues and CC cells of patients with CC, which was consistent with the expression in TCGA database. It was also concluded that *EGOT* was bound up with TNM staging and lymphatic metastasis.

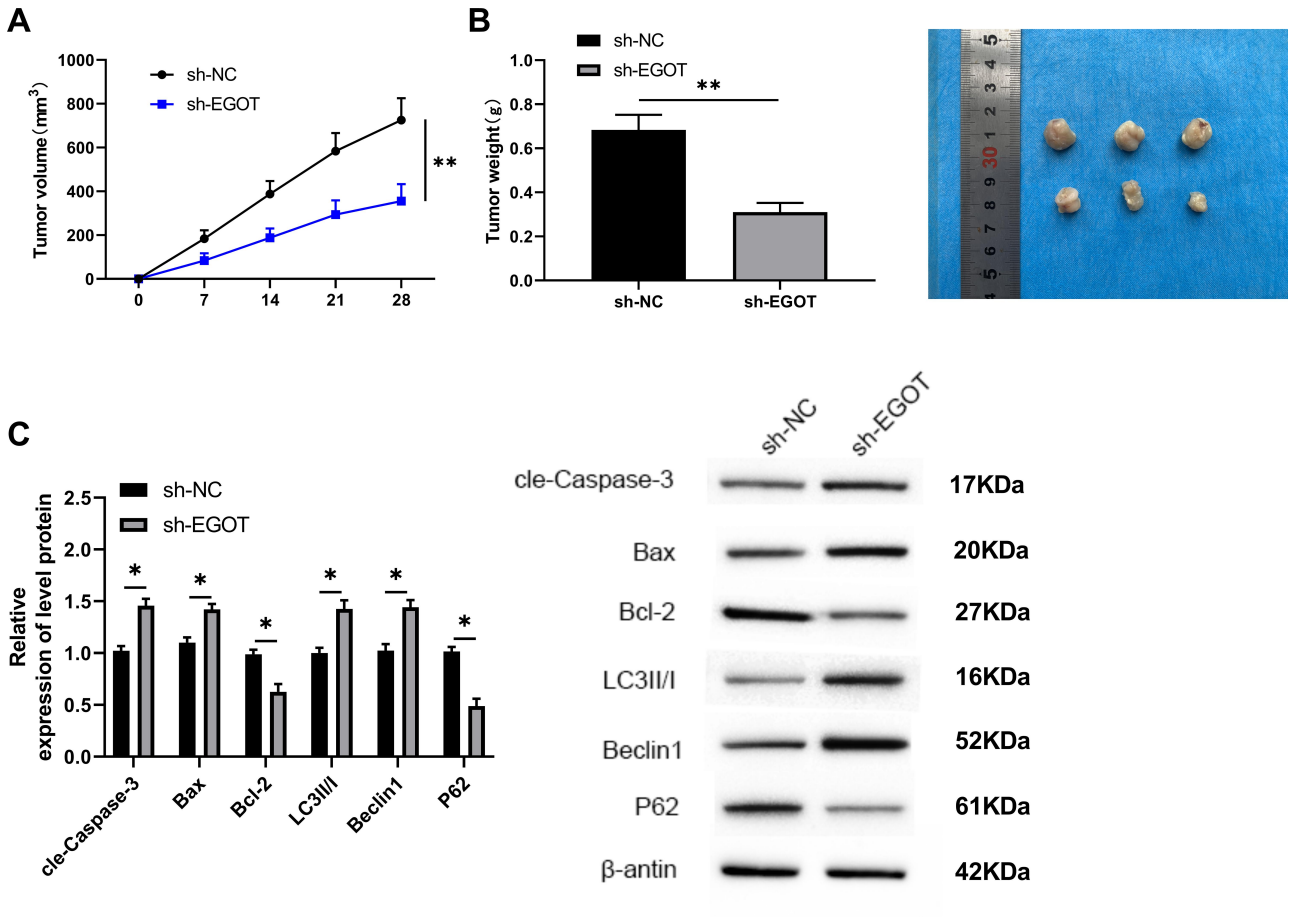

Figure 5 *EGOT* could inhibit growth of tumor volume in nude mice. (**A**) Changes of tumor volume in nude mice within 28 days. (**B**) Changes of tumor quality in nude mice after death for 28 days. (**C**) WB was used to detect the changes of apoptosis-related proteins and autophagy-related proteins in tumor tissues of nude mice. **P*<0.05, ***P*<0.01.

Serum detection is a widely used diagnostic standard in diagnosing tumors at present.[30] Early research has also revealed that detecting the lncRNA in serum can be used as a potential index for diagnosis of CC.[31] To determine the diagnostic value of *EGOT* in CC, we drew a ROC curve. The results showed that *EGOT* had certain diagnostic value in diagnosing patients with CC, lymphatic metastasis and TNM staging, especially in diagnosing tumor and lung tumor patients, and the area under the curve was greater than 0.9, which was a better clinical potential diagnostic index. In addition, we also analyzed the relationship between *EGOT* and five-year survival of patients with CC. Through the analysis, we found that *EGOT* and TNM staging were independent factors for the prognosis of patients with CC. We also found that *EGOT* had a certain value in predicting five-year survival of CC patients with an area under the ROC curve greater than 0.6, but it was still not ideal.

Studies have shown that *EGOT* has the ability to regulate autophagy, and facilitates the apoptosis of tumor cells by adjusting autophagy.[32] However, whether *EGOT* has the same effect in CC has not been confirmed by relevant studies. Therefore, we established the overexpression vector of pcDNA-*EGOT* to explore the value of *EGOT* in CC. Through experiments, we concluded that transfection of pcDNA-*EGOT* promoted the growth and invasiveness of CC cells and inhibited apoptosis. This showed that *EGOT* participated in the development of CC. The ratio of LC3-II/I is an important marker of autophagy. P62 is also considered as a selective autophagy substrate. More and more evidences show that p62 can act as a receptor for selective autophagy products of various ubiquitinated substrates.[33] In WB experiment, we found that the expressions of cle-Caspase-3, Bax, LC3II/I and Beclin1 were obviously decreased, and the expressions of p62 and Bcl-2 were increased in cells transfected with pcDNA-*EGOT*, which indicated that *EGOT* promoted the

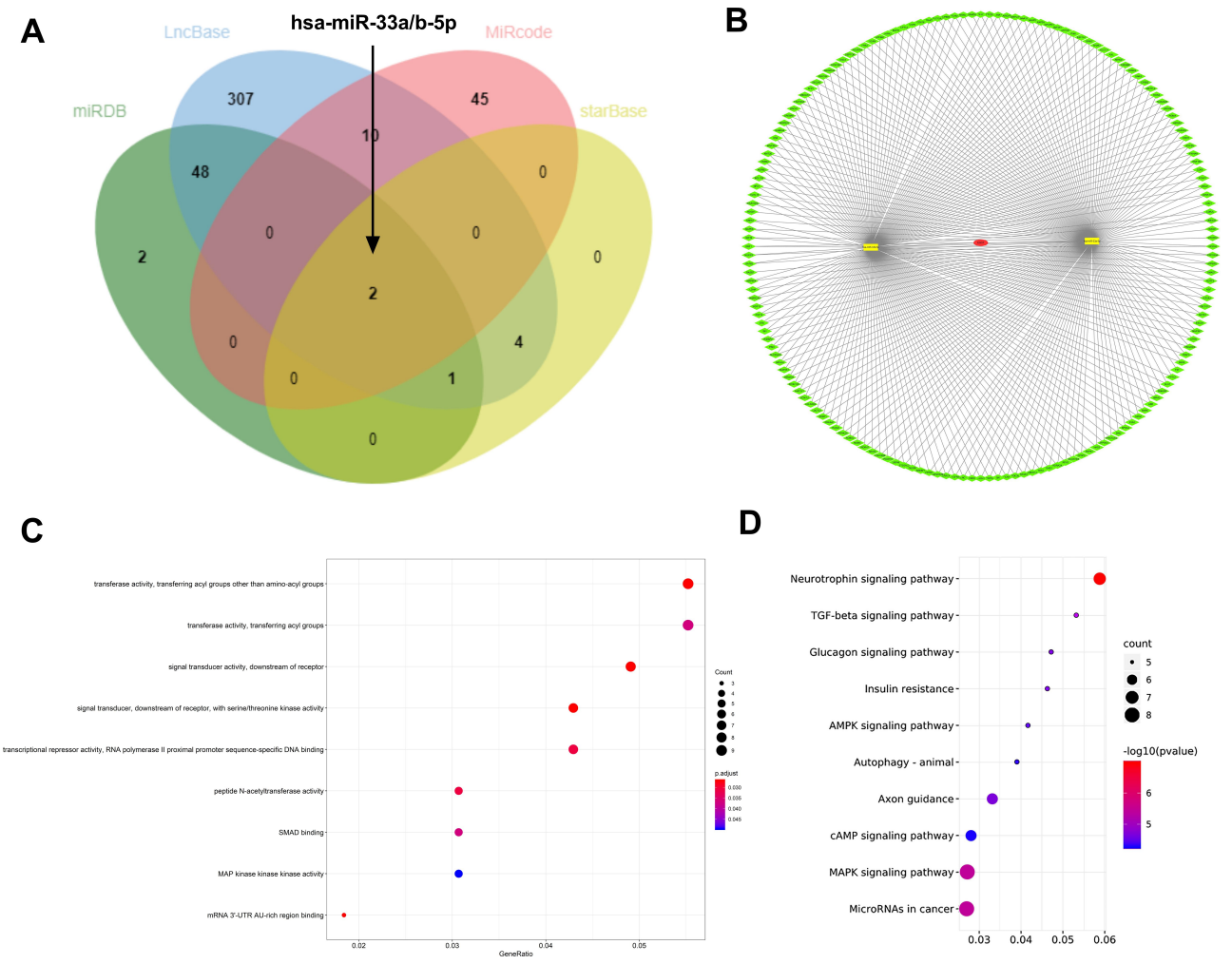

Figure 6 *EGOT* function analysis. (**A**) Common potential microRNA of *EGOT* predicted by several online sites. (**B**), *EGOT*-microRNA-mRNA network map drawn by Cytoscape (red was lncRNA, yellow was microRNA, and green was mRNA). (**C**) Analysis of potential GO function of target gene. (**D**) Analysis of potential KEGG signaling pathway of target gene.

development of CC by inhibiting autophagy. In order to further verify our conclusion, we also carried out in vivo experiments. The findings showed that the tumor size and mass of nude mice were significantly decreased after knocking down *EGOT*, and the relative expression of autophagy protein and apoptosis protein in tumor tissue was contrary to that in vitro, which verified that *EGOT* could regulate CC.

At the end of the study, we predicted the potential microRNA-mRNA of *EGOT* to further seek the potential mechanisms of lncRNA. Early studies have shown that lncRNA can be used as a microRNA sponge to regulate downstream target genes and participate in tumorigenesis. Through prediction, we found that microRNA-33a-5p and microRNA-33b-5p had targeted binding sites with *EGOT*, Then we predicted microRNA-33a-5p and microRNA-33b-5p downstream target genes and conducted enrichment analysis, and found that there was a connection between *EGOT* and autophagy signaling pathway. However, the specific relationship has not been proved, which is also our main research direction in the future.

Compared with the study of Ni et al,[29] this study was designed to analyze the diagnostic value of LncRNA *EGOT* in CC, and the ceRNA map was mapped by miR and its downstream mRNA which could bind to LncRNA *EGOT*. In addition, we also carried out a nude mouse experiment to verify the inhibitory effect of LncRNA *EGOT* on tumor growth in vivo. However, there are some deficiencies in this research. First, it is not clear whether *EGOT* can mediate microRNA to regulate autophagy of CC. Second, autophagy inhibitors are not used to

intervene in cells or nude mice in this study. Therefore, we hope to carry out more basic research in the future to improve our research conclusions.

Conclusion

To sum up, *EGOT* is highly expressed in CC and has high diagnostic value. In addition, inhibition of *EGOT* can promote autophagy of CC cells and inhibit cell growth and metastasis, which is expected to become a potential therapeutic index.

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Disclosure

The authors report no conflicts of interest in this work.

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