Circular RNA mitochondrial translation optimization 1 correlates with less lymph node metastasis, longer disease-free survival, and higher chemotherapy sensitivity in gastric cancer

Cheng Chang¹,² | Anrui Zheng¹ | Pinfa Wang¹ | Xiaojun Teng¹

¹Department of Gastroenterology, Wuhan Hospital of Traditional Chinese medicine, Wuhan, China
²Department of Gastroenterology, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group, Huangshi, China

Correspondence
Anrui Zheng, Department of Gastroenterology, Huangshi Central Hospital, Affiliated Hospital of Hubei Polytechnic University, Edong Healthcare Group, Huangshi, 43 Wuhan Road, Xisaishan District, Huangshi 435000, China. Email: anqiaolianben@163.com

Abstract
Objective: Circular-mitochondrial translation optimization 1 (circ-MTO1) inhibits the progression of gastric cancer by regulating the growth, apoptosis, and invasion of tumor cells. However, its clinical potential as a biomarker for gastric cancer remains to be further evaluated. This study aimed to assess circ-MTO1 expression and its correlation with clinical features and prognosis in gastric cancer patients, as well as the effect of circ-MTO1 on the sensitivity to chemotherapy in gastric cancer cells.

Methods: Circ-MTO1 in tumor and adjacent tissues of 97 gastric cancer patients undergoing resection was examined by reverse transcription-quantitative polymerase chain reaction. HGC-27 and NCI-N87 cells transfected by circ-MOT1 overexpression plasmid (OE-circ-MOT1) and negative control (OE-NC) were treated with 0–6.4 μM oxaliplatin. Relative cell viability was detected using Cell Counting Kit-8.

Results: Circ-MTO1 was insufficiently expressed in gastric tumor tissue (median (interquartile range): 0.403 (0.288–0.518)) compared with adjacent tissue (median (interquartile range): 1.000 (0.715–1.524)) (p < 0.001). Besides, tumor circ-MTO1 was correlated with less lymph node metastasis (p = 0.014) and low TNM stage (p = 0.039), while was not correlated with demographic features or other clinical characteristics (all p > 0.05). Furthermore, tumor circ-MTO1 high expression was independently correlated with prolonged disease-free survival (DFS) (p = 0.013, adjusted hazard ratio (95% confidential interval): 0.314 (0.126–0.782)), but was not correlated with overall survival (p > 0.05). Lastly, in gastric cancer cells, OE-circ-MTO1 apparently decreased relative cell viabilities at oxaliplatin concentrations of 0.4, 0.8, 1.6, and 3.2 μM (all p < 0.05).

Conclusion: Circ-MTO1 correlates with less lymph node metastasis, prolonged DFS, and improved chemotherapy sensitivity in gastric cancer.

KEYWORDS
Circ-MTO1, DFS, Gastric cancer, Oxaliplatin sensitivity, TNM stage

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1 | INTRODUCTION

Gastric cancer, originating from gastric mucosa epithelium, is a worldwide health burden since it is one of the top leading causes of cancer-related mortality in humans, with over 950,000 newly diagnosed cases around the world annually (approximately 60% of new cases in east Asia such as China, Japan, and Korea). Over the past decades, progresses on the screening and diagnosis of gastric cancer bring an increased number of early-stage gastric cancer patients. These early-stage patients, together with intermediate patients with gastric cancer, are recommended to be treated with gastric resection. Besides, neoadjuvant or adjuvant treatments such as chemoradiotherapy are supposed to be applied to creating conditions for surgical resection. In addition, a huge number of studies are devoted to making contributions to the treatment for advanced gastric patients including chemotherapy, targeted therapy, and immunotherapy. In spite of these, patients still commonly experience dissatisfied survivals. Therefore, finding biomarkers for monitoring the progression and prognosis of gastric cancer is important for more accurate treatment and better prognosis.

Circular RNAs (Circular RNAs) are evolutionarily conserved molecules with covalently closed continuous loop structures that possess potential bioactivities in various cancers. For example, circular RNAs modulate the proliferation, progression, tumorigenesis, and chemosensitivity of pancreatic cancer. Besides, circ_0016760 regulates the miRNA_1287/G antigen 1 signaling axis in lung cancer. Circular RNA mitochondrial translation optimization 1 (Circ-MTO1) has been shown to be an anti-tumor gene in cancers including gastric, colorectal, hepatoacellular, and prostate cancers. As for gastric cancer, previous studies exhibit that circ-MTO1 suppresses its development and progression by regulating microRNA (miR)-3200-5p/phosphatidylethanolamine binding protein 1 (PEBP1) axis and miR-199a-3p/pro-apoptotic WT1 regulator (PAWR) axis. Clinically, insufficient circ-MTO1 expression is correlated with frequent lymph node metastasis, advanced TNM stage, and shortened overall survival (OS) in patients with colorectal cancer. Besides, circ-MTO1 high expression is correlated with decreased pathological T/N stage, as well as longer disease-free survival (DFS) and OS in prostate cancer patients. However, the clinical potential of circ-MTO1 as a biomarker for gastric cancer patients remains to be further evaluated.

This study aimed to assess the expression of circ-MTO1, its correlation with clinical characteristics, and prognosis in postoperative patients with gastric cancer, as well as the effect of circ-MTO1 on oxaliplatin sensitivity in gastric cancer cell lines.

2 | MATERIALS AND METHODS

2.1 | Patients

Ninety-seven patients with gastric cancer who underwent surgical resection in our hospital from January 2016 to December 2019 were retrospectively included in this study. All patients were screened out from the electronic medical record system (EMRS), according to the following criteria: (i) pathologically confirmed as gastric cancer; (ii) received surgical resection without neoadjuvant therapy; (iii) had freshly frozen specimens including tumor and adjacent tissue; (iv) had complete clinicopathological information and follow-up records; and (v) without other primary cancers or malignant diseases. Approval was obtained from the Ethical Inspection Committee, and the informed consent was signed by patients or their relatives.

2.2 | Data acquisition

Clinical features were collected from the EMRS, including demographics, smoke and drink status, complications, helicobacter pylori infection status, tumor location, pathological grade, tumor size, clinical T stage, clinical N stage, clinical TNM stage, and adjuvant treatment (chemotherapy with XELOX regimen or S-1 monotherapy, with or without radiotherapy). In addition, follow-up information of patients was also reviewed to abstract the main time points for estimation of progression-free survival (PFS) and overall survival (OS).

2.3 | Circ-MTO1 determination

The freshly frozen tumor and adjacent tissues of patients were acquired from the sample library of the hospital, which were available for reverse transcriptase quantitative polymerase chain reaction (RT-qPCR). Briefly, total RNA was extracted from tumor or adjacent tissues by TRIzol™ Reagent (Thermo Fisher Scientific), followed by the removal of linear RNA with RNase R (Epicentre), and reverse transcription into cDNA using PrimeScript™ RT reagent Kit (Perfect Real Time) (Takara). Subsequently, qPCR was conducted by TB Green™ Fast qPCR Mix (Takara) to quantify the circ-MTO1 expressions, which were calculated by 2^ΔΔCT method with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference. Primers for PCR were designed according to previous studies.

2.4 | Cell culture

The gastric cancer cell lines including HGC-27 and NCI-N87 were purchased from Cell Blank of Chinese Academy of Sciences (Shanghai). The cells were all cultured in the 90% RPMI1640 medium (Gibco) with 10% Fetal Bovine Serum (Gibco) and were maintained in at 37°C in a humidified atmosphere of 5% CO2.

2.5 | Plasmid transfection, treatment, and cell viability determination

The pCD5-ciR vector (GENESEED) was applied to construct the circ-MTO1 overexpression (OE) plasmid (OE-circ-MTO1) and negative control (OE-NC) plasmid; then, the constructed plasmids were
transfected into the HGC-27 and NCI-N87 cells using Lipofectamine™
3000 Transfection Reagent (Thermo Fisher Scientific). The result-
ing cells were termed as OE-circ-MTO1 and OE-NC, respectively.
Meanwhile, the cells without transfection were used as blank controls.
After transfection, all cells including OE-circ-MTO1, OE-NC, and blank
cells were treated with oxaliplatin (Sigma-Aldrich) at the following
concentrations: 0, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 μM. After incubation for
48 h, the relative cell viability in each cell group was detected using Cell
Counting Kit-8 (Beyotime) as per the instruction of the kit.

2.6 | Statistical analysis

Data were analyzed by SPSS 26.0 (IBM Corp.), and figures were plot-
ted by GraphPad Prism 7.01 (GraphPad Software Inc.). Comparison
analysis was determined by t test, Wilcoxon signed rank test, Wilcoxon
rank sum test, or Kruskal-Wallis H rank sum test. Correlation analysis
was checked by Spearman rank correlation test. PFS and OS were dis-
played using Kaplan-Meier method and determined by log-rank test.
Multivariable Cox’s proportional hazard regression model analysis was
applied to estimate the prognostic role of variables. p Value <0.05 was
used as threshold to identify the statistical significance.

3 | RESULTS

3.1 | Clinical characteristics

The mean age of gastric cancer patients was 60.5 ± 11.5 years, among
which female and male proportions were 45.4% and 54.6%, respec-
tively. Regarding pathological grade, the number of gastric cancer pa-
tients with grade 1, grade 2, and grade 3 was 11 (11.3%), 72 (74.2%),
and 14 (14.4%), respectively. With respect to clinical TNM stage, the
number of gastric cancer patients with TNM stage I, II, and III was 20
(20.6%), 35 (36.1%), and 42 (43.3%), respectively. More detailed demo-
graphic and clinical characteristics were presented in Table 1.

3.2 | Circ-MTO1 expression in tumor tissue and
adjacent tissue

Circ-MTO1 expression was obviously lower in tumor tissue than in ad-
jacent tissue of gastric cancer patients (p < 0.001). The median (IQR)
value of circ-MTO1 expression in tumor tissue was 0.403 (0.288–
0.518), while that in adjacent tissue was 1.000 (0.715–1.524) (Figure 1).

3.3 | Correlation of tumor circ-MTO1 with
clinical features

Tumor circ-MTO1 expression was negatively correlated with clinical
N stage (p = 0.014) and clinical TNM stage (p = 0.039) (Figure 2E-2F).
However, no correlation of tumor circ-MTO1 with tumor location,
pathological grade, tumor size, or clinical T stage was observed
(all p > 0.05) (Figure 2A-2D). Besides, no correlation of tumor circ-
MTO1 with age, gender, or other clinical characteristics apart from
tumor features was observed in gastric cancer patients (all p > 0.05;
Table 2).

| TABLE 1 Clinical characteristics |
|---------------------------------|
| Items                          | Gastric carcinoma patients (N = 97) |
| Age (years), mean±SD           | 60.5±11.5                             |
| Gender, No. (%)                |                                        |
| Female                         | 44 (45.4)                             |
| Male                           | 53 (54.6)                             |
| Current smoke, No. (%)         | 27 (27.8)                             |
| Current drink, No. (%)         | 37 (38.1)                             |
| Complications                  |                                        |
| Hypertension, No. (%)          | 25 (25.8)                             |
| Hyperlipidemia, No. (%)        | 26 (26.8)                             |
| DM, No. (%)                    | 14 (14.4)                             |
| Helicobacter pylori infection, No. (%) | 64 (66.0)                          |
| Negative                       | 33 (34.0)                             |
| Positive                       |                                        |
| Tumor location, No. (%)        | 26 (26.8)                             |
| Cardia                         | 59 (60.8)                             |
| Gastric body                   | 12 (12.4)                             |
| Gastric antrum                 |                                        |
| Pathological grade, No. (%)    |                                        |
| Grade 1                        | 11 (11.3)                             |
| Grade 2                        | 72 (74.2)                             |
| Grade 3                        | 14 (14.4)                             |
| Tumor size (cm), median (IQR)  | 3.0 (2.5–4.0)                         |
| Clinical T stage, No. (%)      |                                        |
| T1                             | 7 (7.2)                               |
| T2                             | 18 (18.6)                             |
| T3                             | 71 (73.2)                             |
| T4                             | 1 (1.0)                               |
| Clinical N stage, No. (%)      |                                        |
| N0                             | 50 (51.5)                             |
| N1                             | 25 (25.8)                             |
| N2                             | 18 (18.6)                             |
| N3                             | 4 (4.1)                               |
| Clinical TNM stage, No. (%)    |                                        |
| Stage I                        | 20 (20.6)                             |
| Stage II                       | 35 (36.1)                             |
| Stage III                      | 42 (43.3)                             |
| Adjuvant treatment             |                                        |
| Chemotherapy, No. (%)          | 74 (76.3)                             |
| Radiotherapy, No. (%)          | 9 (9.3)                               |

Abbreviations: DM, diabetes mellitus; IQR, interquartile range; SD,
standard deviation.
3.4 | Correlation of tumor circ-MTO1 with accumulating DFS and OS

According to the median of circ-MTO1 expression in gastric tumor tissues (Figure 1), tumor circ-MTO1 expression was divided by high (over 0.403) and low (below 0.403) expressions. In gastric cancer patients, tumor circ-MTO1 high expression was associated with better accumulating DFS ($p = 0.027$) (Figure 3A). In terms of accumulating OS, no correlation of tumor circ-MTO1 with OS was found ($p > 0.05$) (Figure 3B).

**FIGURE 1** Circ-MTO1 expression in gastric cancer patients. Comparison of circ-MTO1 expression in adjacent tissue and tumor tissue of gastric cancer patients. Circ-MTO1, circular RNA mitochondrial translation optimization 1

3.5 | Analysis of independent factors relating to DFS and OS

From multivariate Cox's proportional hazard regression model analyses for DFS (Figure 4A), high expression of tumor circ-MTO1 ($p = 0.013$, adjusted hazard ratio (HR) (95% confidential interval (CI)): 0.314 (0.126–0.782)), and adjuvant chemotherapy ($p = 0.004$, adjusted HR (95%CI): 0.081 (0.015–0.453)) were independent factors for better DFS, while male ($p = 0.018$, adjusted HR (95%CI): 3.461 (1.236–9.691)) and higher clinical TNM stage ($p = 0.002$, adjusted HR (95%CI): 4.550 (1.744–11.869)) were independent factors for worse DFS. As to OS, adjuvant chemotherapy ($p < 0.001$, adjusted HR (95%CI): 0.037 (0.006–0.236)) was an independent factor for better OS, while higher clinical TNM stage ($p = 0.006$, adjusted HR (95%CI): 5.086 (1.592–16.248)) was an independent factor for worse OS (Figure 4B).

**FIGURE 2** Association between tumor circ-MTO1 and clinical characteristics. The association of tumor circ-MTO1 expression with tumor location (A), pathological grade (B), clinical T stage (D), clinical N stage (E), and clinical TNM stage (F) in gastric cancer patients. Circ-MTO1, circular RNA mitochondrial translation optimization 1; T, tumor; N, lymph node; TNM, tumor-node-metastasis

3.6 | The effect of circ-MTO1 on oxaliplatin sensitivity in gastric cancer cell lines

Since circ-MTO1 independently correlated with PFS, we further explored the effect of circ-MTO1 on oxaliplatin sensitivity in gastric cancer cell lines; then, plasmid transfection was conducted in HGC-27 and NCI-N87 cells followed by the oxaliplatin treatment. In terms of HGC-27 cells, the relative cell viability of OE-circ-MTO1 group was lower than that of OE-NC group at oxaliplatin concentrations of 0.8 μM, 1.6 μM, and 3.2 μM (all $p < 0.05$)
(Figure 5A). Besides, the same trend was observed in NCI-N87 cells at oxaliplatin concentrations of 0.4 μM, 0.8 μM, and 1.6 μM (all p < 0.05) (Figure 5B).

**TABLE 2** Correlation of circ-MTO1 with characteristics apart from tumor features

| Items                         | Circ-MTO1 expression | P value |
|-------------------------------|----------------------|---------|
| Age                           |                      |         |
| <60 years                     | 0.384 (0.221–0.480)  | 0.293   |
| ≥60 years                     | 0.429 (0.297–0.574)  |         |
| Gender                        |                      |         |
| Female                        | 0.407 (0.295–0.532)  | 0.865   |
| Male                          | 0.395 (0.238–0.502)  |         |
| Current smoke                 |                      |         |
| No                            | 0.383 (0.292–0.497)  | 0.541   |
| Yes                           | 0.448 (0.261–0.589)  |         |
| Current drink                 |                      |         |
| No                            | 0.375 (0.256–0.493)  | 0.245   |
| Yes                           | 0.406 (0.318–0.544)  |         |
| Hypertension                  |                      |         |
| No                            | 0.407 (0.297–0.502)  | 0.520   |
| Yes                           | 0.384 (0.185–0.527)  |         |
| Hyperlipidemia                |                      |         |
| No                            | 0.406 (0.254–0.509)  | 0.754   |
| Yes                           | 0.387 (0.308–0.527)  |         |
| DM                            |                      |         |
| No                            | 0.398 (0.295–0.509)  | 0.935   |
| Yes                           | 0.424 (0.180–0.568)  |         |
| Helicobacter pylori infection |                      |         |
| Negative                      | 0.405 (0.297–0.522)  | 0.855   |
| Positive                      | 0.384 (0.233–0.514)  |         |
| Adjuvant chemotherapy         |                      |         |
| No                            | 0.410 (0.318–0.560)  | 0.338   |
| Yes                           | 0.390 (0.223–0.513)  |         |
| Adjuvant radiotherapy         |                      |         |
| No                            | 0.405 (0.303–0.528)  | 0.113   |
| Yes                           | 0.226 (0.187–0.464)  |         |

Abbreviations: Circ-MTO1, circular RNA mitochondrial tRNA translation optimization 1; DM, diabetes mellitus; IQR, interquartile range.

**FIGURE 3** Association between tumor circ-MTO1 and prognosis. The association of tumor circ-MTO1 expression with accumulating DFS (A) and OS (B) in gastric cancer patients. Circ-MTO1, circular RNA mitochondrial translation optimization 1; DFS, disease-free survival; OS, overall survival

**DISCUSSION**

The main findings were listed as follows: 1) Circ-MTO1 was down-regulated in tumor tissue compared with adjacent tissue of gastric cancer patients. 2) Tumor circ-MTO1 was correlated with less lymph node metastasis and lower clinical TNM stage in gastric cancer patients. 3) Circ-MTO1 high expression was independently correlated with prolonged DFS, but was not correlated with OS in gastric cancer patients. 4) Circ-MTO1 enhanced the sensitivity to oxaliplatin in gastric cancer cell lines.

CircRNAs, widely presenting in transcriptomes, play vital roles in the regulation of various biological activities. Circ-MTO1 is one of the commonly studied circRNAs, which acts as a cancer-suppressor gene involved in a variety of cancers. Circ-MTO1 suppresses the growth and invasion of gastric, colorectal, and prostate cancer cells. Moreover, it is insufficiently expressed in hepatocellular tumor tissues. In this study, circ-MTO1 was down-regulated in gastric tumor tissue compared with adjacent tissue, which could be explained by that overexpression of circ-MTO1 inhibited the proliferation of gastric cancer cells via multiple mechanisms such as regulating miR-199a-3p/PAWR axis, miR-3200-5p/PEBP1 axis, miR-9/p21 axis, and Wnt/β-catenin signaling pathway. The viability of gastric cancer cells was decreased, leading to its down-regulation in gastric tumor tissue compared with adjacent tissues.

Circ-MTO1 is clinically correlated with tumor characteristics. For example, a study shows that insufficient circ-MTO1 expression is associated with advanced lymph node metastasis and TNM stage in colorectal cancer patients. Another study exhibits that circ-MTO1 is associated with decreased pathological T stage and N stage in patients with prostate cancer. Partly in line with these reports, our study presented that tumor circ-MTO1 was correlated with less severe lymph node metastasis and clinical TNM stage in gastric cancer patients. The possible reasons might be that (1) circ-MTO1 suppressed the invasion and migration of gastric cancer cells by up-regulating PAWR via sponging miR-199a-3p, leading to the less severe lymph node metastasis in gastric cancer patients; (2) circ-MTO1 also attenuated the proliferation and invasion, as well as promoted the apoptosis of gastric cancer cells via regulating the tumor necrosis factor receptor-associated factor 4/Eg5 axis, resulting in lower TNM stage in gastric cancer patients.
As for prognosis of cancer patients, circ-MTO1 is associated with their better survivals.19-21 For instance, circ-MTO1 expression is associated with longer OS in patients with colorectal cancer and hepatocellular carcinoma.19,21 Furthermore, circ-MTO1 is independently correlated with favorable DFS and OS in prostate cancer patients.20 The current study also displayed that tumor circ-MTO1 was correlated with prolonged DFS and was an independent factor affecting DFS in gastric cancer patients. The

### Multivariate Cox's proportional hazard regression model analysis for DFS

| Variables                        | P value | Adjusted HR (95%CI)       |
|----------------------------------|---------|---------------------------|
| Circ-MTO1 high vs. low           | 0.013   | 0.314 (0.126-0.782)       |
| Age ≥60 years vs. <60 years      | 0.739   | 1.162 (0.480-2.814)       |
| Gender male vs. female           | 0.018   | 3.461 (1.236-9.691)       |
| Current smoke                    | 0.816   | 0.882 (0.305-2.547)       |
| Current drink                    | 0.829   | 1.104 (0.451-2.702)       |
| Hypertension                     | 0.937   | 1.036 (0.433-2.478)       |
| Hyperlipidemia                   | 0.137   | 0.476 (0.179-1.265)       |
| Diabetes mellitus                | 0.483   | 0.624 (0.167-2.327)       |
| Helicobacter pylori infection    | 0.280   | 0.568 (0.203-1.587)       |
| Higher pathological grade        | 0.129   | 2.165 (0.799-5.866)       |
| Tumor size > 3 cm vs. ≤ 3 cm     | 0.784   | 0.872 (0.329-2.312)       |
| Higher clinical TNM stage        | 0.002   | 4.550 (1.744-11.869)      |
| Adjuvant chemotherapy            | 0.004   | 0.081 (0.015-0.453)       |
| Adjuvant radiotherapy            | 0.341   | 0.523 (0.137-1.989)       |

### Multivariate Cox's proportional hazard regression model analysis for OS

| Variables                        | P value | Adjusted HR (95%CI)       |
|----------------------------------|---------|---------------------------|
| Circ-MTO1 high vs. low           | 0.314   | 0.527 (0.152-1.834)       |
| Age ≥60 years vs. <60 years      | 0.126   | 0.400 (0.124-1.295)       |
| Gender male vs. female           | 0.839   | 1.151 (0.297-4.470)       |
| Current smoke                    | 0.407   | 0.463 (0.075-2.856)       |
| Current drink                    | 0.235   | 2.371 (0.571-9.849)       |
| Hypertension                     | 0.336   | 0.582 (0.194-1.752)       |
| Hyperlipidemia                   | 0.156   | 0.344 (0.079-1.504)       |
| Diabetes mellitus                | 0.129   | 0.175 (0.018-1.658)       |
| Helicobacter pylori infection    | 0.462   | 1.708 (0.410-7.107)       |
| Higher pathological grade        | 0.086   | 3.705 (0.833-16.485)      |
| Tumor size > 3 cm vs. ≤ 3 cm     | 0.055   | 4.121 (0.970-17.514)      |
| Higher clinical TNM stage        | 0.006   | 5.086 (1.592-16.248)      |
| Adjuvant chemotherapy            | <0.001  | 0.037 (0.006-0.236)       |
| Adjuvant radiotherapy            | 0.697   | 0.699 (0.115-4.238)       |

**FIGURE 4** Multivariate regression analysis for DFS and OS. Factors affecting DFS (A) and OS (B) analyzed by multivariate Cox's proportional hazard regression model. DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidential interval; Circ-MTO1, circular RNA mitochondrial translation optimization 1; TNM, tumor-node-metastasis
explanations might be as follows: (1) Tumor circ-MTO1 was associated with less severe tumor metastasis and TNM stage, thus prolonging DFS in gastric cancer patients. (2) Circ-MTO1 enhanced the sensitivity of chemotherapy which could bring a favorable treatment efficacy, leading to a subsequently longer DFS. This could be supported by the result that circ-MTO1 increased the sensitivity of gastric cancer cell lines to oxaliplatin in this study. Furthermore, no correlation of circ-MTO1 with OS in gastric cancer patients was observed in this study. This was attributed by that the number of death events was too small, leading to the low statistical power. Further study with long follow-up duration was supposed to be conducted in the future.

It could not be ignored that there were a few limitations in this study. Firstly, the sample size was relatively small, which might lead to poor statistical power. Further study with a larger sample size was supposed to be performed. Secondly, specific mechanism about how circ-MTO1 regulated gastric cancer progression was still unclear and was required to be extensively explored in the future. Lastly, since our study was a cohort study, selection bias and confounding factors such as diversified resection treatments and tumor status might exist.

Conclusively, circ-MTO1 correlates with less lymph node metastasis, prolonged DFS, and increased chemotherapy sensitivity in gastric cancer. This study provides evidences for selecting optimized treatment strategies and improving prognosis for gastric cancer patients.

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CONFLICT OF INTEREST
No potential conflict of interest was reported by the authors.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available in this article.

FIGURE 5  The influence of circ-MTO1 on the sensitivity to oxaliplatin. The relative cell viability of OE-circ-MTO1, OE-NC, and blank under oxaliplatin treatment in HGC-27 (A) and NCI-N87 (B) cell lines. Circ-MTO1, circular RNA mitochondrial translation optimization 1

ORCID
Anrui Zheng https://orcid.org/0000-0002-9438-2171

REFERENCES
1. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res*. 1992;52(24):6735–6740.
2. Machlowska J, Baj J, Sitarz M, et al. Gastric cancer: epidemiology, risk factors, classification, genomic characteristics and treatment strategies. *Int J Mol Sci*. 2020;21(11):4012.
3. Lyons K, Le LC, Pham YT, et al. Gastric cancer: epidemiology, biology, and prevention: a mini review. *Eur J Cancer Prev*. 2019;28(5):397-412.
4. Hsu A, Raufi AG. Advances in systemic therapy for gastric cancer. *Gastrointest Endosc Clin N Am*. 2021;31(3):607–623.
5. Zhu J, Wang SM, Chen R, et al. Progress on screening for gastric cancer. *Zhonghua Zhong Liu Za Zhi*. 2020;42(7):603–608.
6. Lee A, Chung H. Endoscopic resection of undifferentiated-type early gastric cancer. *J Gastrointest Cancer*. 2020;204(3):345-354.
7. Aversa JG, Diggs LP, Hagerty BL, et al. Multivisceral resection for locally advanced gastric cancer. *J Gastrointest Surg*. 2021;25(3):609-622.
8. Marsh AM, Buicko JL. Gastric Resection. Treasure Island, FL: StatPearls; 2021.
9. Wang JB, Li P, Liu XL, et al. An immune checkpoint score system for prognostic evaluation and adjuvant chemotherapy selection in gastric cancer. *Nat Commun*. 2020;11(1):6352.
10. Tanaka O, Sugiyama A, Omatsumi T, et al. Hemostatic radiotherapy for inoperable gastric cancer: a pilot study. *Br J Radiol*. 2020;93(1111):20190958.
11. Shitara K, Van Cutsem E, Bang YJ, et al. Efficacy and Safety of Pembrolizumab or Pembrolizumab Plus Chemotherapy vs Chemotherapy Alone for Patients With First-line, Advanced Gastric Cancer: The KEYNOTE-062 Phase 3 Randomized Clinical Trial. *JAMA Oncol*. 2020;6(10):1571-1580.
12. Iwasa S, Kudo T, Takahari D, et al. Practical guidance for the evaluation of disease progression and the decision to change treatment in patients with advanced gastric cancer receiving chemotherapy. *Int J Clin Oncol*. 2020;25(7):1223-1232.
13. Kawazoe A, Shitara K, Boku N, et al. Current status of immunotherapy for advanced gastric cancer. *Jpn J Clin Oncol*. 2021;51(1):20-27.
14. Patel TH, Cecchini M. Targeted Therapies in Advanced Gastric Cancer. *Curr Treat Options Oncol*. 2020;21(9):70.
15. Hess DT, Beesley H, Carter CO, et al. Surgical management of obstructing clot at the jejunoojejunostomy after gastric bypass: a single center experience and literature review. *Surg Obes Relat Dis*. 2021;17(4):765-770.

16. Rong Z, Xu J, Shi S, et al. Circular RNA in pancreatic cancer: a novel avenue for the roles of diagnosis and treatment. *Theranostics*. 2021;11(6):2755-2769.

17. Drula R, Braicu C, Harangus A, et al. Critical function of circular RNAs in lung cancer. *Wiley Interdiscip Rev RNA*. 2020;11(5):e1592.

18. Tucker D, Zheng W, Zhang DH, et al. Circular RNA and its potential as prostate cancer biomarkers. *World J Clin Oncol*. 2020;11(8):563-572.

19. Ge Z, Li LF, Wang CY, et al. CircMTO1 inhibits cell proliferation and invasion by regulating Wnt/beta-catenin signaling pathway in colorectal cancer. *Eur Rev Med Pharmacol Sci*. 2018;22(23):8203-8209.

20. Hu Y, Guo B. Circ-MTO1 correlates with favorable prognosis and inhibits cell proliferation, invasion as well as miR-17-5p expression in prostate cancer. *J Clin Lab Anal*. 2020;34(3):e23086.

21. Han D, Li J, Wang H, et al. Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. *Hepatology*. 2017;66(4):1151-1164.

22. Song R, Li Y, Hao W, et al. Circular RNA MTO1 inhibits gastric cancer progression by elevating PAWR via sponging miR-199a-3p. *Cell Cycle*. 2020;19(22):3127-3139.

23. Hu K, Qin X, Shao Y, et al. Circular RNA MTO1 suppresses tumorigenesis of gastric carcinoma by sponging miR-3200-5p and targeting PEBP1. *Mol Cell Probes*. 2020;52:101562.

24. Liu DY, Li Z, Zhang K, et al. Circular RNA CircMTO1 suppressed proliferation and metastasis of osteosarcoma through miR-630/KLF6 axis. *Eur Rev Med Pharmacol Sci*. 2021;25(1):86-93.

25. Zhang H, Shen Y, Li Z, et al. The biogenesis and biological functions of circular RNAs and their molecular diagnostic values in cancers. *J Clin Lab Anal*. 2020;34(1):e23049.

26. Liu Y, Dong Y, Zhao L, et al. Circular RNAMTO1 suppresses breast cancer cell viability and reverses monastrol resistance through regulating the TRAF4/Eg5 axis. *Int J Oncol*. 2018;53(4):1752-1762.