Cancer Management and Research

Dovepress

open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

ENO1 Acts as a Prognostic Biomarker Candidate and Promotes Tumor Growth and Migration Ability Through the Regulation of Rab1A in Colorectal Cancer

This article was published in the following Dove Press journal:
Cancer Management and Research

Zhengwu Cheng[1,*]
Xinyu Shao[2,*]
Menglin Xu[3,*]
Chunli Zhou[2]
Junfeng Wang[1]

[1]Department of Gastrointestinal Surgery, The First Affiliated Hospital of Wannan Medical College, Wuhu 241000, People's Republic of China; [2]Department of Gastroenterology, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou 215006, People's Republic of China; [3]Department of Oncology, The First Affiliated Hospital of Wannan Medical College, Wuhu 241000, People's Republic of China

*These authors contributed equally to this work

Background: Colorectal carcinoma (CRC) is one of the most common malignancies with a dismal 5-year survival rate. The glycolytic enzyme α-enolase (*ENO1*) is overexpressed in multiple cancers and is involved in tumor cell proliferation and metastasis. However, its clinical significance, biological role, and underlying molecular mechanisms in CRC are still unclear. The aim of the present study was to investigate the potential role of *ENO1* in the initiation and development of CRC.

Patients and methods: The in situ expression of ENO1 in CRC and adjacent normal tissues was examined by immunohistochemistry. The effects of *ENO1* on the in vitro proliferation and migration of CRC cell lines were investigated by MTT, colony formation, and Transwell assays. Finally, the in vivo tumorigenic capacity of ENO1 was assessed in a mouse model.

Results: ENO1 was overexpressed in CRC tissues and significantly correlated with the clinicopathological parameters. Furthermore, Rab1A was also overexpressed in CRC tissues and was positively correlated to that of ENO1. The high expression levels of both ENO1 and Rab1A led to significantly worse prognosis of CRC patients compared to either alone. Furthermore, knockdown of ENO1 significantly inhibited CRC cells proliferation and migration in vitro and reduced xenograft growth in vivo via the concomitant downregulation of Rab1A.

Conclusion: The ENO1/Rab1A signaling axis is involved in CRC progression and is a potential biomarker for the treatment of CRC.

Keywords: colorectal cancer, *ENO1*, *Rab1A*, prognosis, tumor growth

Correspondence: Junfeng Wang
Department of Gastrointestinal Surgery, The First Affiliated Hospital of Wannan Medical College, No. 2 Zheshan West Road, Jinghu District, Wuhu, Anhui Province 241000, People's Republic of China
Email wujianyjs02@163.com

Chunli Zhou
Department of Gastroenterology, The Affiliated Suzhou Hospital of Nanjing Medical University, No. 242 Guangji Road, Suzhou, Jiangsu Province 215006, People's Republic of China
Email zhouchunli079@163.com

Introduction

Colorectal cancer (CRC) is a common malignant tumor of the digestive system and one of the leading causes of cancer-related deaths worldwide.[1,2] Despite significant progress in the systematic treatment of CRC, the long-term survival of patients with advanced metastatic disease remains poor due to distant metastasis and resistance to chemotherapy.[3,4] Therefore, novel therapeutic targets are urgently required to improve the survival and prognosis of CRC patients.

Cancer cells undergo characteristic changes in glucose metabolism in order to support their unrestricted proliferation and metastasis.[5,6] Enolase 1 (*ENO1*) is a conserved glycolytic enzyme that catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate, an ATP-generating step that is pivotal in cancer cell

proliferation and metastasis.[7,8] In addition, *ENO1* is also involved in several physiological processes, such as cell growth, hypoxia tolerance, and autoimmunity.[9,10] Previous studies have reported ENO1 overexpression in several cancers, such as breast,[11] neck,[12] lung,[13] prostate,[14] and gastric cancer,[15] which is closely linked with cancer progression and poor patient prognosis.

One study showed that *ENO1* acts as an oncogene in CRC by regulating the mTOR pathway,[16] which is also targeted by *Rab1A* to promote CRC genesis and metastasis.[17] However, it is unclear whether *ENO1* and *Rab1A* interact during colon cancer initiation and progression. Herein, we analyzed the relationship between ENO1 and Rab1A expression in CRC patients, and the effects of *ENO1* knockdown on colon cancer cell proliferation and metastasis in vitro and in vivo. Our findings indicate that *ENO1* is essential for CRC progression, and its effects are likely mediated by the upregulation of *Rab1A*. This study provides a solid theoretical and experimental basis for the therapeutic targeting of *ENO1* in CRC.

Patients and Methods

Human CRC Tissues and Cell Lines

The tumor and adjacent normal tissues were resected from CRC patients between 2011 and 2013 at the Department of General Surgery, the Affiliated Suzhou Hospital of Nanjing Medical University. The study was approved by the Affiliated Suzhou Hospital of Nanjing Medical University Ethics Committee and the patient consent was written informed consent.

The human CRC cell lines SW620, LOVO, and RKO were purchased from the Chinese Academy of Sciences (Shanghai, China), and HCT116 was obtained from the College of Life Sciences, Soochow University, which was approved by the Affiliated Suzhou Hospital of Nanjing Medical University Ethics Committee. All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS; Gibco, USA) at 37°C in a humidified atmosphere containing 5% CO_2.

Immunohistochemistry (IHC)

The paraffin-embedded tissues were immersed in boiling citrate buffer (Gene Tech, Shanghai, China, GT100202) for antigen retrieval, followed by a 15-mins incubation with 3% hydrogen peroxide (Yonghua Chemical Technology Co. LTD, Changshu China), and blocking with 5% FBS (Beyotime Inc, NanTong, China) for another 15 mins. The suitably treated sections were then incubated with primary antibodies against ENO1(A1033; 1:100 dilution; ABclonal; Wuhan, China) or Rab1A (Ab97956; 1:75 dilution; Abcam, Cambridge, MA, USA) at room temperature for 2–3 hrs and stained using a tissue staining kit (Zhongshan Biotechnology, Beijing, China) according to the manufacturer's protocol. Five random high-power fields were observed per section, and the staining intensity was scored as 0 (no staining), 1 (weak), 2 (moderate), and 3 (strong), and the percentage of positively stained cells as 1 (<25%), 2 (25–50%), 3 (51–75%), and 4 (>75%). The total score was calculated by multiplying the staining intensity score with the staining percentage score, and the samples were accordingly stratified into the low expression (− or +) and high expression (++ or +++) groups (0 = −; 1–4 = +; 5–8 =++; 9–12 = +++), as described in our previous study.[18]

Short Hairpin RNA Transfection

SW620/HCT116 cell lines stably expressing ENO1-specific shRNA or scrambled control shRNA were generated by lentiviral shRNA transduction. The shRNA sequences specific for human ENO1 were synthesized by Gene Pharma (Shanghai GenePharma Co., Ltd., Shanghai, China). The SW620/HCT116 cells were also transfected with shRab1A or control shRNA using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, as described in our previous study.[19]

Protein Extraction and Western Blotting

SW620/HCT116 were lysed for 30 mins in ice-cold RIPA lysis buffer for protein extraction according to the manufacturer's protocol. Equal amounts of protein per sample (10 μg) were separated by SDS-PAGE and then transferred to nitrocellulose membranes. After blocking with 5% non-fat milk for 1 hr at room temperature, the membranes were incubated overnight with polyclonal rabbit anti-human ENO1(A1033; 1:1000 dilution; ABclonal; Wuhan, China) or Rab1A (1:2000, Abcam, USA, Ab97956) and mouse anti-human GAPDH antibodies, followed by HRP-conjugated secondary antibodies for 1 hr at room temperature. The immunoreactive bands were visualized by chemiluminescence and quantified using ImageJ software.

MTT Assay

Cell viability was assessed using an MTT assay kit (Amresco, USA) according to the manufacturer's protocol. The cells were harvested, resuspended in complete medium, and seeded into 96-well culture plates. After culturing

for 24, 48, 72, 96, and 120 hr, the MTT solution was added and the cells were incubated at 37°C for another 4 hrs. The supernatant was aspirated, and the formazan crystals were dissolved in 150 μL DMSO. After a 10-mins incubation at 37°C, the absorbance at 490 nm was measured. Each sample was tested in five replicate wells, and the experiment was repeated thrice.

Cell Migration Assay

The in vitro migration of the ENO1 or control shRNA-transfected SW620/HCT116 cells was evaluated in 24-well Transwell plates (Corning Incorporated, USA) according to the manufacturer's protocol. The cells were re-suspended in serum-free RPMI 1640 and seeded in the upper chamber of each well, while the lower chamber was filled with 500 μL complete medium. After a 24-hr incubation at 37°C, the cells remaining on the upper surface of the membrane inserts were removed with a cotton swab, and the migrated cells on the lower surface were fixed with methanol, stained with 0.5% crystal violet, and counted in five randomly selected fields under an inverted microscope (scale bar = 200μm). Each experiment was repeated thrice.

Colony Formation Assay

The cells were seeded in six-well plates at the density of 800 cells per well in complete medium. After 3 weeks of culture, the resulting colonies were fixed and stained with crystal violet (0.1%, w/v in 20 nM 4-morpholinepropanesulfonicacid), and the macroscopic colonies were counted. The experiments were repeated thrice, and each sample was tested in triplicates.

Xenografts

Nude mice (BALB/c, SPF grade, 16–18 g, 3–5 weeks old, male) were purchased from Shanghai SLRC laboratory Animal Co (Shanghai, China) and housed in a pathogen-free environment under a 12-hrs light/dark cycle. The mice were subcutaneously injected with 5×10^6 GC cells that were, respectively, transfected with control-shRNA or ENO1-shRNA. The two groups of nude mice were individually marked (n = 3 mice per group) after 3 days of feeding according to the weight and the day was recorded as 0. Body weight and tumor size were measured twice a week. The mice were sacrificed on day 35, and the tumors were dissected. All animal experimental procedures were approved by the Animal Ethics Committee of the Suzhou Municipal Hospital (Suzhou, China), and performed in accordance with the guidelines of the Care and Use of Laboratory Animals in Suzhou Municipal Hospital, which was described in our previous study.[20]

Statistical Analysis

Data were presented as the mean ± S.E.M. of three independent experiments. The groups were compared using Student's *t*-test and Chi-square test as appropriate. Survival analysis was performed by Kaplan–Meier method and compared by the log-rank test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with the SPSS17.0 software (SPSS Inc, Chicago, IL, USA).

Results

ENO1 Expression Levels are Higher in CRC Tissues Compared to Adjacent Tissues

The in situ ENO1 expression in paired CRC and adjacent normal tissues was evaluated by IHC, and the percentage staining and intensity scores were multiplied to obtain the total staining score (Figure 1A). ENO1 levels were significantly higher in CRC tissues compared to that in paired adjacent tissues ($P < 0.001$, Figure 1B). Furthermore, ENO1 expression was slightly higher in patients with lymph node invasion compared with those without ($P = 0.09$, Figure 1C). Similar trends were seen in ENO1 expression levels between the TNM I-II and TNM III-IV stage tissues ($P = 0.07$, Figure 1D).

ENO1 Expression Is Associated with the Clinicopathological Status of CRC Patients

The relationship between ENO1 expression levels and the clinicopathological parameters of 135 CRC patients is summarized in Table 1. Overexpression of ENO1 was significantly associated with the depth of tumor invasion (P =0.014), lymph node invasion ($P < 0.001$), neural invasion ($P = 0.016$), and TNM staging ($P < 0.001$). However, no significant differences were observed between the ENO1-positive and ENO1-negative groups in terms of other clinicopathological factors, such as age, gender, tumor size, degree of differentiation, venous invasion, and tumor location ($P > 0.05$).

ENO1 Expression Is Significantly Correlated to Rab1A Expression Levels in CRC

Previous studies have reported that *ENO1* promotes CRC cell proliferation and metastasis through the mTOR pathway, and that *Rab1A* is an mTORC1 activator in CRC. However, the association between *ENO1* and *Rab1A* has not been elucidated so far. To this end, we analyzed the

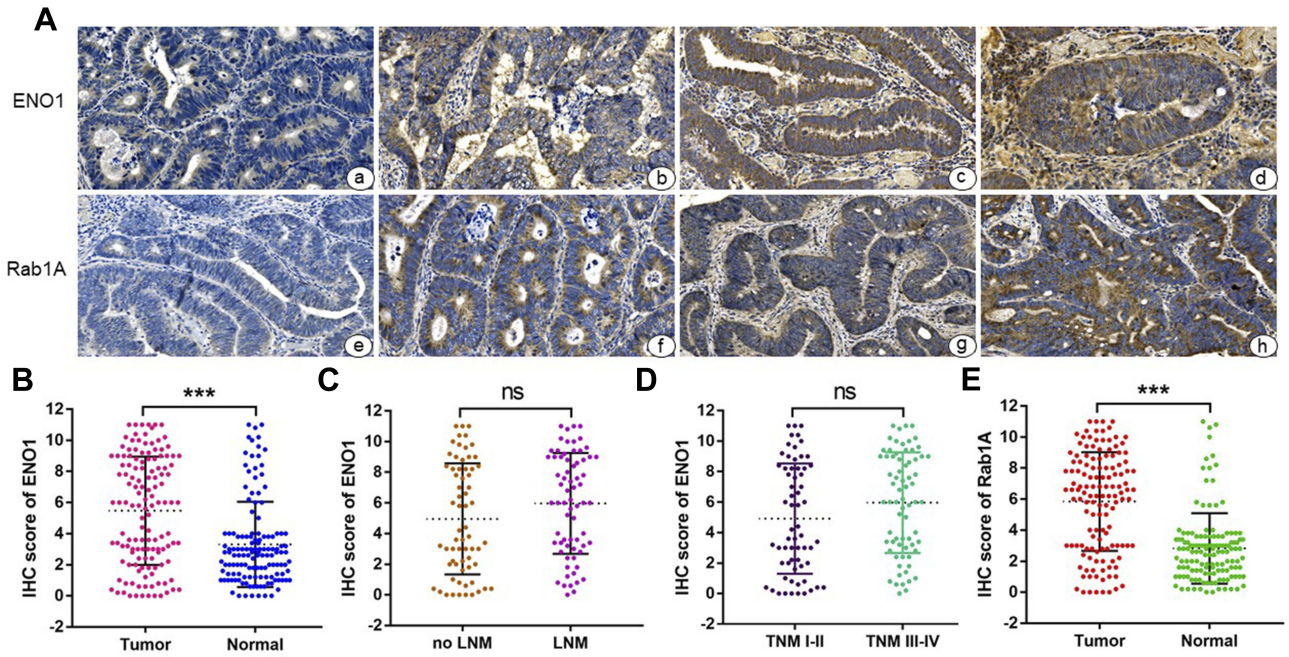

Figure 1 Expression of ENO1/Rab1A in CRC tissues. (**A**) Immunohistochemical (IHC) staining of ENO1/Rab1A in 135 pairs of primary human CRC and adjacent normal tissues (200×). The expression of ENO1 protein was negative (a), weak (b), positive (c), or strongly positive (d). The expression of Rab1A protein was negative (e), weak (f), positive (g), or strongly positive (h). (**B**) Staining scores of ENO1 in CRC tissues and adjacent normal tissues. (**C**) Staining scores of ENO1 in lymph node invasion-positive and negative patients. (**D**) Staining scores of ENO1 in TNM I-II and TNM III-IV stage tissues. (**E**) Staining scores of Rab1A in CRC tissues and adjacent normal tissues. ***P < 0.001. ns: not significant. Scale bar = 100 μm.

expression levels of Rab1A in the CRC and adjacent normal tissues by IHC (Figure 1A), and found that Rab1A was significantly overexpressed in the former (P < 0.001, Figure 1E). Interestingly, a scatter plot indicated that ENO1 expression levels were positively correlated to that of Rab1A in CRC patients (P < 0.001, Figure 2A). Furthermore, subgroup analysis based on TNM staging showed that ENO1 expression was closely associated with that of Rab1A in both TNM I-II (P < 0.001, Figure 2B) and TNM III-IV (P = 0.044, Figure 2C) patients.

ENO1/Rab1A Overexpression Correlates with Poor Prognosis in CRC Patients

Based on the findings so far, we next analyzed the correlation between ENO1/Rab1A expression and patient prognosis and found that overexpression of ENO1 (P < 0.001, Figure 2D) as well as Rab1A (P < 0.001, Figure 2E) led to worse prognosis compared with that of the corresponding negative groups. In addition, high levels of both ENO1 and Rab1A indicated significantly worse prognosis compared to the overexpression of either alone (P < 0.001, Figure 2F). Univariate analysis further showed that the depth of invasion, lymph node metastasis, degree of differentiation, venous invasion, neural invasion, TNM stage, Rab1A expression, and ENO1 expression correlated significantly to poor prognosis (P < 0.05, Table 2). However, only lymph node metastasis, TNM stage, Rab1A expression, and ENO1 expression were independent risk factors as per the multivariate analysis (P < 0.05, Table 2).

ENO1/Rab1A Expression in Four Colorectal Cancer Cell Lines and shRNA-Mediated Deletion of the ENO1 gene in HCT116 and SW620 Cells

ENO1/Rab1A expression levels in four human CRC cell lines (HCT116, SW620, LOVO, and RKO) were analyzed by Western blotting. ENO1/Rab1A levels were relatively high in the HCT116 and SW620 cells, and relatively low in RKO cells (Figure 3A–C). Therefore, we knocked down ENO1 in the HCT116 and SW620 cells, and confirmed the efficiency of shRNA-mediated knockdown by Western blotting, which showed significantly decreased ENO1 expression in cells transfected with ENO1-shRNA compared to that in the control-shRNA-transfected cells (P < 0.05, Figure 3D and E). Thus, effective and specific suppression of ENO1 expression was achieved in HCT116 and SW620 cells.

Table 1 Association Between ENO1 Expression and Clinicopathological Factors in 135 Patients with CRC

	ENO1		
	Negative	**Positive**	***P***
Gender			
Male	37	40	0.487
Female	24	34	
Age (years)			
≤60	24	33	0.601
>60	37	41	
Size (cm)			
≤5	29	30	0.486
>5	32	44	
Depth of invasion			
T1–2	17	8	0.014*
T3–4	44	66	
Lymph node invasion			
Negative	42	24	<0.001***
Positive	19	50	
Degree of differentiation			
Well	51	60	0.822
Poor	10	14	
Venous invasion			
Negative	48	48	0.057
Positive	13	26	
Neural invasion			
Negative	48	43	0.016*
Positive	13	31	
TNM staging			
I–II	42	22	<0.001***
III–IV	19	52	
Tumor location			
Right	16	18	0.813
Left	17	18	
Rectum	28	38	

Notes: *$P < 0.05$. ***$P < 0.001$.
Abbreviations: CRC, colorectal cancer; TNM, tumor-lymph node-metastasis.

ENO1 Knockdown Inhibits in vitro Migration and Proliferation of CRC Cells

The effect of *ENO1* on the proliferation and migration of CRC cells was evaluated by the Transwell, MTT and colony formation assays as appropriate. ENO1 knockdown significantly decreased the proliferative ability of the HCT116/SW620 cells both in terms of their viability ($P < 0.05$, Figure 3F and G) and colony-forming capacity ($P < 0.01$, $P < 0.05$; Figure 4B, E, and F). Furthermore, ENO silencing markedly decreased the in vitro migration of HCT116/SW620 cells compared to the control cells ($P < 0.01$; Figure 4A, C, and D). Taken together, ENO1 promotes colon cancer cell growth and metastasis in vitro.

ENO1 Knockdown Retards the Growth of CRC Cells in vivo

As stated above, the expression of ENO1 could increase the proliferation ability in vitro. To determine the physiological role of ENO1, we subcutaneously implanted HCT116 cells transfected with control or ENO1 shRNA into 3–4 weeks nude mice, and measured tumor size and body weight twice a week. Compared to the control group mice, the subcutaneous tumors derived from ENO1-knockdown cells grew much slower and had relatively stable weight ($P < 0.05$, Figure 5A and B), which was significantly lower than that of control mice ($P < 0.05$, Figure 5D). Furthermore, the body weight of the mice injected with ENO1-knockdown cells was slightly greater compared to that of the control group mice ($P > 0.05$, Figure 5C), and became significantly greater after subtracting the respective tumor weights ($P < 0.05$, Figure 5E). Taken together, ENO1 promotes the growth of CRC cells in vivo.

Effects of ENO1 on Colon Cancer Cell Proliferation and Metastasis are Mediated by Targeting Rab1A

Previous studies have reported that both *ENO1* and Rab1A regulate the mTOR signaling pathway in CRC. To determine whether Rab1A mediates the effects of ENO1 on CRC cells, we analyzed the expression levels of Rab1A after ENO1 knockdown and found a significant decrease in the former following ENO1 knockdown ($P < 0.05$, Figure 5F–H). Thus, ENO1 mediates its effects in CRC cells by upregulating Rab1A.

Discussion

Colorectal cancer is one of the most commonly diagnosed cancers worldwide, and the second leading cause of cancer-related deaths.[21] Despite advancements in treatment strategies, including chemotherapy, the prognosis is still poor.[22] The key challenge in improving CRC prognosis is the molecular heterogeneity of these tumors.[16] Therefore, it is essential to identify novel therapeutic targets in order to improve the survival of CRC patients. Abnormal glucose metabolism is one of the hallmarks of cancer since

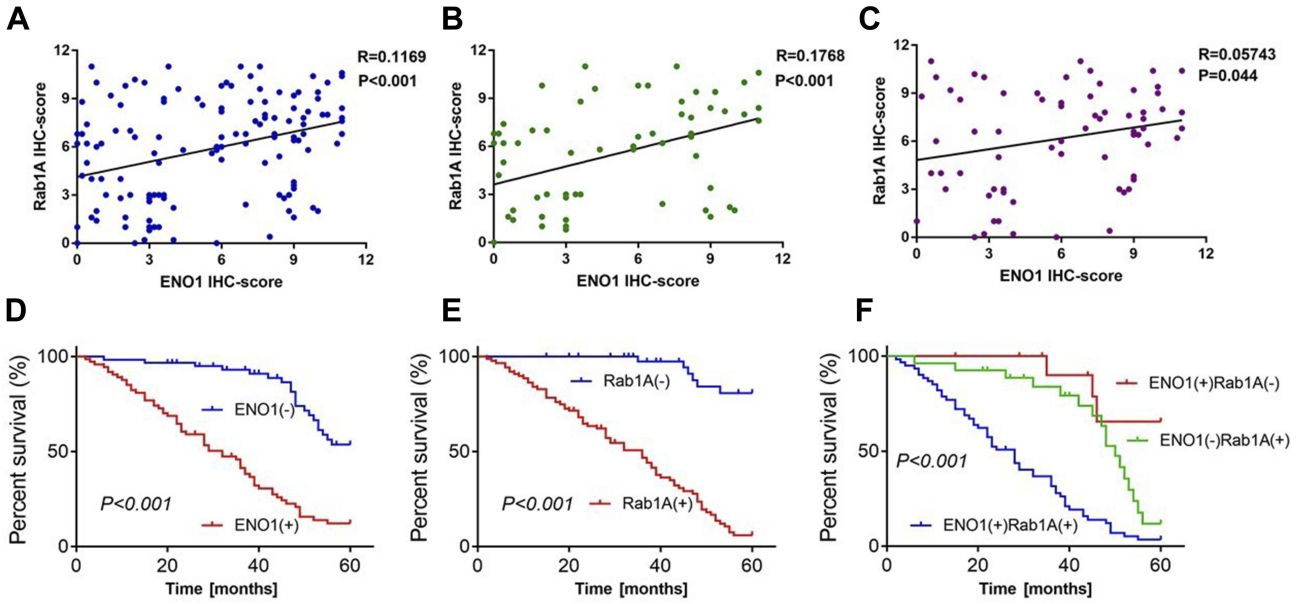

Figure 2 Expression association and Kaplan–Meier survival analysis of ENO1/Rab1A in 135 CRC patients. Scatter plots showing the association between ENO1 and Rab1A expression as per IHC-scores in all CRC patients (**A**), TNM I–II stage patients (**B**), and TNM I–II patients (**C**). Post-surgery Kaplan–Meier survival analysis of 135 CRC patients stratified according to ENO1 (**D**) and Rab1A (**E**) expression levels. (**F**) Survival analysis of $ENO1^{hi}/Rab1A^{hi}$, $ENO1^{lo}/Rab1A^{hi}$ and $ENO1^{hi}/Rab1A^{lo}$ patients.

tumor cells acquire characteristic changes in glucose metabolism to support their proliferation and metastasis.[23] Therefore, metabolic manipulation, such as targeting key glycolytic enzymes, is a promising therapeutic approach towards cancer.[24]

ENO1 is a conserved glycolytic enzyme that catalyzes the formation of phosphoenolpyruvate from 2-phosphoglycerate, an ATP-generating step that supports cancer cell proliferation and metastasis.[25] In addition, *ENO1* is also involved in important cellular functions and has a significant role in tumor initiation and regression.[26] Recent studies show that *ENO1* is a diagnostic marker in many tumors,[25–27] and its overexpression correlates positively with cancer progression and poor prognosis.[28,29] However, little is known regarding the role of ENO1 in CRC. A previous study showed that ENO1 is overexpressed in CRC tissues compared to normal colon tissues, and promotes CRC cell proliferation and migration, indicating that ENO1 plays an oncogenic role in CRC.[16] This hypothesis was substantiated by the finding that *ENO1* regulated the mTOR pathway in CRC cells.[16] Another

Table 2 Results of Univariate and Multivariate Analyses of Patients' Survival in CRC by Cox's Proportional Hazard Model

Factor	Univariate Analysis			Multivariate Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender (Male/Female)	0.876	0.561–1.366	0.559			
Age (≤60 or >60 years)	1.084	0.696–1.687	0.722			
Size of cancer (≤5 or >5 cm)	0.654	0.418–1.023	0.063			
Depth of invasion (T1-2/T3-4)	0.223	0.102–0.487	<0.001***	0.566	0.243–1.317	0.186
Lymph node metastasis (negative/positive)	0.179	0.108–0.298	<0.001***	8.051	1.653–39.213	0.010*
Degree of differentiation (poor/well)	0.461	0.270–0.787	0.005**	0.634	0.360–1.118	0.115
Venous invasion (negative/positive)	0.374	0.263–0.595	<0.001***	0.680	0.414–1.116	0.127
Neural invasion (negative/positive)	0.544	0.346–0.856	0.008	0.883	0.539–1.447	0.622
TNM stage (I–II/III–IV)	0.157	0.093–0.264	<0.001***	0.035	0.007–0.182	<0.001***
ENO1 expression (low/high)	0.206	0.123–0.345	<0.001***	0.336	0.189–0.598	<0.001***
Rab1A expression (low/high)	0.071	0.031–0.166	<0.001***	0.121	0.050–0.295	<0.001***

Notes: *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$.
Abbreviations: CRC, colorectal cancer; TNM, tumor-lymph node-metastasis.

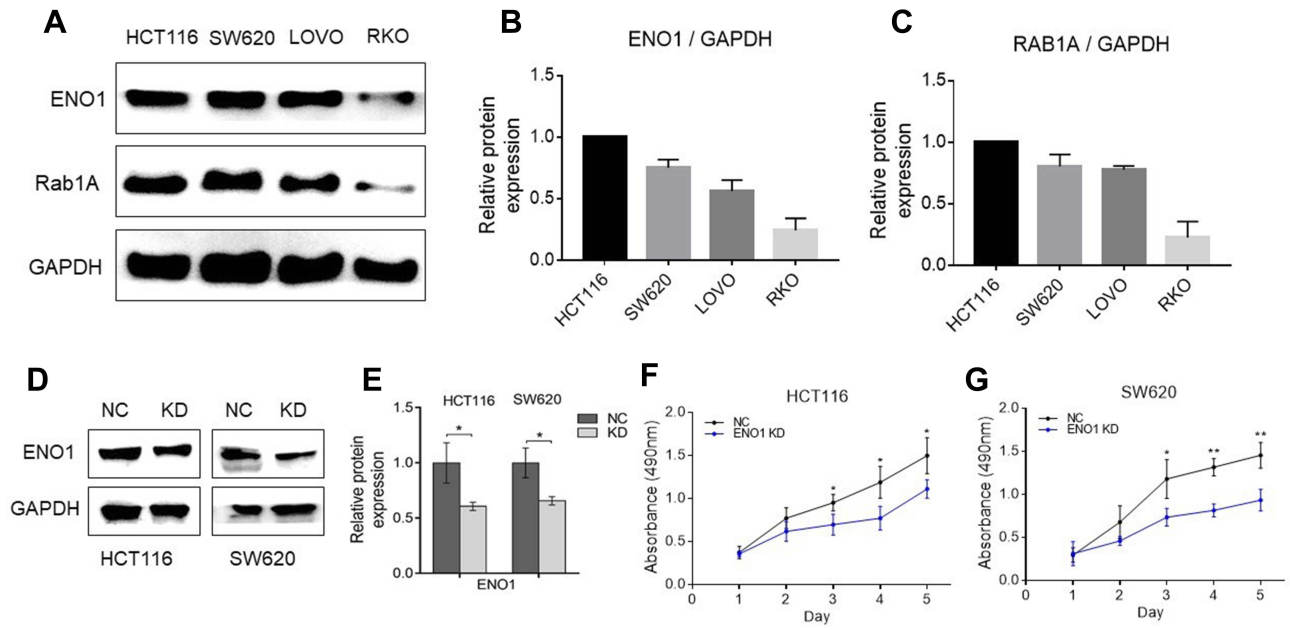

Figure 3 Effect of ENO1 knockdown on the proliferation of CRC cells. (**A**) Western blot analysis showing ENO1/Rab1A expression levels in four CRC cell lines. (**B, C**) The intensity of protein bands as determined by densitometric analysis. (**D**) Immunoblot showing ENO1 expression levels in the shENO1 (KD) and control shRNA (NC) cells. (**E**) Quantification of the protein bands in terms of grayscale values. (**F, G**) MTT assay analysis of the proliferation ability of SW620 and HCT116 cells transfected with control shRNA (NC) or shENO1 (KD). *P < 0.05, **P < 0.01.

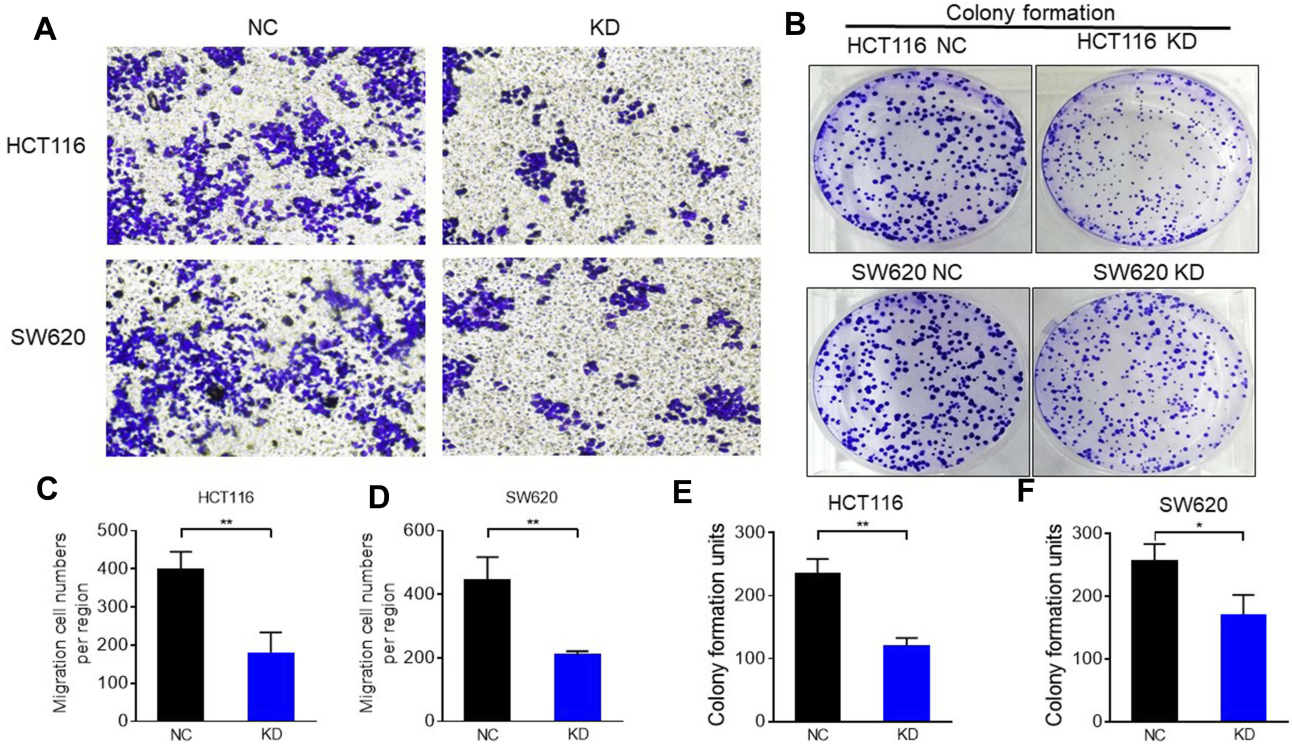

Figure 4 Effects of ENO1 knockdown on the migration and colony-forming capacity of CRC cells. (**A**) Representative images of Transwell assay showing the migration ability of SW620 and HCT116 cells transfected with control shRNA (NC) or shENO1 (KD) (magnification ×200). (**C, D**) Migration rate in terms of the relative number of migratory cells; data are represent as the mean ± S.E.M. (n = 5). (**B**) Representative images showing colonies formed by SW620 and HCT116 cells transfected with control shRNA (NC) or shENO1 (KD); (**E, F**) Number of colonies in the different groups. Data are presented as the mean ± S.E.M. (n = 3). *P < 0.05, **P < 0.01.

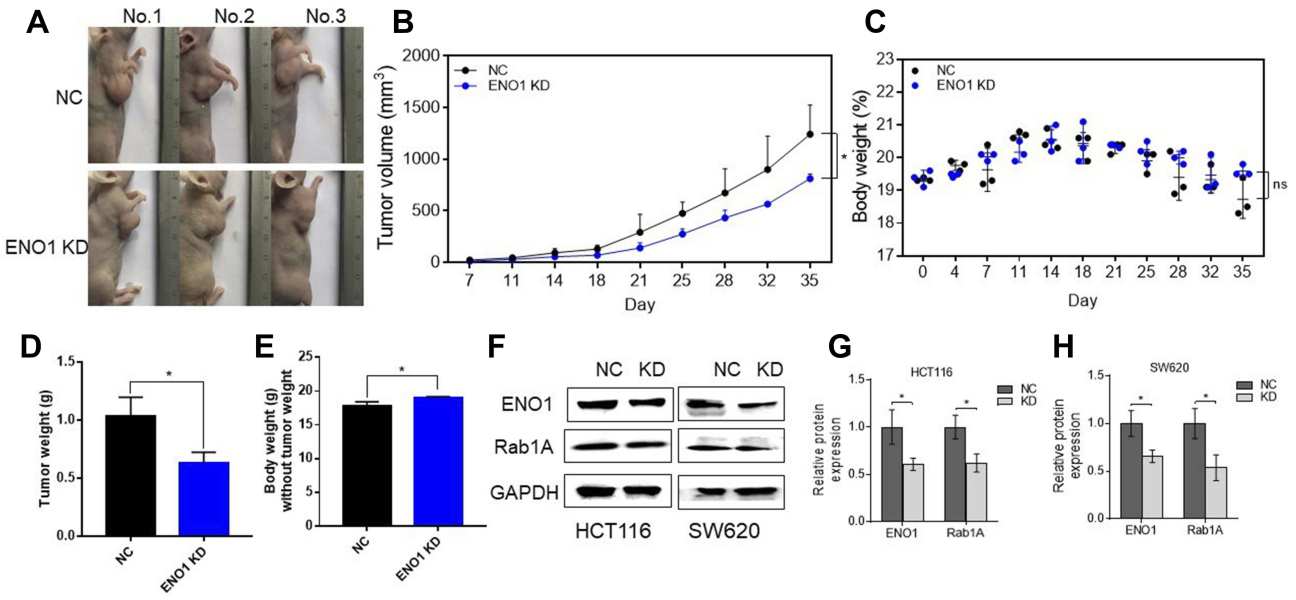

Figure 5 Knockdown of ENO1 suppressed CRC tumor growth in vivo and downregulated Rab1A expression in CRC cells. (**A**) Representative images of nude mice inoculated with CRC cells transfected with control shRNA (NC) or shENO1 (ENO1 KD). (**B**) Tumor volumes in the different groups measured twice a week over 5 weeks. Data are presented as the mean ± S.E.M. (**D**) Tumor weight of different groups. Data are presented as the mean ± S.E.M. (**E**) The body weight of the mice after subtraction of tumor weight. Data are presented as the mean ± S.E.M. (**F**) Immunoblot showing expression levels of ENO1 and Rab1A in the SW620 cells/HCT116 cells transfected with shENO1 (KD) and control shRNA (NC). (**G, H**) Quantitative analysis of the band gray values in HCT116 cells (**G**) and SW620 cells (**H**). *$P < 0.05$.

recent study reported that *Rab1A* promotes CRC genesis and metastasis by targeting the mTOR pathway.[17] However, a potential molecular crosstalk between *ENO1* and *Rab1A* in CRC cell remains unclear and needs to be elucidated.

Previous studies have shown that ENO1 is highly expressed in multiple cancers and correlated with clinicopathological parameters.[25,30] Consistent with this, the expression of ENO1/Rab1A was significantly higher in the CRC tissues compared to that in paired adjacent normal tissues. Furthermore, ENO1 overexpression was positively associated with Rab1A expression levels, indicating a functional link between the two in CRC cells. In addition, ENO1 expression was also slightly higher in patients with lymph node invasion compared with those without, which suggested a metastatic function of ENO1. Finally, enhanced ENO1 expression was closely linked with the depth of tumor invasion lymph node invasion, neural invasion, and TNM staging, indicating that ENO1 is involved in CRC initiation and progression as well. Previous reports have demonstrated that ENO1 overexpression correlates to poor survival in gastric cancer.[31] In our study also, the overexpression of ENO1 or Rab1A led to a worse prognosis, which further worsened when both were overexpressed. Furthermore, multivariate analysis indicated that lymph node metastasis, TNM stage, Rab1A expression, and ENO1 expression were independent risk factors of CRC.

ENO1 silencing significantly inhibits the growth and migration abilities of gastric cancer[23] and colon cancer cell lines.[16] Consistent with this, knockdown of ENO1 expression in CRC cells inhibited their proliferation and migration in vitro, and reduced their growth in vivo. These results suggested that ENO1 acts as an oncogenic factor and promotes CRC genesis and metastasis. Mechanistically, the oncogenic potential of *ENO1* has been linked to the regulation of the mTOR pathway,[16] which is also targeted by *Rab1A* during CRC initiation and regression.[17] In our study as well, Rab1A expression level was significantly decreased following ENO1 knockdown, which suggested that *ENO1* promotes the proliferation and migration of CRC cells by upregulating *Rab1A*.

Conclusions

ENO1 is overexpressed in CRC tissues and correlates significantly with lymph node invasion, TNM staging, and other clinicopathological factors. It is also positively correlated with high Rab1A expression in CRC tissues, and ENO1/Rab1A co-overexpression led to worse prognosis. ENO1 knockdown significantly inhibited CRC cells proliferation and migration in vitro and xenograft growth in vivo by targeting Rab1A. Thus, *ENO1* is a potential therapeutic target against CRC.

Acknowledgment

This study was supported by the Nature and Science Fund from Wannan Medical College, CN (Grant Nos. WK2019F07, WK2019F09), Nanjing Medical University Science and Technology Development Fund (NMUB2018231), Suzhou Special Project of Diagnosis and Treatment for key Clinical Disease (LCZX201814) and Suzhou Science and Technology Development Project (SYSD2018144).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. *CA Cancer J Clin*. 2014;64(2):104–117. doi:10.3322/caac.21220
2. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7–34. doi:10.3322/caac.21551
3. Anemone A, Consolino L, Conti L, et al. In vivo evaluation of tumour acidosis for assessing the early metabolic response and onset of resistance to dichloroacetate by using magnetic resonance pH imaging. *Int J Oncol*. 2017;51(2):498–506. doi:10.3892/ijo.2017.4029
4. Zhao M, Liu Q, Gong Y, et al. GSH-dependent antioxidant defense contributes to the acclimation of colon cancer cells to acidic microenvironment. *Cell Cycle*. 2016;15(8):1125–1133. doi:10.1080/15384101.2016.1158374
5. Jin S, DiPaola RS, Mathew R, White E. Metabolic catastrophe as a means to cancer cell death. *J Cell Scie*. 2007;120(Pt 3):379–383. doi:10.1242/jcs.03349
6. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab*. 2016;23(1):27–47. doi:10.1016/j.cmet.2015.12.006
7. Kang HJ, Jung SK, Kim SJ, Chung SJ. Structure of human alpha-enolase (hENO1), a multifunctional glycolytic enzyme. *Acta Crystallogr D Biol Crystallogr*. 2008;64(Pt 6):651–657. doi:10.1107/S0907444908008561
8. Zhu X, Miao X, Wu Y, et al. ENO1 promotes tumor proliferation and cell adhesion mediated drug resistance (CAM-DR) in Non-Hodgkin's Lymphomas. *Exp Cell Res*. 2015;335(2):216–223. doi:10.1016/j.yexcr.2015.05.020
9. Bae S, Kim H, Lee N, et al. α-Enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *J Immunol*. 2012;189(1):365–372. doi:10.4049/jimmunol.1102073
10. Gao J, Zhao R, Xue Y, et al. Role of enolase-1 in response to hypoxia in breast cancer: exploring the mechanisms of action. *Oncol Rep*. 2013;29(4):1322–1332. doi:10.3892/or.2013.2269
11. Tu SH, Chang CC, Chen CS, et al. Increased expression of enolase alpha in human breast cancer confers tamoxifen resistance in human breast cancer cells. *Breast Cancer Res Treat*. 2010;121(3):539–553. doi:10.1007/s10549-009-0492-0
12. Tsai ST, Chien IH, Shen WH, et al. ENO1, a potential prognostic head and neck cancer marker, promotes transformation partly via chemokine CCL20 induction. *Eur J Cancer*. 2010;46(9):1712–1723. doi:10.1016/j.ejca.2010.03.018
13. Pernemalm M, De Petris L, Branca RM, et al. Quantitative proteomics profiling of primary lung adenocarcinoma tumors reveals functional perturbations in tumor metabolism. *J Proteome Res*. 2013;12(9):3934–3943. doi:10.1021/pr4002096
14. Duijvesz D, Burnum-Johnson KE, Gritsenko MA, et al. Proteomic profiling of exosomes leads to the identification of novel biomarkers for prostate cancer. *PLoS One*. 2013;8(12):e82589. doi:10.1371/journal.pone.0082589
15. Bai Z, Ye Y, Liang B, et al. Proteomics-based identification of a group of apoptosis-related proteins and biomarkers in gastric cancer. *Int J Oncol*. 2011;38(2):375–383. doi:10.3892/ijo.2010.873
16. Zhan P, Zhao S, Yan H, et al. alpha-enolase promotes tumorigenesis and metastasis via regulating AMPK/mTOR pathway in colorectal cancer. *Mol Carcinog*. 2017;56(5):1427–1437. doi:10.1002/mc.22603
17. Thomas JD, Zhang YJ, Wei YH, et al. Rab1A is an mTORC1 activator and a colorectal oncogene. *Cancer Cell*. 2014;26(5):754–769. doi:10.1016/j.ccell.2014.09.008
18. Wang ZK, Cheng ZW, Chen SJ, et al. Aberrant expression of Rab1A and its prognostic significance in human colorectal cancer. *Eur Rev Med Pharmacol Sci*. 2018;22(14):4509–4517. doi:10.26355/eurrev_201807_15505
19. Cheng Z, Shao X, Xu M, et al. Rab1A promotes proliferation and migration abilities via regulation of the HER2/AKT-independent mTOR/S6K1 pathway in colorectal cancer. *Oncol Rep*. 2019;41(5):2717–2728. doi:10.3892/or.2019.7071
20. Shao X, Kuai X, Pang Z, et al. Correlation of Gli1 and HER2 expression in gastric cancer: identification of novel target. *Sci Rep*. 2018;8(1):397. doi:10.1038/s41598-017-17435-6
21. Bak Y, Kwon T, Bak IS, Hong J, Yu DY, Yoon DY. IL-32theta inhibits stemness and epithelial-mesenchymal transition of cancer stem cells via the STAT3 pathway in colon cancer. *Oncotarget*. 2016;7(6):7307–7317. doi:10.18632/oncotarget.7007
22. Zhang Z, Dong X, Yang X, et al. Expression and clinical significance of absent in melanoma 2 in colorectal cancer. *Biomed Pharmacother*. 2017;94:843–849. doi:10.1016/j.biopha.2017.07.161
23. Sun L, Lu T, Tian K, et al. Alpha-enolase promotes gastric cancer cell proliferation and metastasis via regulating AKT signaling pathway. *Eur J Pharmacol*. 2019;845:8–15. doi:10.1016/j.ejphar.2018.12.035
24. Boroughs LK, DeBerardinis RJ. Metabolic pathways promoting cancer cell survival and growth. *Nat Cell Biol*. 2015;17(4):351–359. doi:10.1038/ncb3124
25. Yu L, Shi J, Cheng S, et al. Estrogen promotes prostate cancer cell migration via paracrine release of ENO1 from stromal cells. *Mol Endocrinol*. 2012;26(9):1521–1530. doi:10.1210/me.2012-1006
26. Song Y, Luo Q, Long H, et al. Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma. *Mol Cancer*. 2014;13:65. doi:10.1186/1476-4598-13-65
27. Yang J, Zhou M, Zhao R, et al. Identification of candidate biomarkers for the early detection of nasopharyngeal carcinoma by quantitative proteomic analysis. *J Proteomics*. 2014;109:162–175. doi:10.1016/j.jprot.2014.06.025
28. Cappello P, Rolla S, Chiarle R, et al. Vaccination with ENO1 DNA prolongs survival of genetically engineered mice with pancreatic cancer. *Gastroenterology*. 2013;144(5):1098–1106. doi:10.1053/j.gastro.2013.01.020
29. Takashima M, Kuramitsu Y, Yokoyama Y, et al. Overexpression of alpha enolase in hepatitis C virus-related hepatocellular carcinoma: association with tumor progression as determined by proteomic analysis. *Proteomics*. 2005;5(6):1686–1692. doi:10.1002/pmic.200401022
30. Liu YQ, Huang ZG, Li GN, et al. Effects of alpha-enolase (ENO1) over-expression on malignant biological behaviors of AGS cells. *Int J Clin Exp Med*. 2015;8(1):231–239.
31. Qiao H, Wang Y, Zhu B, et al. Enolase1 overexpression regulates the growth of gastric cancer cells and predicts poor survival. *J Cell Biochem*. 2019. doi:10.1002/jcb.29179