The expression of long-chain non-coding RNA DMTF1v4 in colorectal cancer tissue and its relationship with clinicopathological features

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Abstract
Emerging studies have showed that long-chain non-coding RNA DMTF1v4 might participate in the process of multidrug resistance phenotype of gastric cancer. However, its expression and function in colorectal cancer (CRC) is still unknown. In this study, we discovered that DMTF1v4 was generally 5.15 ± 1.67 times upregulated in CRC tissues compared to the adjacent normal tissues. Moreover, the expression level of DMTF1v4 was closely related to the distant metastasis of tumor, but it was not related to age, sex, tumor location, tumor staging, depth of invasion, lymph node metastasis, and differentiation level. Survival analysis showed that the overall survival rate of patients with high expression of DMTF1v4 was 45.0% in cancer tissues, which was significantly lower than 82.5% of DMTF1v4 low expression patients ($\chi^2 = 11.562, P < 0.01$). The results of univariate COX regression analysis showed that DMTF1v4, TNM (tumor, node, metastasis) staging, distant metastasis, and tumor differentiation were closely related to the prognosis of patients ($P < 0.05$). Multivariate COX regression analysis showed that DMTF1v4 and distant metastasis could be independent prognostic factors for CRC patients. In conclusion, this study revealed that DMTF1v4 might promote the development of CRC, which can be used as an independent factor to judge the prognosis of CRC.

Keywords
clinicopathology, colorectal cancer, DMTF1v4, long-chain non-coding RNA, prognosis

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Introduction
Colorectal cancer (CRC) is one of the most common malignant tumors in the world. In China, the morbidity and mortality of CRC is located in the third and the fifth of malignant tumors, respectively, and it shows an upward trend. Currently, the primary treatment for colon cancer is the surgical removal of tumor tissue, and the survival rate of advanced colon cancer is also dependent on chemotherapy. The 5-year survival rate of patients with early colon cancer is about 90%, but the overall survival rate of patients with advanced and metastatic cancer is still not significantly increased, only about 15%. Studies have shown that colon cancer is associated with red meat, obesity, lack of physical exercise, and so on. Long-chain non-coding RNA (lncRNA) is a non-coding RNA > 200 nt in length. It is an important member of mammalian transcriptome that has...
attracted much attention in recent years. It can be transcribed by genetic events such as chromosomal rearrangement and transposable element insertion. They indicated that changes in lncRNA play an important role in the occurrence, development, and prognosis of CRC. Therefore, lncRNA can be an early marker of early diagnosis, early treatment and prognosis of intestinal cancer, and a potential biological therapy target molecule, which plays an important role in the diagnosis and treatment of CRC. LncRNA DMTF1v4 is a key lncRNA molecule that affects the multidrug resistance phenotype of gastric cancer. However, the expression of lncRNA DMTF1v4 in CRC and its relationship with clinical prognosis has not been reported in China at present. In this study, we examined the expression of lncRNA DMTF1v4 in paired cancer tissues and paracancerous tissues of 80 patients with CRC, and analyzed the role of lncRNA in the development and progression of CRC and its relationship with clinical prognosis. The aim of this study is to provide a therapeutic strategy and experimental basis for molecular targeted therapy of CRC.

Materials and methods

Research object

Eighty patients with CRC underwent surgical resection of the tumor from February 2013 to August 2014 in our hospital, all of whom had paired cancer tissues and paracancerous tissues (margin of 5–10 cm away from cancer tissues). All patients underwent radical surgery, and no preoperative radiotherapy or chemotherapy was performed, and the postoperative specimens were clearly diagnosed as CRC by histopathology. The collected samples were placed in liquid nitrogen cooling, and then transferred to –80°C preservation. Clinical data collection included age, sex, tumor location, TNM (tumor, node, metastasis) staging, and tumor grade. The follow-up was started on the date of surgery. Follow-up was performed every 3 months in the first 2 years after operation. Follow-up was performed every 6 months in the third year after operation. The 3-year overall survival of CRC patients was calculated by August 2017. All patients signed informed consent.

Cell lines, reagents, and instruments

High glucose DMEM (Dulbecco’s modified eagle medium), optimum serum-free medium, fetal bovine serum, lipofectamine 2000, and Trizol reagent were purchased from Life Technologies, USA; PrimeScript reverse transcription kit and SYBR Premix Ex Taq kit were purchased from Japan TaKaRa company; and ABI 7500 real-time polymerase chain reaction (PCR) instrument was purchased from Life Technologies, USA.

RNA extraction and reverse transcription (RT) reaction

Tissue RNA extraction. Tissue RNA was extracted with Trizol reagent for cancer tissues and paracancerous tissues from 50 to 100 mg of CRC patients.

Cell RNA extraction. FHC and CRC cells were routinely cultured in high glucose DMEM medium containing 10% fetal bovine serum and 5% CO2, 37°C and 90% humidity, and logarithmic growth phase cells were treated with Trizol reagent to extract cell RNA. The absorbance of sample was determined by spectrophotometer (A260/280nm). The samples with A260/280nm value of 1.8–2.1 were reverse transcribed into cDNA according to the instructions of PrimeScript reverse transcription kit. The samples were stored at –20°C.

Using real-time fluorescent quantitative RT-PCR to detect the expression of lncRNA DMTF1v4

The Primer Premier 5.0 software was used to design the primers, and the primers were synthesized by Shanghai Bioengineering Technology Service Co., Ltd. DMTF1v4 primer sequence is F: ACCCACAGACAACGTGACC, R: GCCGC CCTATTGTTGGCCA. GADPH primer sequence is F: GCACCGTCAAGGCTGACCC, R: TGG TGAAGACGCCAGTGA. The total reaction system of PCR was 20 μL, including 10 μmol/L the upstream and downstream primers 0.8 μL each, SYBRPremix reagent 10 μL, ROX correction solution 0.4 μL, template DNA 2 μL, and ddH2O (sterile ultra-pure water) make up to 20 μL. Cycle parameters are as follows: pre-denaturation at 95°C for 2 min, followed by denaturation at 95°C for 5 s, 58°C for 34 s, 80°C for 34 s, a total of 40 cycles. Using GADPH as an internal reference, the relative expression of DMTF1v4 was detected by real-time RT-PCR. The qRT-PCR and data acquisition and analysis were carried out by using ABI7500 real-time fluorescence quantitative PCR instrument.
instrument and its own software, and the relative expression level of the target gene was calculated by $2^{-\Delta\Delta Ct}$ method—Formula: $\Delta Ct = Ct_{DMTF1v4} - Ct_{GADPH}$; $\Delta\Delta Ct = \Delta Ct_{\text{Test group}} - \Delta Ct_{\text{Control group}}$

The patients were divided into DMTF1v4 low expression group and DMTF1v4 high expression group according to the median relative expression level. The relationships between DMTF1v4 expression and clinicopathological parameters such as age, gender, tumor location, staging, depth of invasion, lymph node metastasis, and differentiation were analyzed.

**Statistical analysis**

SPSS21.0 statistical software was used to analyze the data normally; the data which accorded with the normal distribution were represented by $\bar{x} \pm s$, and the measurement data were tested by two independent-samples t test. The number of cases of missed visits and surviving until the end of observation was set as censored data, and the survival rate was determined by the log-rank test in Kaplan–Meier survival analysis. COX regression analysis evaluated the effect of DMTF1v4 expression and clinical parameters on the overall survival rate of patients. $P < 0.05$ was considered statistically significant.

**Results**

**Differential expression of DMTF1v4 in CRC and its relationship with clinicopathological parameters**

The results of qRT-PCR showed that the relative expression level of DMTF1v4 in cancer tissues was $5.15 \pm 1.67$ times of that in paracancerous tissues; the difference was statistically significant ($t = 5.481, P < 0.05$) (Figure 1).

Eighty pairs of tissue samples were divided into high expression group ($\geq 2.56$, 40 cases) and low expression group ($< 2.56$, 40 cases) by the median of DMTF1v4 relative expression (2.56).

**Relationship between DMTF1v4 and clinicopathological parameters**

The expression level of DMTF1v4 was closely related to the distant metastasis of tumor, but it was not related to age, sex, tumor location, staging, depth of invasion, lymph node metastasis, and differentiation level (Table 1).

**The relationship between DMTF1v4 and the survival prognosis of CRC**

At the end of the follow-up, the median follow-up time was 27.5 (3–37) months, and six cases lost in following up. The survival analysis showed that the overall survival rate of DMTF1v4 overexpression was 45.0% in cancer tissues, which was significantly lower than 82.5% of DMTF1v4 low-expression patients ($\chi^2 = 11.562, P < 0.01$).

The results of univariate COX regression analysis showed that DMTF1v4, TNM staging, distant metastasis, and tumor differentiation were closely related to the prognosis of patients ($P < 0.05$). Multivariate COX regression analysis showed that DMTF1v4 and distant metastasis could be independent prognostic factors for CRC patients (Table 2).

**Discussion**

CRC is colon cancer which is a complex disease caused by uncontrolled proliferation and uncontrolled growth of tumor cells and unlimited
diffusion of tumor cells that result from a variety of factors, involving immunity and multiple steps. At present, more and more researchers are concerned about the impact of lncRNA on tumor development, which might provide a new field of vision for the study of tumor biology.

Our study revealed that DMTF1v4 is overexpressed in CRC patients. Furthermore, the expression level of DMTF1v4 was closely related to the distant metastasis of tumor, but it was not related to age, sex, tumor location, staging, depth of invasion, lymph node metastasis, and differentiation level. These results suggested that DMTF1v4 might be a oncogene in CRC.

Takahashi et al. showed that pvt-1 oncology (pvt-1) was an independent risk factor, which was closely related to the prognosis of CRC; Kam et al. found that molecular beacons based on CCAT-1 (colon cancer associated transcript 1) can be used as specific CRC diagnostic tools.

### Table 1. Relationship between the expression of DMTF1v4 and clinicopathological parameters in CRC patients (n (%)).

| Parameter                  | DMTF1v4 low expression group (n = 40) | DMTF1v4 high expression group (n = 40) | χ²   | P     |
|----------------------------|--------------------------------------|----------------------------------------|------|-------|
| Sex                        |                                      |                                        |      |       |
| Male                       | 21 (52.5)                            | 20 (50.0)                              | 0.715| 0.526 |
| Female                     | 19 (47.5)                            | 20 (50.0)                              |      |       |
| Age (year)                 |                                      |                                        |      |       |
| <70                        | 22 (55.0)                            | 19 (47.5)                              | 1.043| 0.274 |
| ≥70                        | 18 (45.0)                            | 21 (52.5)                              |      |       |
| Tumor location             |                                      |                                        |      |       |
| Colon                      | 23 (57.5)                            | 24 (60.0)                              | 1.672| 0.306 |
| Rectum                     | 17 (42.5)                            | 16 (40.0)                              |      |       |
| TNM staging                |                                      |                                        |      |       |
| I–II                       | 20 (50.0)                            | 19 (47.5)                              | 0.814| 0.503 |
| III–IV                     | 20 (50.0)                            | 21 (52.5)                              |      |       |
| Depth of invasion          |                                      |                                        |      |       |
| T1–T2                      | 8 (20.0)                             | 6 (15.0)                               | 1.155| 0.168 |
| T3–T4                      | 32 (80.0)                            | 34 (85.0)                              |      |       |
| Lymph node metastasis      |                                      |                                        |      |       |
| N0                         | 20 (50.0)                            | 22 (55.0)                              | 1.327| 0.106 |
| N1–N2                      | 20 (50.0)                            | 18 (45.0)                              |      |       |
| Distant metastasis         |                                      |                                        |      |       |
| M0                         | 35 (87.5)                            | 26 (65.0)                              | 9.104| 0.005 |
| M1                         | 5 (12.5)                             | 14 (35.0)                              |      |       |
| Tumor differentiation      |                                      |                                        |      |       |
| G1–G2                      | 36 (90.0)                            | 34 (85.0)                              | 0.683| 0.149 |
| G3                         | 4 (10.0)                             | 6 (15.0)                               |      |       |

### Table 2. The effect of DMTF1v4 expression level and clinicopathological parameters on the overall survival rate of CRC patients.

| Risk factor                  | Classification (cases) | Univariate analysis | Multivariate analysis |
|------------------------------|------------------------|---------------------|-----------------------|
|                              |                        | HR (95% CI)         | P                     |
| DMTF1v4                      | High (n = 40)/low (n = 40) | 4.21 (1.72–10.89)   | 0.002                 |
| Sex                          | Female (n = 39)/male (n = 41) | 0.84 (0.38–2.12)    | 0.825                 |
| Age                          | ≥70 (n = 39)/<70 (n = 41) | 1.79 (0.73–4.46)    | 0.194                 |
| Tumor location               | Rectum (n = 33)/colon (n = 47) | 0.51 (0.22–1.53)   | 0.252                 |
| TNM staging                  | III–IV (n = 41)/I–II (n = 39) | 4.34 (1.63–11.78)  | 0.007                 |
| Lymph node metastasis        | N1–N2 (n = 38)/N0 (n = 42) | 1.31 (0.59–2.94)    | 0.662                 |
| Distant metastasis           | M1 (n = 19)/M0 (n = 61) | 8.02 (3.59–17.63)   | 0.000                 |
| Tumor differentiation        | G3 (n = 10)/G1–G2 (n = 70) | 2.43 (1.02–5.94)    | 0.047                 |

HR: hazard ratios; CI: confidence interval.
this study, multivariate COX regression analysis was used to find that DMTF1v4 high expression was closely related to poor prognosis in CRC patients, and could be an independent risk prognostic factor.

In summary, lncRNA DMTF1v4 plays an important role in the occurrence and development of CRC. LncRNA DMTF1v4 can promote the development of CRC, which can be used as an independent factor to judge the prognosis of CRC and can provide a new molecular target for the treatment of CRC.

There are still some shortcomings in this experiment. First, this study found that the expression level of DMTF1v4 was not related to the stage of tumor, which may be due to the insufficient sample size of the study, and it requires further clarification of the sample size. Second, the patient’s follow-up period was only 3 years. The follow-up duration of oncology should generally be extended to 5 years and follow-up is needed to enhance the stability of the results.

Author’s contribution
Shutong Zhuang and Yanjuan Cai contributed equally.

Declaration of conflicting interests
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