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Exploring the correlation between Tom1L1 and the efficacy of neoadjuvant chemotherapy for locally progressive mid-low rectal cancer

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Abstract

Objective To investigate the specificity of Target of Myb1-Like1 (Tom1L1) expression in colorectal adenocarcinoma tissues and analyze the predictive value of Tom1L1 in the efficacy of neoadjuvant chemotherapy for patients with rectal adenocarcinoma.

Methods The cancerous tissues and paracancerous normal tissues of 102 patients diagnosed with colorectal adenocarcinoma without treatment were selected; quantitative polymerase chain reaction (qPCR), Western blot and immunohistochemistry (IHC) were adopted to validate the expression level of Tom1L1 in the two groups. Furthermore, 34 patients with locally progressive mid-low rectal adenocarcinoma, who were treated with neoadjuvant Xelox chemotherapy prior to the operation, IHC was adopted to detect the expression of Tom1L1 protein in patients before and after neoadjuvant chemotherapy and to analyze the relationship between the expression level of Tom1L1 and the sensitivity of neoadjuvant therapy.

Results The results of qPCR, Western blot and IHC showed that the expression of Tom1L1 in colorectal adenocarcinoma tissues was significantly higher than that in paracancerous normal tissues, with a statistically significant difference ($P < 0.01$); Neoadjuvant chemotherapy was significantly more effective in patients with low expression of Tom1L1 protein than in those with high expression of Tom1L1 protein, with a statistically significant difference ($P < 0.05$).

Conclusions Tom1L1 is highly expressed in colorectal adenocarcinoma tissues; neoadjuvant Xelox chemotherapy can have an impact on Tom1L1 expression in progressive rectal cancer; patients with locally progressive mid-low rectal adenocarcinoma who have low Tom1L1 expression are more sensitive to neoadjuvant chemotherapy.

Keywords Target of Myb1-Like1, Neoadjuvant chemotherapy, Mid-low rectal adenocarcinoma, Biomarker

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Background

Neoplasms remain the main killer worldwide [1–3]. According to the 2022 Cancer Report released by the International Agency for Research on Cancer, colorectal cancer ranked third in terms of morbidity of common cancers in the world, with 1.9 million new cases; it ranked second in terms of mortality, contributing to 904,000 deaths [4–8]. According to the statistics of the National Cancer Center of China in 2020, colorectal cancer leapt to second place in terms of morbidity, with approximately 555,000 new cases; it ranked second in terms of mortality, contributing to approximately 280,000 deaths, thereby posing an enormous threat to the life and health of human beings [8]. China has become the country with the newest cases of colorectal cancer in the world, and there is an increasing trend of colorectal cancer seen in younger age groups. The proportion of young people suffering from rectal cancer is relatively high, accounting for approximately 10–15% [9]. The research conducted by Nielsen et al. revealed that approximately 50% of colorectal cancer (CRC) patients will eventually develop metastatic disease [10]. Two autopsy studies reported the incidence of bone metastases (BM) in colorectal cancer (CRC) patients, with findings ranging from 1.7% in Hugen et al.'s research [11] to 23% in the study conducted by Katoh et al. [12]. The mechanism of bone metastasis in colorectal cancer may be linked to Batson's venous plexus, a valveless network that connects the pelvic and thoracic veins to the vertebral system, allowing cancer cells to spread directly to the bones, particularly the spine [13, 14]. The specific location colon or rectum might also affect the risk of BM [15].

Colorectal cancer in locally progressive stages (stages II and III) has high recurrence and metastasis rates. Large amounts of research data have confirmed the high clinical value of neoadjuvant therapy for such cancer. A study by Santini et al., which included 269 colorectal cancer patients with bone metastases, found that poorly differentiated tumors were associated with the shortest time to bone metastases (6 months), while well-differentiated tumors had the longest time (33 months) [16].Neoadjuvant therapy [17–19] for colorectal cancer helps to reduce tumor sizes, treat clinical micro-metastasis, lower pathological stages, and local recurrence rates; increase the chance of surgery; improve the R_0 resection rate; and preserve the anal sphincter [20]. In recent years, the prediction and evaluation of the efficacy after neoadjuvant therapy for locally progressive colorectal cancer has attracted much attention. Many studies have evaluated the accuracy of clinical symptoms, serum biomarkers, transrectal ultrasound, computed tomography, magnetic resonance imaging (MRI), and other imaging histological characteristics in evaluating the efficacy of neoadjuvant therapy in patients. However, these methods have relatively low sensitivity and specificity and cannot really achieve accurate prediction [21–23].

Tom1L1 (Target of Myb1-Like1) protein is one of the members of the Tom family. Tom1L1 is the most widely known and recently studied protein in the Tom family, which consists of three members: Tom1, Tom1L1, and Tom1L2. All the members in the Tom family contain an N-terminal VHS domain, a middle GAT domain, and a C-terminal VHS domain [24]. Studies show that the GAT domain of Tom1L1 is able to bind ubiquitin, suggesting that Tom1L1 may be involved in ubiquitination and sorted into multivesicular bodies (MVBs) [25]. It has been demonstrated that Tom1L1 can interact with HRS and TSG101/VPS23, thereby sorting EGFR into MVBs via endocytosis, which are then delivered to lysosomes for degradation [25]. In addition, Tom1L1 is essential for the aggregation of Fyn into the clathrin structure on the plasma membrane, and Tom1L1 and Fyn modulate the production of PIP3 by PI3K on the plasma membrane to control EGFR signal transduction, thereby modulating Akt activation. Apart from this, Tom1L1 also facilitates the endocytosis of EGFR [26].

In this study, we compared the differential genes of colorectal cancer patients before and after chemotherapy, and the resulting differential genes were analyzed for survival based on clinical data, and genes with differential expression in colorectal cancerous tissues and paracancerous normal tissues were screened as the study objects, from which Tom1L1 genes were selected for validation analysis.

Moreover, the specificity of Tom1L1 expression in colorectal cancerous tissues and paracancerous normal tissues and its correlation with clinicopathological characteristics were validated at the gene and protein levels, respectively, via quantitative polymerase chain reaction (qPCR), Western blot, and immunohistochemistry staining (IHC). Furthermore, IHC was applied to evaluate the expression of Tom1L1 before and after neoadjuvant chemotherapy with reference to endoscopy, imaging, and pathology so as to analyze its sensitivity and predictive value for neoadjuvant chemotherapy.

Data and methods

Bioinformatics analysis

The GSE15781 dataset was selected from the GEO database for comparing the differential genes of patients before and after chemotherapy, and the differential genes obtained were analyzed for survival based on the clinical data of rectal cancer patients in the TCGA database, and the differential genes with prognostic significance were screened. And based on these genes, a Signature model was established, which is a RiskScore

formula containing multiple genes, with weights for each gene, and a negative number means that the gene is a protective gene, and a positive number means that the gene is a risk gene. The model is specified as Riskscore = (0.103)*PHLDA3 + (-0.8249)*ARHGAP8 + (-0.6402)*TOM1L1 + (-0.15)*RAD51 + (-0.1544)*RAB-11FIP4 + (-0.1961)*OVOL2 + (0.0685)*G0S2. Nomogram modeling was performed for genes within the model. Column line plots provide a graphical representation of these factors, allowing the prognostic risk of individual patients to be calculated from the points associated with each risk factor, giving the above methodology, TOM1L1 was used as the focus of this project. The results are shown in Fig. 2.

UALCAN is an interactive portal for in-depth analyses of TCGA gene expression data on 37 tumors. In this study, it was used to compare the differential expression of Tom1L1 in colorectal cancer and the association of Tom1L1 with selected clinical phenotypes of colorectal cancer. R language (version 4.2.3) was used for correlation analysis of data.

Tom1L1 enrichment analysis: the top 300 co-expressed genes with the highest correlation with Tom1L1 ($p < 0.05$) were screened using the "corrplot" package of R language; Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses were performed based on the "clusterprofiler" package.

Patients' data

The flowchart of the experimental research is presented in Fig. 1. A total of 102 colorectal cancer patients without any preoperative treatment were enrolled from August 1 2021 to December 13 2023 at the First Affiliated Hospital of Hebei North University. Tissues in the center of the tumor and paracancerous normal intestinal tissues greater than 10 cm away from the tumor were selected from the patients' surgically resected specimens and defined as the "cancer group" (experimental group) and the "paracancerous normal group" (control group), respectively; 34 progressive rectal cancer patients with complete clinical information, who underwent further surgical treatment after preoperative neoadjuvant chemotherapy, were selected as the study subjects; preoperative biopsy wax blocks and postoperative pathological tissue wax blocks were collected. The patients were divided into the pretreatment group and the post-treatment group, with one group serving as the control group of the other.

Inclusion criteria: (1) definite diagnosis by preoperative auxiliary examinations such as electronic enteroscopy, pelvic MRI, and thoracic + abdominal + pelvic enhanced CT; (2) diagnosis of colon cancer or rectal cancer with a pathological type of adenocarcinoma by biopsy pathology or pathology consultation in our hospital; (3) no treatment prior to the operation; (4) completion of open or laparoscopic Total Mesorectal Excision (TME) for rectal cancer, with smooth surgical procedures; (5) complete pathological data such as biopsy pathology slides and wax blocks prior to neoadjuvant chemotherapy; (6) tumor staging and tumor regression grading conducted in accordance with the eighth edition of the American Joint Committee on Cancer (AJCC) criteria; (7) TME performed 6–8 weeks after the completion of neoadjuvant chemotherapy.

Exclusion criteria: (1) history of combined multiple primary tumors or other malignant tumors; (2) previous antitumor therapy; (3) patients refusing surgery or changing the surgical plan for various reasons; (4) patients refusing to participate in the study; (5) patients failing to complete neoadjuvant chemotherapy, changing

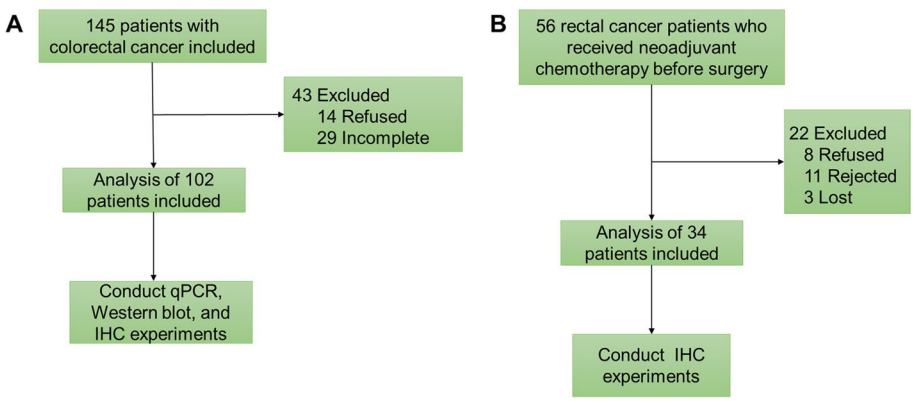

Fig. 1 The flow chart of this study. **A** 145 colorectal cancer patients were enrolled, and ultimately 102 were included for analysis and experimentation; **B** 56 rectal cancer patients who underwent preoperative neoadjuvant therapy were enrolled, and 34 patients were ultimately included in the IHC experiment

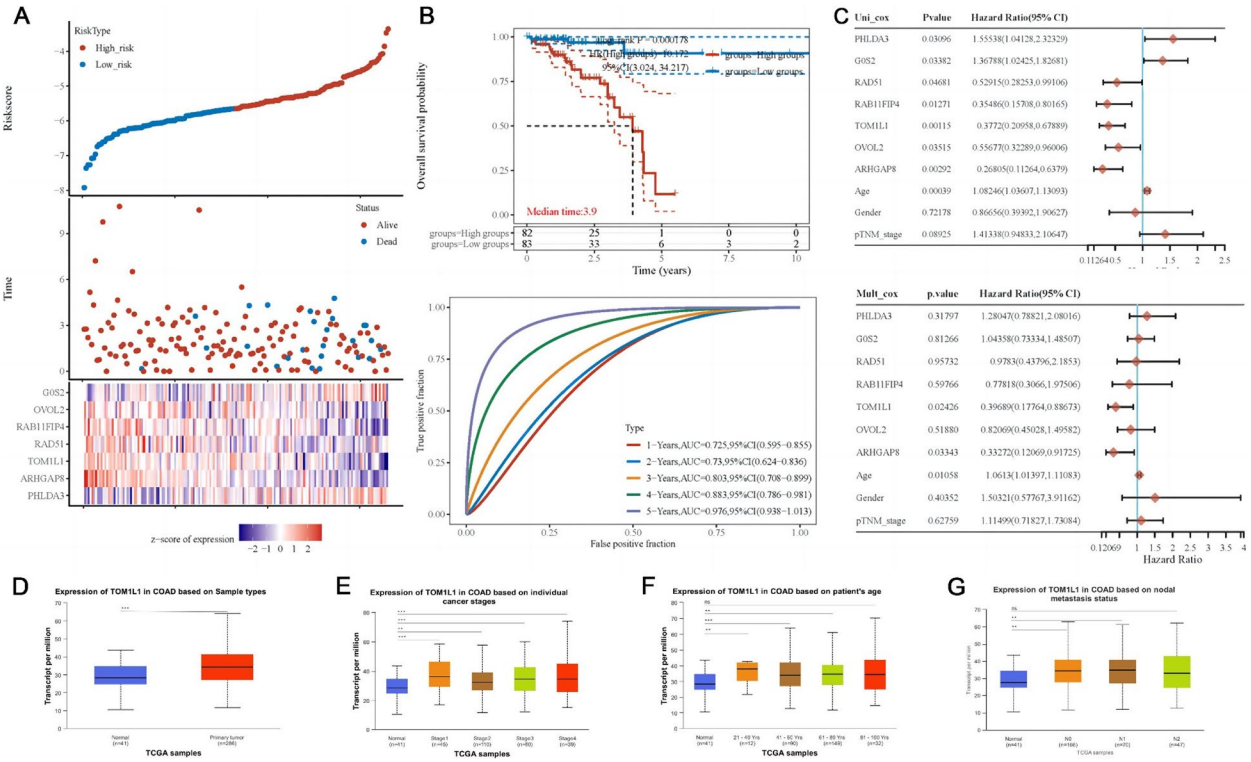

Fig. 2 Statistical analysis of bioinformatics analysis. **A** and **B** show the risk group scatterplot, the survival time,survival state scatterplots and risk model predictive ROC Curves; **C** The third figure shows the Single and multifactorial cox analyses of p-value, risk coefficient HR, and confidence intervals for gene expression and clinical characteristics; **D** The fourth figure shows the differences in Tom1L1 expression between normal tissues and tumor tissues ($P < 0.01$); **E** and **F** show the differences in Tom1L1 expression among different clinical stages($P < 0.01$) and different age groups of tumor patients($P < 0.01$); **(G)** The last figure shows the differences in Tom1L1 expression when considering whether lymph node metastasis occurred ($P < 0.01$)

the treatment plan after chemotherapy, or failing to receive surgery for various reasons.

All enrolled patients or their families provided informed consent. The study was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Hebei North University. (IRB: K2022058).

Main reagents

TOM1L1 primary antibody (Invitrogen PA5-30,348); horseradish enzyme-labeled rabbit anti-goat IgG (catalog No. ZB-2306) was purchased from Beijing Zhong Shan Golden Bridge Biotechnology Co., Ltd (Beijing, China); RIPA (catalog No. R0278) and PVDF membrane (catalog No. P2120) were purchased from Merck KGaA (New Jersey, USA); skimmed milk powder (catalog No. LP0033B) was purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China); Hifair III 1st Strand cDNA Synthesis Kit—gDNA Digester Plus Reverse Transcription Kit (catalog No. 11139ES60) and Hieff qPCR SYBR Green Master Mix—Low Rox Plus Kit (catalog No. 11202ES50) were purchased from Yeasen Biotechnology Co., Ltd (Shanghai, China); Pierce BCA Protein Quantification Kit (catalog No. 23225) was purchased from Thermo Fisher Scientific (California, USA); Trizol (catalog No. 15596026) was purchased from Thermo Fisher Scientific (California, USA); Mini Amp Thermal Cycler (catalog No. A37834) and 7500 Real-Time Fluorescence Quantitative PCR Analyzer (catalog No. 4351104) were purchased from Thermo Fisher Scientific (California, USA); 10% neutral-buffered formalin solution (catalog No. BLC-01) was purchased from Beijing Jiuzhou Bailin Biotechnology Co., Ltd (Beijing, China); xylene (catalog No. X821391) and anhydrous ethanol (catalog No. E809061) were purchased from Shanghai Macklin Biochemical Technology Co., Ltd (Shanghai, China); TOM1L1 antibody [N2C2] (catalog No. GTX115333) was purchased from Internal GeneTex; sodium citrate antigen repair solution (catalog No. C1031), goat serum for blocking (catalog No. SL038), 1×PBS buffer (catalog No. P1020), neutral balsam (catalog No. ZLI-9555), and antigen repair solution (catalog No. C1031) were purchased from Beijing Solarbio Science & Technology Co., Ltd (Beijing, China); rabbit 2-step kit (catalog No. PV-6001) reagent 1(endogenous peroxidase blocker)

and reagent 2(enzyme-labeled goat anti-rabbit IgG polymer), DAB chromogenic solution (ZLI-9017), and horseradish enzyme-labeled rabbit anti-goat IgG (catalog No. ZB-2306) were purchased from Beijing Zhong Shan Golden Bridge Biotechnology Co., Ltd (Beijing, China); hematoxylin (catalog No. BS915) was purchased from Biosharp Biotechnology Co., Ltd (Anhui, China); embedding machine (catalog No. KD-BL111), microtome (catalog No. KD-2258), and biotissue spreading and baking machine (catalog No. KD-T) were purchased from Zhejiang Jinhua Kedi Instrumental Equipment Co., Ltd (Zhejiang, China); LED fluorescence microscope (catalog No. DM 2500) was purchased from Leica Microsystems (Shanghai) Trading Co, Ltd (Shanghai, China).

Methods of neoadjuvant chemotherapy

Patients received a total of 4 cycles of preoperative adjuvant chemotherapy with the Xelox regimen as follows: 130 mg/m^2 of oxaliplatin, administered intravenously on the first day; 2000 mg/m^2 of capecitabine, administered orally from day 1 to day 14, followed by intermittent discontinuation from day 15 to day 21, with every 3 weeks as 1 cycle. Evaluation of surgical treatment was carried out after the completion of the 4-cycle neoadjuvant chemotherapy.

Surgical approaches and specimen collection

TME was performed by the same surgical team on the patients in each group, and specific surgical approaches (Dixon surgery or Miles surgery) were selected according to the distance of the tumor from the anal verge; for the cancer group and the paracancerous normal group, within 30 min of separation of the surgical specimens from the body, 200 mg of paracancerous normal tissues in the non-necrotic area in the center of the cancerous foci and those greater than 10 cm away from the tumor were taken and rinsed to remove residual blood and dirt from the tissue surface with precooled PBS solution or saline. Then, absorbent paper was used to quickly absorb residual from the tissue surface. Afterward, the tissues were loaded into sterile cryogenic tubes, then immediately frozen in liquid nitrogen, and finally transferred to a -80 ℃ refrigerator or liquid nitrogen for preservation for later qPCR and Western blot experiments. The same specimens were prepared and then soaked and fixed with a histological fixative for standard pathological sampling and wax block preparation for later immunohistochemistry staining. After sampling, the surgical specimens were soaked in a histological fixative and sent to the Department of Pathology for standard pathological sampling and wax block preparation by the same pathologist.

qPCR to detect Tom1L1 mRNA expression in the *cancer* group and the paracancerous normal group

Total RNA was extracted from colorectal tissues and cells using the Trizol reagent method in accordance with the current routine protocol. Hifair III 1st Strand cDNA Synthesis Kit—gDNA Digester Plus Reverse Transcription Kit was used to transform 2 μg of RNA into first strand cDNA via reverse transcription. This step should be performed on ice throughout. The primer information of human GAPDH and Tom1L1 was found in the NCBI database and synthesized by Beijing Tsingke Biotech Co., Ltd, including Tom1L1 upstream primer sequence 5'- AACCAGAGTCTGCCACCTTTGC -3'; Tom1L1 primer sequence 5'- CTCCTTTGTGCTGGTGAGCATC -3'; GAPDH upstream primer: 5'-CGGATTTGGTCG TATTGGG-3'; downstream primer: 5'-CTGGAAGAT GGTGATGGGATT-3'. Next, PCR assay was carried out using the Hieff qPCR SYBR Green Master Mix—Low Rox Plus Kit under appropriate conditions in accordance with the manufacturer's instructions. Relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method by taking GAPDH as gene expression normalization, and relative mRNA expression was expressed as $2^{-\Delta\Delta Ct}$.

Western blot to detect Tom1L1 expression in the *cancer* group and the paracancerous normal group

Proteins from tissues (NCM460, MC38, HCT116, HT29, RKO, SW620, CT26, HT29-LV-NC, HT29-LV-shPDIA3, MC38-LV-NC, and MC38-LV-shPDIA3) were lysed with RIPA lysis buffer containing protease and phosphatase inhibitors. Protein quantification was determined using the Pierce BCA Protein Quantification Kit, and quantitative analysis was performed in accordance with published protocols. 10% SDS-PAGE separating gel and 5% SDS-PAGE stacking gel were placed in an electrophoresis tank containing 1×electrophoresis solution; proteins were added; the power cord was then connected. Electrophoresis was stopped when the bromophenol blue indicator reached the bottom of the separating gel. Afterward, a 10% SDS-PAGE separating gel protein sample was transferred to PVDF membrane. The power cord was connected, and the voltage of the transfer tank was adjusted to 110 V for a 60-min membrane transfer. After the completion of transfer, the membrane was blocked with 5% skimmed milk powder for 1 h at room temperature. The PVDF membrane was placed in the antibody dilution and refrigerated overnight at 4 °C. Then, the PVDF membrane was incubated with a secondary antibody for 90 min at room temperature. The immune complexes were detected using a fully automated chemiluminescence image analysis system.

Immunohistochemistry to detect the expression level of Tom1L1 protein in the neoadjuvant therapy pretreatment and post-treatment groups

Appropriate-size tissues were put into the tissue embedding box and then fixed with a 10% neutral-buffered formalin solution. The fixed tissues were embedded with paraffin and then cut into tissue sections in 4 μ m using a microtome. The tissue sections were deparaffinized with xylene and ethanol and then subjected to antigen repair using sodium citrate antigen repair solution. Afterward, an endogenous peroxidase blocker was added dropwise to the tissue surface and then 5% goat serum for blocking was used for blocking. The sections were incubated with ERp57 Rabbit mAb overnight at 4 °C, followed by incubation with enzyme-labeled goat anti-rabbit IgG polymer for 20 min at room temperature. The prepared DAB chromogenic solution was added dropwise to the tissues to cover all the tissues, and staining was terminated when the reaction solution was brownish-yellow. The cells were restained with hematoxylin. Differentiation with hydrochloric acid alcohol, followed by counterstaining. Dehydration with graded alcohols, and clearing with xylene. Neutral balsam was used for mounting. Images of tissue sections were taken using a Leica microimaging system. Staining judging criteria: the gray density analysis method was used. Immunohistochemical images were identified and analyzed using ImageJ-Fiji (National Institutes of Health, NIH) software to derive the AOD value, which was then statistically analyzed using GraphPad Prism 9.0 statistical software.

Tom1L1 was localized in the cytoplasm and positively expressed as brownish-yellow or brown. All the sections were read by the same two associate senior physicians from the Department of Pathology of the First Affiliated Hospital of Hebei North University; five characteristic fields of view were jointly selected for simultaneous observation under low magnification and high magnification, and the staining intensity and the proportion of stained cells were evaluated respectively for tumor regression grading. The specific criteria were as follows. Staining intensity: 0 points for no staining, 1 point for light yellow, 2 points for brownish-yellow, and 3 points for brown; the proportion of stained cells: 0 points for $\leq 5\%$, 1 point for $>5\%$ and $\leq 25\%$, 2 points for $>25\%$ and $\leq 50\%$, and 3 points for $>50\%$. When the above two scores were multiplied, a score of ≤ 3 was regarded as low expression, and a score of >3 was regarded as high expression.

Tumor regression response was evaluated using the AJCC tumor regression grade (TRG) system as follows. TRG0: complete regression with no visible tumor cells under the microscope; TRG1: only single or small foci of tumor cells observed under the microscope; TRG2: significant regression, but the residual tumor was more than single or small foci of tumor cells; TRG3: extensive residual tumor without significant regression.

Statistical processing

Statistical methods for bioinformatics analysis

Wilcoxon t-test was used to analyze the differential expression of cancer tissues and normal tissues in the TCGA database COAD; ANOVA analysis or Kruskal–Wallis test was used to compare the differences between Tom1L1 and different clinical stages and patients' ages; Pearson correlation analysis was performed for correlation analysis to screen Tom1L1-related genes. Scatter plots, histograms, and heat maps were drawn using GraphPad Prism 8.4.3 and the R language ggplot2 package, with statistical significance set as * for $P<0.05$, ** for $P<0.01$, and *** / **** for $P<0.001$.

Statistical methods for experimental data

Statistical analysis was performed using SPSS 25.0 software; measurement data were expressed as mean ± standard deviation; comparisons between two groups were analyzed using t-test, and when the sample size was large, they were analyzed using rank sum test; enumeration data were expressed as percentages; in terms of comparisons between groups, chi-square test was used for $n>40$, corrected chi-square test was adopted for $n>40$ and $T\leq 5$, and Fisher's exact probability method was used for $n\leq 40$. Data were analyzed and plotted using GraphPad Prism 8.4.3. Test level $\alpha = 0.05$ and $P<0.05$ were considered statistically significant.

Results

Results of Tom1L1 bioinformatics analysis

Based on the heterogeneity of adenocarcinoma, we chose the COAD database. The expression level of Tom1L1 in tumors is significantly higher than that in normal tissues, and there was noticeable difference between the expression level of Tom1L1 and the clinical stage of the tumor. In addition, overall, both the age of the patient and lymph node metastasis shadow may affect the expression level of Tom1L1 ($P<0.01$). The results are shown in Fig. 2.

Tom1L1 mRNA expression in the colorectal *cancer* group and the paracancerous normal group

The expression of Tom1L1 mRNA in the cancer group and the paracancerous normal group was plotted as an expression distribution heat map. The results showed that the relative expression of Tom1L1 mRNA in colorectal cancerous tissues was higher than that in paracancerous normal tissues, indicating a statistically significant difference ($P<0.01$). The results are shown in Fig. 3-A.

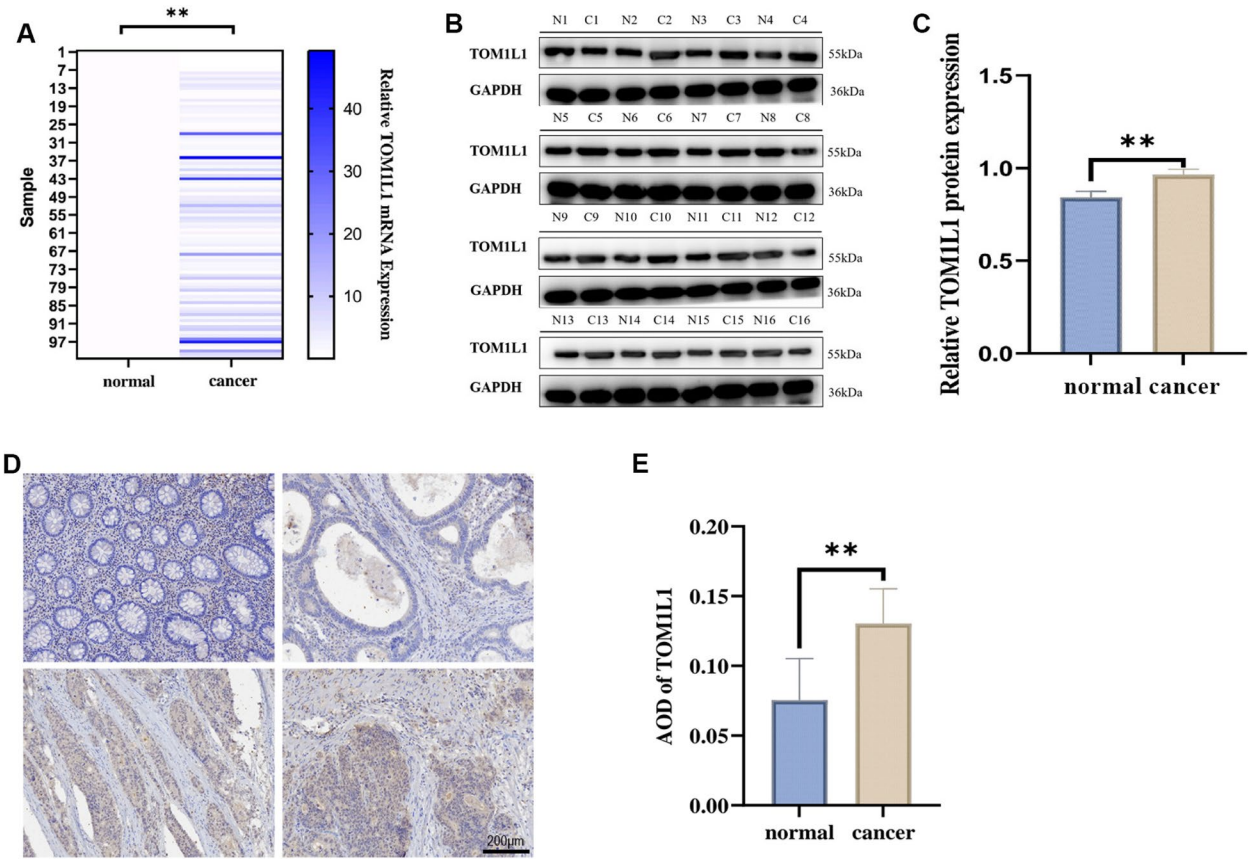

Fig. 3 Statistical analysis of Tom1L1 expression in the paracancerous normal and cancer group. **A** qPCR shows Tom1L1 mRNA expression in the paracancerous normal group and the cancer group of colorectal cancer patients, as determined ($P < 0.01$); **B** Western blot shows the expression level of Tom1L1 protein in the paracancerous normal group and the cancer group of 16 patients detected; **C** The third figure shows the statistical analysis of Western blot results; **D** Immunohistochemistry shows the differentiation degree of colorectal adenocarcinoma: normal adenocarcinoma cells, highly differentiated adenocarcinoma cells, which are morphologically close to the source tissues, with a high degree of maturity and a low degree of malignancy, moderately differentiated adenocarcinoma cells, which retain the morphological characteristics of the source tissues, with irregularly arranged glandular or adenoidal structures and poorly differentiated adenocarcinoma cells, with the cells irregularly arranged in multilayers, presenting nuclear mitosis, poor differentiation, and extreme immaturity(scale bar: 200 μm); **E** The last figure shows the expression level of Tom1L1 protein in the paracancerous normal group and the cancer group detected by IHC ($P < 0.01$)

Tom1L1 protein expression in the colorectal *cancer* group and the paracancerous normal group

The results of the Western blot assay suggested that the expression level of Tom1L1 protein was higher in 16 cases of colorectal cancerous tissues than that in paracancerous normal tissues, indicating a statistically significant difference ($P < 0.01$). The results are shown in Fig. 3-B and Fig. 3-C.

IHC showed the following findings: Tom1L1 was localized in the cytoplasm; in the paracancerous normal group, glandular cells were of uniform size and regular arrangement, and Tom1L1 expression was stable and low. In colorectal cancerous tissues, adenocarcinoma cells were of different sizes and morphologies, with irregularly arranged glandular or adenoidal structures; cells were mostly irregularly arranged in multilayers; nuclei were of different sizes and showed mitotic figures. The expression level of Tom1L1 was significantly higher than that in the paracancerous normal group, indicating a statistically significant difference ($P < 0.01$). The results are shown in Fig. 3-D and Fig. 3-E.

Relationship between Tom1L1 protein expression and clinicopathological characteristics of colorectal tumors

Among the 102 cases of colorectal cancer, there were 5 cases (4.9%) in stage I, 8 cases (7.8%) in stage II, 71 cases (69.6%) in stage III, and 18 cases (16.7%) in stage IV. The relationship between clinicopathological characteristics and Tom1L1 expression in colorectal cancer patients was analyzed. The results suggested that Tom1L1 expression was significantly associated with the pathological stage of the tumor, lymph node metastasis, and ki-67 expression ($P < 0.01$), yet not significantly associated with tumor

sizes, tumor sites, degree of differentiation, liver metastasis, and MSI status ($P > 0.05$) (Table 1).

Neoadjuvant chemotherapy in 34 patients with locally progressive mid-low rectal *cancer*

Among the 34 enrolled patients, before neoadjuvant chemotherapy, tumor biopsies of 24 patients showed high expression of Tom1L1 protein; after neoadjuvant chemotherapy, high expression was found in 17 patients. The high expression rate of Tom1L1 protein was 70.59% (24/34) in the pretreatment group and 50.00% (17/34) in the post-treatment group. Although the difference in the high expression rate between the two groups was not statistically significant ($P = 0.136$), there was still a significant difference in Tom1L1 protein before and after treatment in terms of the overall expression level ($P < 0.01$). In the pretreatment group, the effective rate of neoadjuvant chemotherapy was 90.00% (9/10) in patients with low expression of Tom1L1 protein and only 45.83% (11/24) in those with high expression, indicating a statistically significant difference ($P = 0.024$). Results of MRI, enteroscopy, ultrasound endoscopy, and pathological HE staining before and after neoadjuvant therapy are shown in Fig. 4 and Fig. 5.

Expression of Tom1L1 protein in patients with locally progressive mid-low rectal *cancer* before and after neoadjuvant chemotherapy

After neoadjuvant chemotherapy, there was a significant difference in the expression level of Tom1L1 protein in the pretreatment and post-treatment groups with different efficacy (Fig. 6-A and Fig. 6-B). Among the 34 patients, there were 8 TRG0 cases, 6 TRG1 cases, 6 TRG2 cases, and 14 TRG3 cases. Compared with the pretreatment group, the expression of Tom1L1 protein was reduced in the post-treatment group in terms of TRG0, TRG1, and TRG2 ($P < 0.01$, $P < 0.01$, and $P < 0.05$), whereas in terms of TRG3, there was no statistically significant difference in Tom1L1 protein expression

Table 1 Relationship between Tom1L1 expression and clinicopathological characteristics in 102 colorectal tumor patients. The results suggested that Tom1L1 expression was significantly associated with the pathological stage of the tumor, lymph node metastasis, and ki-67 expression ($P < 0.01$), yet not significantly associated with tumor sizes, tumor sites, degree of differentiation, liver metastasis, and MSI status ($P > 0.05$)

Item parameter	n	Tom1L1			
		High expression (%)	Low expression (%)	χ^2	*P*
Tumor size				1.958	0.162
< 5 cm	49	35 (71.43)	14 (28.57)		
> 5 cm	53	44 (83.02)	9 (16.98)		
Tumor site				0.266	0.606
Colon	44	33 (75.00)	11 (25.00)		
Rectum	58	46 (79.31)	12 (20.69)		
Degree of differentiation				1.242	0.265
High and medium differentiation	89	71(79.76)	18(20.24)		
Low differentiation	13	8(61.54)	5(38.46)		
TNM stage				15.653	0.000
I + II	13	4 (30.77)	9 (69.23)		
III + IV	89	75 (84.27)	14 (15.73)		
Ki-67				29.110	0.000
≥ 50%	66	62 (93.94)	4 (6.06)		
< 50%	36	17 (47.22)	19 (52.78)		
Lymph node metastasis				24.693	0.000
+	82	66 (80.49)	16 (19.51)		
-	20	13 (65.00)	7 (35.00)		
Liver metastasis				0.075	0.784
+	18	13 (72.22)	5 (27.78)		
-	84	66 (78.57)	18 (21.43)		
MSI				1.568	0.208
+	15	14 (93.33)	1 (6.67)		
-	87	65 (74.71)	22 (25.28)		

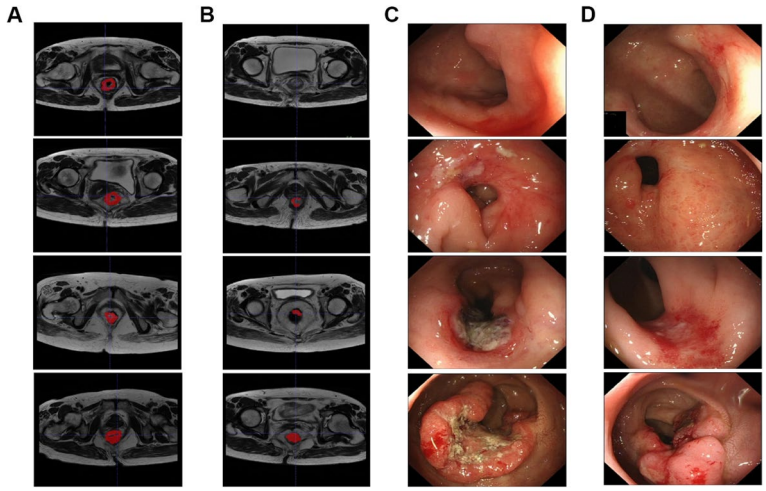

Fig. 4 Nuclear magnetic target area and enteroscopy before and after neoadjuvant chemotherapy. **A** and **B** are images of the nuclear magnetic target area of tumors before and after treatment based on TRG0–TRG3, respectively (the red area is marked tumor site); **C** and **D** are tumor endoscopic images before and after treatment based on tumor regression grades TRG0–TRG3, respectively

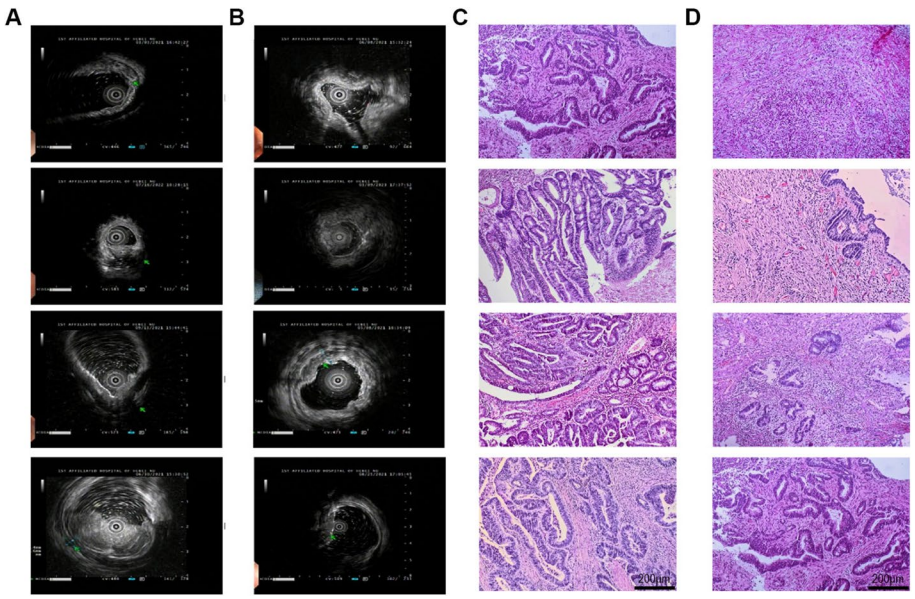

Fig. 5 Ultrasound endoscopy and pathological HE staining before and after neoadjuvant chemotherapy. **A** and **B** are ultrasound endoscopic images of tumors before and after treatment based on tumor regression grades TRG0–TRG3, respectively; **C** and **D** are pathological HE staining images of tumors before and after treatment based on tumor regression grades TRG0–TRG3, respectively

between the pretreatment and post-treatment groups ($P > 0.05$). The results are shown in Fig. 6-C.

Among the patients, 41.17% (14/34) responded positively (TRG0–TRG1), and 58.82% (20/34) responded to neoadjuvant chemotherapy (TRG0–TRG2); in terms of TRG0–TRG1 (significant tumor regression), TRG0–TRG2 (effective treatment), and TRG2–TRG3 (insignificant tumor regression), there was a significant difference in Tom1L1 protein expression in both the pretreatment and post-treatment groups ($P < 0.01$, $P < 0.01$, and $P < 0.05$). The results are shown in Fig. 6-D.

Discussion

Main interpretation

For locally progressive mid-low rectal cancer, current guidelines recommend a combination of neoadjuvant

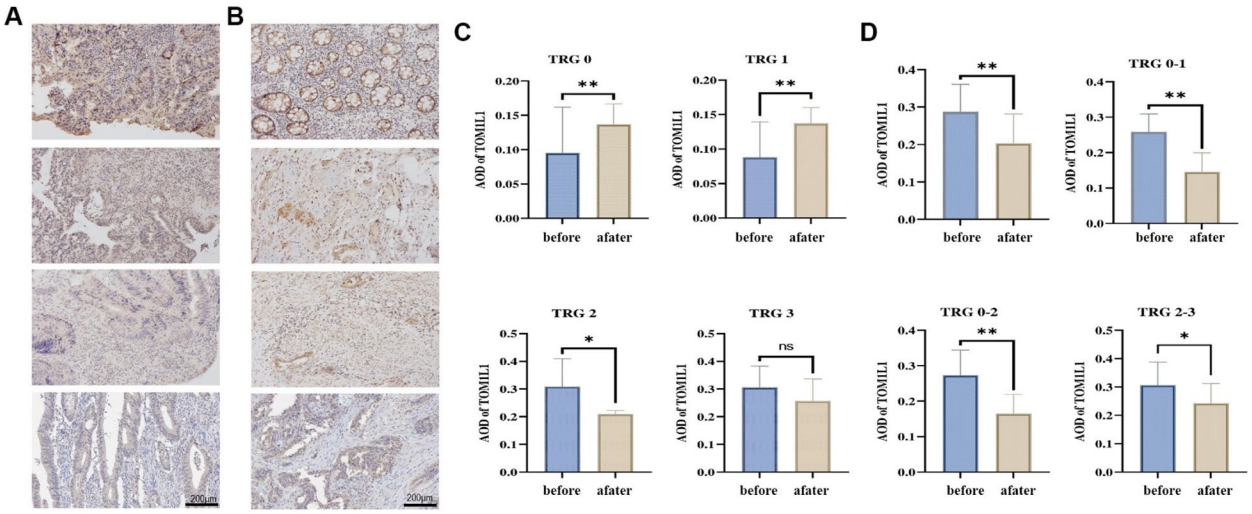

Fig. 6 Tom1L1 protein expression in the pretreatment and post-treatment groups. **A** and **B** are images of Tom1L1 protein expression before and after treatment based on tumor regression grades TRG0–TRG3, respectively(scale bar: 200 μm); **C** The third figure shows the expression of Tom1L1 protein in TRG0, TRG1, TRG2, and TRG3 in the pretreatment and post-treatment groups. Compared with the pretreatment group, the expression of Tom1L1 protein was reduced in the post-treatment group in terms of TRG0, TRG1, and TRG2 ($P < 0.01$, $P < 0.01$, and $P < 0.05$), whereas in terms of TRG3, there was no statistically significant difference in Tom1L1 protein expression between the pretreatment and post-treatment groups ($P > 0.05$); **D** The last figure shows the expression level of Tom1L1 protein in the pretreatment and post-treatment groups detected by IHC, and there was a significant decrease in the expression level of Tom1L1 protein after neoadjuvant therapy, indicating a statistically significant difference ($P < 0.01$); the analysis of Tom1L1 protein expression in the pretreatment and post-treatment groups in terms of TRG0–TRG1 (significant tumor regression), TRG0–TRG2 (effective treatment), and TRG2–TRG3 (insignificant tumor regression) ($P < 0.01$, $P < 0.01$, and $P < 0.05$)

chemoradiotherapy (nCRT), TME, and postoperative adjuvant chemotherapy. However, patients undergoing radical surgery for rectal cancer may face various risks, such as anastomotic leakage, temporary or permanent fistula, organ excision, and urinary damage and dysfunction. Research data show the following findings: the perioperative complication incidence of radical surgery for rectal cancer is about 20% to 30% [27, 28]; among patients undergoing surgery, no residual tumor cells are found in surgically resected specimens in 20% to 30% of the patients [29], i.e., pathological complete remission (pCR) is achieved following nCRT, and the prognosis of this group of patients is significantly better, with the 5-year overall survival rate of approximately 90% [30]. In general, whether clinical complete remission (cCR) is achieved after neoadjuvant therapy can be judged by rectal MRI, endoscopy, serological markers, and other auxiliary examinations [31, 32]. In recent years, domestic and foreign experts have obtained more refined diagnostic criteria for cCR determination based on extensive clinical data [33].

Glynne-Jones et al. [34] revealed that low rectal cancer with small tumor sizes was easily subjected to relatively refined clinical evaluation, but not suitable for clinical evaluation of progressive rectal cancer. Several meta-analyses of rectal cancer [35–37] show local regeneration rates of 31–37% in progressive rectal cancer, indicating a high recurrence rate in high-risk patients with poor prognostic evaluation results of rectal cancer risks (including $cT_{3c\text{-}d}/T_4$, lateral lymph node metastasis, MRF involvement, and WMVI+), to whom the "wait-and-see" strategy is not recommended. A similar retrospective study was conducted by Professor Wang Xishan's team. They found [38] that positive lymph nodes or tumor residuals were found in 13.6% of ypT0N0 patients in 60 rectal cancer patients who underwent radical surgery after nCRT. It can be seen that the cCR and pCR rates are low and that no high-level evidence has been obtained for the applicability and efficacy of the "wait-and-see" strategy [39]. Large-scale prospective studies are needed to confirm the long-term outcomes and to refine chemotherapeutic medication selection, cCR determination, and optimal follow-up protocols for the "wait-and-see" strategy [40]. Some experts and scholars suggest that local excision after neoadjuvant therapy for rectal cancer patients can balance the pros and cons of the first two regimens, which can effectively excise the "primary tumor" and clarify pathological T-stages, but still cannot solve such problems as lymph node positivity, organ preservation, surgical risks, and tumor recurrence [41]. Chiang et al. highlighted that the location of rectal cancer plays a crucial role in influencing the incidence and patterns of

distant metastases in patients who underwent curative surgery without prior neoadjuvant therapy. Additionally, tumor location was identified as a significant factor contributing to local recurrence and bone metastases, among other risk [42].

Tom1L1 plays a role in the onset and development of multiple tumors in the studies that have been confirmed so far. Clément et al. [43, 44] found that Tom1L1 exerted pro-oncogenic activity in breast cancer, Tom1L1 and ERBB2 were co-expressed in breast cancer, overexpression of ERBB2 in human breast cancer resulted in tumor invasion and metastasis, and Tom1L1 regulated ERBB2-induced cellular invasion by interacting with TOLLIP to promote MT1-MMP membrane delivery. Sirvent et al. [45] revealed a completely different function of Tom1L1 in colorectal cancer through mouse xenotransplantation experiments and cell transfection experiments; low expression of Tom1L1 slightly affected Src-induced proliferation of CRC cells in vitro but significantly reduced tumor growth in xenotransplanted nude mice; the results suggested that Tom1L1 promoted Src-induced tumor growth. Studies have found that Tom1L1 interacts with clathrin heavy chain (CHC) in vivo CHC is a structural component of coated vesicles); when Tom1L1 binds to CHC, it negatively regulates Src's mitotic and transforming activity by decreasing the expression level of CHC in cholesterol-rich microvesicles; when Tom1L1 does not bind to CHC, it relocates in the vesicles and promotes Src-driven DNA synthesis [46]. Therefore, we can assume that Tom1L1 (as a new substrate) binding to Src family protein tyrosine kinase can promote Src activity, which in turn promotes cancer cell proliferation, triggers growth invasion mechanism, and induces angiogenesis.

In recent years, miRNA has been widely mentioned as a more studied tumor serum marker with promising research prospects. miRNAs play important regulatory roles in various cellular processes such as cell proliferation, differentiation, and apoptosis, and can be used as a potential biological indicator in colorectal cancer screening [47–49]. Among them, miR-21-5p is expressed significantly different before and after nCRT, and miR-21-5p overexpression is likely to predict good tumor regression response [50, 51]. Controversially, however, in several in vitro studies of CRC cell lines, miR-21-5p overexpression was found to induce chemotherapy resistance [52], and patients with poor prognosis also showed miR-21-5p overexpression [53]. Qi et al. indicated that exosomal miR-21-5p from mesenchymal stem cells (MSCs) promotes tumor growth in osteosarcoma (OS) by suppressing PIK3R1, thereby activating the PI3K/Akt/mTOR signaling pathway [54]. Rectal cancer is highly heterogeneous, and single biomarkers and imaging evaluation methods are not sufficiently sensitive and specific to predict the efficacy of neoadjuvant chemotherapy for rectal cancer [55]. Therefore, for patients with locally progressive rectal cancer, finding an accurate evaluation method to improve cCR and pCR rates is an urgent issue to be addressed. If molecular markers could be found to effectively predict pCR after neoadjuvant therapy for rectal cancer and then to accurately evaluate the TRG grades of neoadjuvant therapy, patients with locally progressive mid-low rectal cancer could be provided with more accurate treatment options, thereby improving their survival and prognosis.

In this study, functional analysis of differential genes screened from the TCGA database suggests that Tom1L1 expression was noticeable difference with clinical tumor stages—lymph node metastasis. Further clinical validation was carried out. To be specific, qPCR was conducted to detect Tom1L1 expression in colorectal cancerous tissues and paracancerous normal tissues. The results suggested that the relative expression of Tom1L1 was higher in colorectal cancerous tissues than that in paracancerous normal tissues. The results of qualitative and semi-quantitative analysis of Western blot and IHC suggested that the expression level of Tom1L1 protein was significantly higher in colorectal cancerous tissues than that in paracancerous normal tissues. For patients with locally progressive mid-low rectal cancer, there was a significant difference in the expression level of Tom1L1 before and after neoadjuvant therapy; a significant difference was also seen between different efficacy grades, which correlated with TRG grades to a certain extent. Furthermore, combined with pathological and histochemical results, it was confirmed that patients with locally progressive mid-low rectal cancer were more sensitive to neoadjuvant chemotherapy.

Limitations

Firstly, this study conducted experiments such as immunohistochemistry and Western blot, but is currently limited to clinical research and has not been extensively studied at the cellular level. Secondly, this study has been experimentally validated, but no research has been conducted on the carcinogenic mechanism or mechanism of action. Thirdly, the number of patients with locally advanced rectal cancer in the middle and low levels is relatively small, so multi center, large sample, and multidisciplinary cooperation can be carried out during the advanced stage, which will be the direction of our team's future research.

Conclusion

In conclusion, this experiment provides valuable biological indicators for the diagnosis and staging of colorectal cancer, as well as for efficacy prediction of neoadjuvant therapy

through bioinformatics prediction screening and clinical validation. We have reached the following three conclusions: Tom1L1 is highly expressed in colorectal cancerous tissues; neoadjuvant Xelox chemotherapy can have an impact on Tom1L1 expression in progressive rectal cancer; patients with locally progressive mid-low rectal cancer who have low Tom1L1 expression are more sensitive to neoadjuvant chemotherapy.

Abbreviations

AJCC	American Joint Committee on Cancer
cCR	Clinical complete remission
CHC	Clathrin heavy chain
IHC	Immunohistochemistry staining
MRI	Magnetic resonance imaging
MSI	Microsatellite instability
MVBs	Multivesicular bodies
nCRT	Neoadjuvant chemoradiotherapy
pCR	Pathological complete remission
qPCR	Polymerase chain reaction
TMB	Tumor mutational load
TME	Total mesorectal excision
TRG	Tumor regression grade

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Authors' contributions
Ming CAI: conception and design, analysis and interpretation of data, drafting of the manuscript. Juxiang GOU: revising it for important intellectual content, final approval of the version to be published. The authors read and approved the final manuscript.

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

Declarations

Ethics approval and consent to participate
Informed consent and approval were obtained from all the patients and the Ethics Committee of The First Affiliated Hospital of Hebei North University (K2022058). The research was conducted in accordance with the guidelines of the Ethics Committee listed in the Ethics Statement. Patients signed informed consent regarding publishing their data and images.

Consent for publication
Written informed consent was obtained from the patient's parent for publication of this case report and the accompanying images. A copy of the written consent obtained from the parent of the patient in this case report is available for review by the editorial office of this journal.

Competing interests
The authors declare no competing interests.

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