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# High Expression of Folate Receptor Alpha (FOLR1) is Associated With Aggressive Tumor Behavior, Poor Response to Chemoradiotherapy, and Worse Survival in Rectal Cancer

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## Abstract

**Objectives:** Recently, molecular medicine targeting Folate Receptor Alpha (FOLR1), which mediates intracellular folate uptake and tumor cell proliferation, has been identified in several malignancies. However, the association between FOLR1 expression and rectal cancer remains unclear. **Methods:** Immunostaining of FOLR1 was performed on biopsy specimens from 172 rectal cancer patients undergoing preoperative chemoradiotherapy (CRT). FOLR1 expression was measured and divided into low (0+-2+) or high (3+-4+) level. Correlations between FOLR1 status and clinicopathologic features, tumor regression grade, disease-specific survival (DSS), local recurrence-free survival, and metastasis-free survival (MeFS) were analyzed, retrospectively. **Results:** High FOLR1 expression was significantly associated with advanced post-treatment tumor and nodal status (T3-4; N1-2, $P = .001$), vascular invasion ($P = .042$), perineural invasion ($P = .012$), and poor regression change after CRT ($P = .001$). In uni- and multi-variable survival analysis, FOLR1 overexpression remained a significant predictor of lower DSS (hazard ratio [HR], 2.328; 95% confidence interval [CI], 1.014-5.344; $P = .046$) and MeFS (HR, 2.177; 95% CI, 1.000-1.1286; $P = .050$). Conclusion: These results indicate that high FOLR1 status is associated with aggressive tumor behavior, poor response to CRT, and worse survival. Therefore, FOLR1 expression at initial biopsy may be useful in predicting outcomes and also be a target for the exploration of FOLR1-based therapeutic agents.

## Keywords

folate receptor alpha, rectal cancer, chemoradiation, response, survival

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**Abbreviations**

CRT, chemoradiotherapy; FOLR1, Folate Receptor Alpha; TRG, tumor regression grading; DSS, disease-specific survival; LRFS, local-recurrence-free survival; MeFS, metastases-free survival; HR, hazard ratio; CIs, confidence intervals

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## Introduction

Preoperative chemoradiotherapy (CRT) followed by surgical resection is recognized as first-line treatment for rectal cancer, providing better local control, higher sphincter preservation, and less toxicity compared to other therapies.[1,2] However, survival after this multimodal therapy has remained stagnant.[3] Previous studies indicated that the response to CRT was associated with survival and rectal cancer patients with a higher grade of tumor regression had a better prognosis.[4] Despite this, response to CRT is highly variable and there are still some patients with minor or even no regressive changes after intense therapy. Therefore, identifying a biomarker that predicts response to CRT may help to guide treatment decisions and thus improve the management of rectal cancer.

Folate Receptor Alpha (FOLR1), a glycosylphosphatidylinositol-linked protein, is a well-characterized folate transporter.[5] Studies have revealed a growth advantage associated with cells transfected with FOLR1, suggesting that folate binding protein may be involved in the control and maintenance of cell proliferation.[6] Thus, FOLR1 overexpression may confer a growth advantage to tumors by increasing folate uptake and may affect cell proliferation via alternative cell signaling pathways.[7] Recently, FOLR1 is a promising biomarker and therapeutic target for ovarian, breast, and lung cancers.[5,8,9] However, there is still limited research investigating the relevance of FOLR1 expression in rectal cancer after preoperative CRT. Previously, Shia et al demonstrated that FOLR1 expression was correlated with younger age, high metastasis, and worse 5-year disease-specific survival (DSS) in the results of the analysis of 130 colorectal cancer patients.[10] However, only 24 rectal cancer were included and there is no information about the use of preoperative therapy or not. As we know, the management for rectal cancer is different to colon cancer and radiation therapy is often needed for rectal cancer. Thus, considering the insufficient evidences of FOLR1 expression in rectal cancer, it motivates us to perform this study to explore FOLR1 expression in our rectal cancer patients after preoperative CRT. To address this question, we aimed to explore FOLR1 expression in rectal cancer tumor samples obtained through biopsy before preoperative CRT. Moreover, we evaluated the effect of FOLR1 expression on clinical outcomes, including response to CRT and survival.

## Materials and Methods

### Ethics Approval

In this study, we used anonymous patient sample information from biobank as approved by Chi-Mei Medical Center Institutional Review Board (IRB: 10801-001). As a rule, inform consent has been signed by every patient before their sample/information collected into biobank. And due to the disconnection between patient ID and their sample/information, we cannot identify the consent signed by that patient anymore. The reporting of this study conforms to STROBE guidelines.[11]

### Patient Demographic Characteristics and Tumor Specimens

Rectal adenocarcinoma tissue specimens from 172 patients who had preoperative CRT and surgery at our medical Center between 1998 and 2004 were eligible for inclusion in the study, retrospectively. Standardized long-course CRT, including 5-fluorouracil-based chemotherapy either orally or intravenously every week from the beginning to the end of radiation therapy (45-50 Gy/25fractions) were applied. Radical surgery with total mesorectal excision (abdominoperineal resection or low anterior resection) was performed 4 to 6 weeks after CRT. Approximately 3 to 4 weeks after surgery, 5-fluorouracil-based adjuvant chemotherapy was administered for at least 4 months. Adjuvant chemotherapy was administered if the pre-treatment (clinical) or post-treatment tumor or nodal status (pathology) was beyond T3 or N1. All patients were regularly monitored after diagnosis until death or last follow-up. Patients who had a previous cancer history, distal metastasis at diagnosis, or were unable to complete a full course of CRT were excluded.

Clinicopathological variables were collected from our cancer registry database and supplemented with a review of medical records which included patient demographics, clinicopathological TNM stage, lymphovascular and perineural invasion, chemotherapy regimen and timing, radiotherapy course and cause of death. The tumor response to CRT was assessed using the standard 5-point tumor regression grading (TRG) system into 3 subgroups: complete response (TRG 4), moderate response (TRG 2+3), and poor/no response (TRG 0+1).[4] All patients were re-staged according to the 7th edition of the American Joint Committee on Cancer staging system. Endpoints represent 5-year DSS, local recurrent-free survival (LRFS), and metastases-free survival (MeFS) rates. Deaths due to cancer were defined as valid events, while deaths secondary to other causes were excluded in the final results.

### Immunohistochemistry Analysis

Immunohistochemical stains were performed on 3-μm thick sections of all biopsies fixed paraffin embedded blocks. The slides were deparaffinized and rehydrated, followed by a heat-induced antigen retrieval method for 20 min using the Leica Epitope Retrieval Solution 2 (Leica Biosystems, Buffalo Grove, IL).

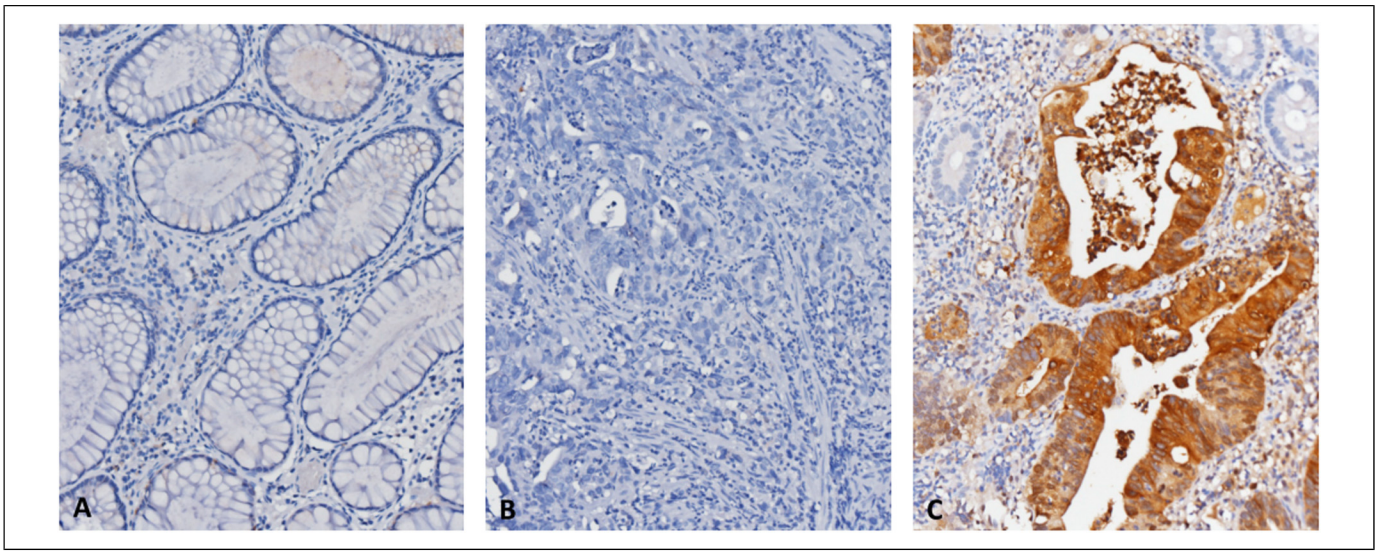


**Figure 1.** Representative FOLR1 protein expression by staining intensity using immunohistochemistry in rectal cancer biopsy tissue microarray (IHE DAB coloration, 400×). The non-neoplastic colonic mucosa (A) reveals no expression of FOLR1 as compared with rectal cancers with low expression (B) and high expression (C) of FOLR1, respectively.

Slides were then incubated for 15 min at a 1:50 dilution in primary antibody of FOLR1 from Novocastra (Leica Microsystems) clone BN3.2 (catalogue #NCL-F-FRalpha).[12] The scoring criteria was based on previous publication and determined independently by our 2 pathologists who were blinded to all clinical outcomes data. As shown in Figure 1, patients were subdivided into "high" (score 3+-4+) and "low" (score 0+-2+) staining groups based on the intensity, the percentage of cells, and staining location.[13]

### *Statistical Analysis*

Statistical analyses and graphics were performed using SPSS for Windows 22.0 (IBM Corporation, Armonk, NY, USA). Comparisons of FOLR1 expression and clinicopathological characteristics were carried out using the chi-squared test. Recurrence/metastasis or deaths due to cancer were defined as events. Kaplan–Meier analysis was used to determine the 5-year DSS, LRFS, and MeFS, with the log-rank test used for comparison. All factors were analyzed using univariate Cox regression to estimate hazard ratios (HRs) with 95% confidence intervals (CIs). Those factors with statistical significance were entered into multivariate analysis. A *P*-value less than .05 was considered statistically significant. Based on a 7-year recruitment period, with maximum follow-up of 5 years, a sample size of 172 participants provided approximately 81% power to detect a 16% effect on the endpoint of the study, assuming an annual event rate in this calculation was 3.1% per year in the population of rectal adenocarcinoma.

## Results

### *Patient and Tumor Characteristics*

As our previous studies described, the clinical and pathological characteristics of study participants are summarized in Table 1.[14,15] About 108 (62.8%) were male and 64 (37.2%) were female. The median age was 63 years (range, 22-88 years). Pre-treatment AJCC tumor staging identified 91 (52.9%) patients with advanced tumor status (cT3-4) and 47 (27.3%) patients with advanced nodal status (cN1-2). After chemoradiation followed by surgery, 86 (50%) patients had advanced tumor status (pT3-4) and 49 (28.5%) patients had advanced nodal status (pN1-2), respectively. In response to CRT, 17 (10%) patients had complete tumor regression (TRG 4), 118 (68.6%) patients had a moderate response (TRG 2-3), and 37 (21.5%) patients had a poor response (TRG 0-1). In terms of other pathological features, 15 (8.7%) patients presented with vascular invasion and 5 (2.9%) patients had perineural invasion.

### *FOLR1 Expression in Rectal Cancers*

Cytoplasmic expression of FOLR1 was successfully scored in all examined cases and examples of these staining intensities are illustrated in Figure 1. Table 1 lists the number of patients with either high or low FOLR1 expression for each clinicopathologic variable. High FOLR1 expression was significantly associated with advanced post-Tx tumor and nodal status (T3-4; N1-2, $P = .001$), vascular invasion ($P = .042$), perineural invasion ($P = .012$), and poor regression change after CRT ($P = .001$). As stated above, higher FOLR1 expression was associated with aggressive tumor behavior and poor sensitivity to CRT.

### *Effects of FOLR1 Expression on Survival*

Table 2 lists the number of cases of DSS, LRFS, and MeFS for each clinicopathological variable, including down stage after

**Table 1.** Associations and Comparisons Between FOLR1 Expression and Clinicopathological Features in 172 Rectal Cancer Patients Receiving Preoperative Chemoradiotherapy.

| Parameter | | No. | FOLR1 expression | | *P*-value |
|---|---|---|---|---|---|
| | | | Low Exp. (0+-2+) | High Exp. (3+-4+) | |
| Gender | Male | 108 | 33 | 31 | .203 |
| | Female | 64 | 67 | 41 | |
| Age | <70 | 106 | 58 | 48 | .249 |
| | ≧70 | 66 | 42 | 24 | |
| Pre-Tx tumor status (Pre-T) | T1-T2 | 81 | 50 | 31 | .439 |
| | T3-T4 | 91 | 50 | 41 | |
| Pre-Tx nodal status (Pre-N) | N0 | 125 | 75 | 50 | .489 |
| | N1-N2 | 47 | 25 | 22 | |
| Post-Tx tumor status (Post-T) | T1-T2 | 86 | 61 | 25 | .001[a] |
| | T3-T4 | 86 | 39 | 47 | |
| Post-Tx nodal status (Post-N) | N0 | 123 | 81 | 42 | .001[a] |
| | N1-N2 | 49 | 19 | 30 | |
| Vascular invasion | Absent | 157 | 95 | 62 | .042[a] |
| | Present | 15 | 5 | 10 | |
| Perineural invasion | Absent | 167 | 100 | 67 | .012[a] |
| | Present | 5 | 0 | 5 | |
| Tumor regression grade | Grade 0-1 | 37 | 12 | 25 | .001[a] |
| | Grade 2-3 | 118 | 74 | 44 | |
| | Grade 4 | 17 | 14 | 3 | |

[a]Statistically significant.

CRT and FOLR1 expression level. Univariate analysis indicated that clinicopathological variable, such as pre-treatment tumor status, was significantly associated with a worse DSS ($P = .0006$).[14,15] Vascular invasion was significantly associated with DSS ($P = .0184$) and LRFS ($P = .0028$). Post-treatment tumor status and tumor regression grade were all negatively associated with DSS, LRFS, and MeFS and were statistically significant ($P \leq .05$ for all). Most importantly, rectal cancer patients with high FOLR1 expression had significantly poor DSS ($P = .0002$), LRFS ($P = .0123$), and MeFS ($P = .0011$), as shown in Figure 2. The results of the multivariate analysis are shown in Table 3. High FOLR1 expression remained a significant predictor of lower DSS (HR, 2.328; 95% CI, 1.014-5.344; $P = .046$) and MeFS (HR, 2.177; 95% CI, 1.000-1.1286; $P = .050$).

## Discussion

Recently, molecular medicine targeting FOLR1 has been reported in many cancer types.[16,17] A recent phase III study demonstrated success in improving survival in ovarian cancer using an anti-FOLR1 antibody, mirvetuximab.[18] However, the role of FOLR1 expression has not been clarified in rectal cancer. To our knowledge, this is the first report investigating the association between FOLR1 expression, clinicopathological features, and response to CRT in rectal cancer. Similar to previous studies, high FOLR1 expression was correlated with aggressive tumor behavior, poor response to CRT, and worse survival. These observations suggest that FOLR1 expression may be useful to predict outcomes and also be a promising target for the exploration of novel therapeutic agents.

Over recent decades, molecular biomarkers have become a source of reliable information and have improved the accuracy of prognosis for cancer patients.[19,20] Folate is a necessary component for DNA synthesis, repair, and methylation, and FOLR1 plays a role in folate metabolism as a membrane-bound protein with high affinity for binding and mediating folate uptake by endocytosis.[21] FOLR1 expression may confer a growth advantage to tumors by increasing folate uptake and also affect cell proliferation via alternative cell signaling pathways such as the JAK-STAT3 pathway.[6,7]

FOLR1 expression is tumor specific and is overexpressed in various epithelial tumors such as ovarian, breast, endometrial, and lung cancers.[22–24] This specific characteristic in pathogenic tissue makes FOLR1 status an ideal biomarker to evaluate tumor behavior and prognosis. Necela et al reported that FOLR1 expression increased the growth of triple negative cell lines, that inhibition of FOLR1 significantly reduced the cell growth of established triple negative breast cancer cell lines, and that the magnitude of the effect was proportional to their original mRNA expression level.[25] For these reasons, FOLR1 has become a biomarker of recent interest in the cancer field; however, there is still limited data on FOLR1 expression in colorectal cancer.

FOLR1 expression in many epithelial cancer types such as ovarian and non-small cell lung carcinoma has been associated with tumor behavior.[8,12] We also analyzed whether FOLR1 expression was associated with tumor behavior and survival. In our results, high FOLR1 expression was significantly associated with advanced post-treatment tumor and nodal status (T3-4; N1-2, $P = .001$), vascular invasion ($P = .042$), perineural invasion ($P = .012$), and poor regression change after CRT ($P = .001$). Moreover, in survival analysis, FOLR1 expression was also associated with lower DSS and MeFS. Similarly, a recent meta-analysis, including 4471 patients, supports these findings and found high FOLR1 expression to be significantly associated with poor overall survival (OS) (HR = 0.78, 95% CI = 0.64-0.94, $P = .009$) and disease-free survival (HR = 1.25, 95% CI = 1.07-1.47, $P = .005$) in cancer patients.[26] Stratified analyses found that breast cancer and endometrial carcinoma patients with FOLR1 overexpression had a poor prognosis. Omote et al also demonstrated that FOLR1 expression was an independent prognostic factor for OS in pancreatic cancer.[27] Patients who had high FOLR1 expression had shorter survival than those without high FOLR1 expression (median, 18.8 vs 21.3 months; $P = .017$). For colorectal cancer, Shia et al reported the expression of FOLR1 was inversely related to the tumor microsatellite instability-high phenotype and directly related to worse 5-year DSS.[10] These intriguing findings

**Table 2.** Univariate Log-Rank Analysis for Important Clinicopathological Variables and FOLR1 Expression.

| | | | DSS | | LRFS | | MeFS | |
|---|---|---|---|---|---|---|---|---|
| Parameter | | No. of case | No. of event | *P*-value | No. of event | *P*-value | No. of event | *P*-value |
| Gender | Male | 108 | 20 | .9026 | 7 | .2250 | 17 | .3520 |
| | Female | 64 | 11 | | 20 | | 14 | |
| Age | <70 | 106 | 19 | .8540 | 18 | .6615 | 20 | .7427 |
| | ≧70 | 66 | 12 | | 9 | | 11 | |
| Pre-Tx tumor status (Pre-T) | T1-T2 | 81 | 10 | .0776 | 10 | .2261 | 11 | .1745 |
| | T3-T4 | 91 | 21 | | 17 | | 20 | |
| Pre-Tx nodal status (Pre-N) | N0 | 125 | 19 | .0711 | 15 | .0070[a] | 19 | .0973 |
| | N1-N2 | 47 | 21 | | 12 | | 12 | |
| Post-Tx tumor status (Post-T) | T1-T2 | 86 | 7 | .0006[a] | 7 | .0040[a] | 8 | .0033[a] |
| | T3-T4 | 86 | 24 | | 20 | | 23 | |
| Post-Tx nodal status (Post-N) | N0 | 123 | 21 | .5998 | 16 | .1320 | 20 | .4634 |
| | N1-N2 | 49 | 10 | | 11 | | 11 | |
| Vascular invasion | Absent | 157 | 25 | .0184[a] | 21 | .0028[a] | 27 | .4470 |
| | Present | 15 | 6 | | 6 | | 4 | |
| Perineural invasion | Absent | 167 | 29 | .2559 | 25 | .0940 | 30 | .9083 |
| | Present | 5 | 2 | | 2 | | 1 | |
| Tumor regression grade | Grade 0-1 | 37 | 13 | .0038[a] | 10 | .0090[a] | 14 | .0006[a] |
| | Grade 2-3 | 118 | 17 | | 17 | | 16 | |
| | Grade 4 | 17 | 1 | | 0 | | 1 | |
| Down stage after CCRT | Non-Sig. | 150 | 29 | .1651 | 24 | .5961 | 30 | .0853 |
| | Sig. (>=2) | 22 | 2 | | 3 | | 1 | |
| FOLR1 expression | Low Exp. | 100 | 9 | .0002[a] | 10 | .0123[a] | 10 | .0011[a] |
| | High Exp. | 72 | 22 | | 17 | | 21 | |

Abbreviations: DSS, disease-specific survival; LRFS, local recurrence-free survival; MeFS, metastasis-free survival.
[a]Statistically significant.

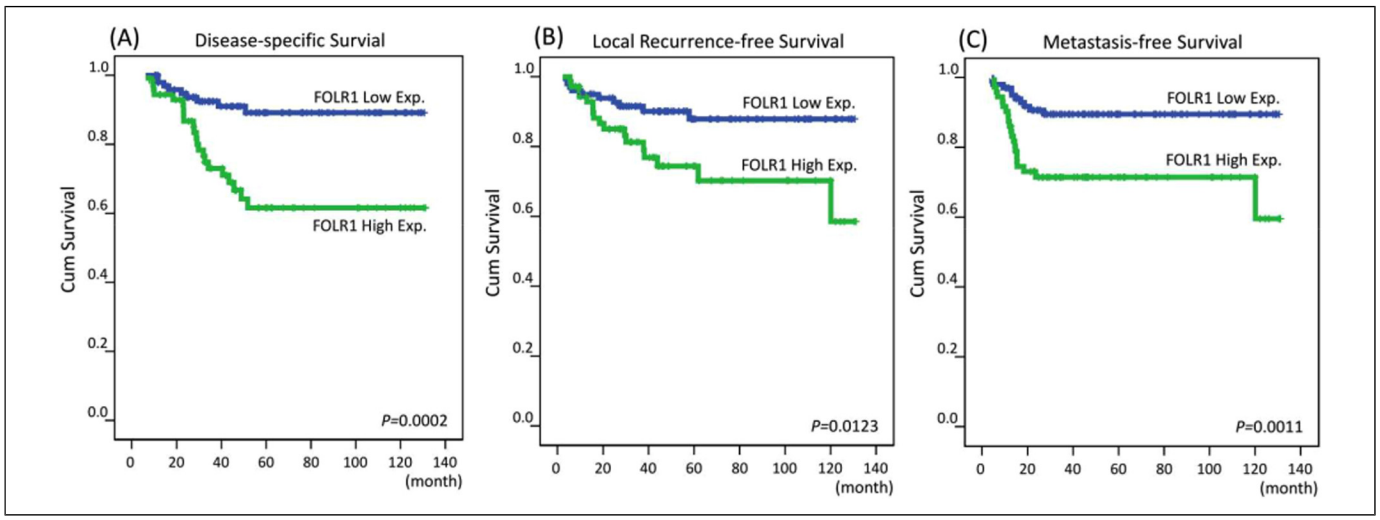


**Figure 2.** Kaplan–Meier analysis of the 5-year (A) disease-specific survival (DSS), (B) local recurrence-free survival (LRFS), and (C) metastasis-free survival (MeFS) for rectal cancer patients between high and low FOLR1 expression.

emphasize the relevance of evaluating FOLR1 expression in rectal cancer in order to provide additional independent prognostic information after preoperative CRT.

It is important to note that high FOLR1 expression was also significantly associated with poor tumor regression after preoperative CRT. It is plausible that FOLR1 expression is associated with enhanced resistance to radiation and chemotherapy. FOLR1, as a cell surface receptor, could regulate cancer cell growth by activating the ERK1/2 signaling pathway, which is essential for the inhibition of radiation-induced cell death.[28,29] In addition, high FOLR1 expression is an indicator of platinum-containing drug resistance in ovarian cancer.[30,31] Fu et al also demonstrated that FOLR1 expression promotes chemotherapy resistance.[32] Downregulation of FOLR1 significantly inhibited

**Table 3.** Multivariate Analysis.

| | DSS | | | LRFS | | | MeFS | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value | HR | 95% CI | *P*-value |
| Tumor regression grade | 1.972 | 0.966-4.016 | .062 | 2.557 | 1.176-5.555 | .018[a] | 1.600 | 1.022-4.115 | .008[a] |
| FOLR1 expression | 2.328 | 1.014-5.344 | .046[a] | 1.339 | 0.560-3.202 | .511 | 2.177 | 1.000-1.1286 | .050[a] |
| Vascular invasion | 1.969 | 0.750-5.167 | .169 | 3.015 | 1.111-8.185 | .030[a] | - | - | - |
| Post-Tx tumor status (Post-T) | 2.274 | 0.927-5.583 | .073 | 1.826 | 0.707-4.721 | .214 | 1.743 | 1.053-5.551 | .201 |
| Pre-Tx nodal status (Pre-N) | - | - | - | 1.257 | 0.549-2.874 | .589 | - | - | - |

Abbreviations: DSS, disease-specific survival; LRFS, local recurrence-free survival; MeFS, metastasis-free survival.
[a]Statistically significant.

cell viability, enhanced sensitivity to oxaliplatin, and promoted cell apoptosis. The mechanism of chemotherapy resistance through stabilizing MDM2 in cooperation with chaperone protein prohibitin 2 was noted in FOLR1 expressing gastric cancer cells.[33] Together, these studies imply that FOLR1 expression is involved in resistance to radiotherapy or chemotherapy, leading to poor response and worse survival.

A limitation of this study was that some early-stage rectal cancer patients received preoperative CRT due to the intention of organ preservation and were included into our analysis. However, this is why we could comprehensively observe the relationship among different FOLR1 expression in early or advanced stage groups and their tumor regression grade. Second, our cancer registry database lacked specific information such as gene mutation status of tumors (KRAS and BRAF), which have been shown to be significant predictors of disease progression and survival. Future studies including these factors are necessary to confirm our findings. Finally, investigating the detailed mechanism may provide essential clues for clinical rectal cancer treatment. Although this study did not provide the mechanism for the role of FOLR1 expression, our results indicated that FOLR1 expression could be a prognostic biomarker to predict the response to chemoradiation and survival of rectal cancer patients administered CCRT. We still continue to determine the mechanism of FOLR1 in our future work.

## Conclusion

In summary, our data provides evidence that FOLR1 expression might be a useful biomarker and promising target for the development of novel therapeutic agents for the management of rectal cancer, like FOLR1-specific monoclonal antibodies or folate-targeted drug treatments. Further studies investigating the mechanisms behind our observations are warranted.

### Acknowledgments

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### Availability of Data and Materials

Clinicopathological datasets are available from the corresponding author upon request.

### Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

### Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

### Ethics Approval and Consent to Participate

In this study, we used anonymous patient sample information from biobank as approved by Chi-Mei Medical Center Institutional Review Board (IRB: CMFHR10801001). As a rule, inform consent has been signed by every patient before their sample/information collected into biobank. And due to the disconnection between patient ID and their sample/information, we cannot identify the consent signed by that patient anymore.

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