MiR-494 Inhibits the Proliferation, Migration and Invasion of Cervical Cancer Cells by Regulating LETMD1

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ABSTRACT

LETMD1 is a differentially expressed gene selected by scientists from cervical cancer (CC) tissues by RT-PCR technology. It has been confirmed that LETMD1 is overexpressed in many human malignant tumors, so it can be used as an early diagnostic marker for malignant tumors and as a target for gene therapy. The purpose of this article is to further explore the effectiveness of miR-494 in inhibiting the proliferation, migration and invasion of CC cells by regulating LETMD1, selecting 40 cases of CC admitted to a hospital from June 2015 to September 2018 Patients, tumor tissue specimens were taken from the primary tumor tissue of CC, and normal tissues near the cutting edge were collected as controls. Normal tissues were confirmed by pathology after surgery that they were not invaded by cancer tissues. The results of the study showed that the expression level of miR-494 increased by 15%, and the prognostic survival rate after surgery increased by 20%, depending on gender, age, tumor size, and tumor site. After high expression of miR-494 in CC patients, the vascular invasion of CC cells was reduced by 33%, and distant metastasis was reduced by 11%, and the survival time of patients was significantly prolonged. After the expression of LETMD1, the proportion of cancer cells decreased by 5%, the proportion of macrophages increased by 2%, and the dendritic cells increased by 3%.

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Introduction

In gliomas, miR-494 can overregulate the PTEN/Akt signaling pathway to promote glioma cell proliferation, invasiveness and migration. In breast cancer, miR-494 exerts a breast cancer inhibitory effect by regulating the WNT/β-catenin signaling pathway (1, 2). MiRNA mainly exerts its biological effects by regulating downstream target genes, so the downstream target genes determine the role of miRNA (3). According to this, the target gene of miR-494 was screened by tetraxetans target gene prediction software, because LETMD1 has an important role in the progress of human cancer, and became a target gene of interest (4).

CC is by far the most definite cause of malignant neoplasms, which seriously harms women's health. In recent years, there have been many studies on CC. Kim examined the expression and function of HOTAIR in CC, detected the expression of HOTAIR in CC tissue (5). Hu explored the effect of different concentrations of salidroside on the viability, cell cycle and apoptosis of CC SIHA cells and its underlying mechanism. Cell growth potential and colony formation were measured by Cell Counting Kit-8, and SIHA cells were detected by an optical microscope (6). Jiang study investigated the effects of over-expressed catenin and T cytokine inhibitors on the proliferation and migration of human CC CASKI cells (7). Munagala studied the anti-tumor activity of ginseng ketone IIA against several types of cancer, indicating that ginseng ketone IIA can effectively inhibit the proliferation of human CC CASKI, SIHA, HeLa and C33a cells (8). Wen evaluated the effectiveness of chemotherapy as an adjuvant treatment for stage IB-IIB CC with moderate risk factors through a retrospective analysis of a radical hysterectomy and pelvic lymph nodes from December 1997 to September 2010 (9).

There are many studies on how miR-494 inhibits the proliferation, migration and invasion of CC cells...
by regulating the LETMD1 factor (10). Zhou Quanquan polymerase chain reaction was used to detect the expression of miR-494 in CC tissues and cell lines (11). Chengyan research investigated the potential role of miR-494 and its targets in regulating CC development and chemotherapy resistance (12). Zamani studied human papillomavirus (HPV) infection and differences in the expression patterns of miR-21 and miR-29a in different grades (13). Qureshi said in the study that with cancer metastasis, the prognosis of CC patients is significantly reduced epithelial to mesenchymal transition (EMT) plays an important role in the progression and metastasis of CC (14).

The primary tumor research in this study is mainly to select 40 female patients with advanced CC who were admitted to a hospital from June 2015 to September 2018. Both tumor sections and cancer tissue sections were were taken from the normal tissue of the primary tumor of female CC. At the same time, by collecting specimens of normal tumor tissue near the edge of the cut tumor as a clinical control, the normal tumor tissue was confirmed by detailed pathological analysis after surgery. The results of this research show that, depending on the patient’s gender, age, tumor size, and primary tumor site, the expression level of miR-494 increased by 15%, and the prognostic survival probability increased by 20%. The vascular infiltration of CC cells was reduced by 33%, the distant metastasis of cells was reduced by 11%, and the survival time of patients was significantly prolonged.

The innovation of this research lies in the application of the luciferase reporter gene to verify the expression of the LETMD1 gene, which improves detection accuracy and data reliability. In addition, this study also innovatively uses the ANOVA analysis method to compare the differences between multiple sets of data, and the test method is used to compare the two sets of data, with P<0.05 as the difference is statistically significant.

Materials and methods

Experimental objects and experimental materials

Participants collected 40 female patients with primary CC who were admitted to a hospital from June 2015 to September 2018. The specimens of tumor tissues were taken from the normal tissues of the primary tumor of CC. At the same time, the specimens of normal tumor tissues near the edges of the cut tumor were collected as experimental controls for cellular tissue invasion (15).

The main reagents and materials used in this experiment are CC cell line Hela cells, Lonza transfection reagent, M-MLV reverse transcriptase, Trizell, SYBR Premix Ex Taq enzyme, PCR primers, EB (ethidium bromide), DTT, 10xPCR buffer, 5xcDNA synthesis buffer, Taq DNA polymerase, Prime Script RT reagent kit, SY BR Premix Extant II, etc. The main instruments used are micropipette, Milli Q Plus super pure water instrument, Light Cycler 480 System, Applied Biosystems, etc. Other auxiliary instruments are needed for this experiment, as shown in Table 1.

<table>
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<th>Name</th>
<th>Amount</th>
<th>Producer</th>
</tr>
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<tbody>
<tr>
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<td>German sigma Co., Ltd.</td>
</tr>
<tr>
<td>Autoclave</td>
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<td>Astell company</td>
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<tr>
<td>Electronic balance</td>
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<td>Sartorius Co., Ltd.</td>
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<td>Ultra low-temperature</td>
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<td>Nanodrop ND-1000</td>
<td>1</td>
<td>Nanodrop Technology</td>
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Extraction of RNA from CC tissue

Taking CC tissue samples and marginal normal tissue samples (1 cm2), grind them thoroughly with a homogenizer, add an appropriate amount of triazole reagent, and mix by pipetting repeatedly, and then let stand at room temperature for 6 minutes. Then add an appropriate volume of chloroform to the liquid (the volume ratio of chloroform to triazole is 3:1). After centrifugation, the red phenol-chloroform layer can be seen, and the RNA is distributed in the uppermost layer (16). Add 1 mL of isopropanol to the tube to precipitate RNA. After mixing, let stand at room temperature for 10 min. Centrifuge at 1500 reprint for 10 min at 4°C. After centrifugation, a white precipitate can be seen placed on the bottom of the EP tube, the supernatant is poured, 70% alcohol is added, the supernatant is decanted after 10 minutes of centrifugation, 70% alcohol is added again to shake and wash, and centrifuged at 10,000 rpm for 10
minutes. Then add 15uL of RNase-free water to fully dissolve. After dilution, measure the RNA concentration by spectrophotometer and store in -80°C refrigerator. The obtained data were quantitatively analyzed by comparing the CT value method (17).

**Cloning and wound healing experiments**

Clone formation experiment: Hela cells transfected with miR-494 and anti-miR-494 were inoculated into 8-well plates 6x10^3 each. After 3 days of culture, the clones formed were fixed with methanol, and then the number of clones formed was counted, as Formula 1.

\[
\text{Clones per board} = \frac{n \times 6 \times 10^3}{16}
\]

Where \( n \) is the number of templates used for PCR.

The wound healing experiment used the wound healing experiment method to detect the miR-494 migration ability of Hela cells. Bioinformatics method predicts miR-494 target gene. Through Turgescent target gene prediction software, LETMD1 was selected as the target gene of miR-494 (18).

**Luciferase reporter gene validates LETMD1 gene**

Luciferase is a reporter gene verification of target genes. According to the Targets cans target gene prediction software, the 565-734 position of the 3'UTR of LETMD1 is a possible point of action for miR-494, which was synthesized in vitro DNA fragments of site mutants (19). The PMIR vector and miR-494 were co-transfected into the pre-cultured Hela cells. After 4 days, the luciferase activity was detected. The comparison between multiple sets of data was analyzed using the anoia analysis method, and the comparison between the two sets of data was using: the test method, with \( P<0.05 \) being considered statistically significant (20).

**Results and discussion**

**Expression of miR-494 in CC Tissues and Cell Lines**

The results of the study showed that the number of benign cell pellets expressed by miR-494 in normal typical CC benign tumor cell tissue (experimental group) was significantly higher than that in normal benign CC tumor tissue (control group) reduced (\( p<0.01 \)). In addition, the expression rate of miR-494 in normal malignant CC cells and tumor cell tissues and the number of cell bodies were significantly reduced (\( p<0.05 \)). The expression of miR-494 in the cells of the experimental group and the control group will affect the viability of the cells, the number of cell growth, the migration ability and the invasion ability. The specific data are shown in Table 2.

<table>
<thead>
<tr>
<th></th>
<th>EG</th>
<th>CG</th>
<th>P</th>
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</thead>
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<tr>
<td>A</td>
<td>99.28±3.41</td>
<td>133.42±5.33</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>B</td>
<td>223±5.66</td>
<td>345±1.28</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>66.35</td>
<td>89.88</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>D</td>
<td>55%</td>
<td>86%</td>
<td>P&lt;0.05</td>
</tr>
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</table>

**Analysis of Independent Risk Factors for Prognosis of CC**

In order to determine whether miR-494 is an independent risk factor for the prognosis of CC, this study used an independent COX model to analyze the influencing factors of CC OS, such as gender, age, tumor size and tumor location. As shown in Figure 1, according to gender, age, different tumor size and tumor location, miR-494 expression level increased by 15%, postoperative prognosis survival probability increased by 20%.

**Figure 1.** Multivariate analysis chart of OS in CC patients

**Analysis of Risk Assessment of CC Patients**

The results showed that patients in the miR-494 high expression group were expected to survive longer, indicating that the prognostic performance of CC patients was directly related to the expression level of miR-494. Vascular invasion, distant metastasis, TNM staging, degree of differentiation, and miR-494 mRNA expression are independent factors that affect the prognostic performance of CC.
patients. As shown in Figure 2, the blood vessels of CC cells infiltration were reduced by 33%, distant metastasis rate was reduced by 11%, and patient survival time was significantly prolonged.

**Figure 2.** CC patient risk assessment analysis chart

**LETMD1 Expression Level Test Results**

The test results show that after the expression of LETMD1, it has a certain effect on the proliferation, differentiation and migration of the Hela cell line. When miR-494 is normally expressed, the proliferation, differentiation, and migration of Hela cell lines gradually increase with time, reaching 1.74 on the fifth day. When miR-494 is not expressed, the proliferation, differentiation, and migration of Hela cell lines are decreasing. It was only 0.91 at 5 days, and when miR-494 regulated the expression of LETMD1, the proliferation, differentiation and migration of the Hela cell line at day 5 was only 0.66. As shown in Figure 3, when miR-494 regulated the expression of LETMD1, the proliferation, differentiation and migration rate of the Hela cell line was 0.22 on the first day, and only increased by 0.44 by the fifth day.

**Figure 3.** Effect of LETMD1 expression on Hela cells

**Analysis of the Activity of Different Cells in CC**

Studies have shown that after miR-494 regulates the expression of LETMD1, the proportion of different cells in CC tissue has also changed. The rate of cancer cell proliferation, migration and invasion was reduced by an average of 15%. CC cells accounted for the most, reaching 54%, and Cyclin E cells accounted for the least, reaching 2%. Others included red blood cells, platelets, macrophages, dendritic cells and intercellular adhesion molecule-1. As shown in Figure 4, after the expression of LETMD1, the proportion of cancer cells decreased by 5%, the proportion of macrophages increased by 2%, and the dendritic cells increased by 3%.

**Figure 4.** The proportion of different cells in CC tissue after miR-494 regulates the expression of LETMD1

Leucine zipper EF-hand domain transmembrane protein 1 (LETMD1) was originally called human CC oncogene (HCCR). It was used by scientists to show the reverse transcription-polymerase chain reaction (RT-PCR) technology from the cervix differentially expressed genes from cancer tissue screening (14). Existing studies have confirmed that LETMD1 is an oncogene, which is overexpressed in a variety of malignant tumors in humans, especially in the early stage of malignant tumors, there is an increase in gene expression levels, so it can be predicted that this gene antibody can be widely used as an early stage of malignant tumor markers for gene diagnosis and treatment and important roles and targets of gene therapy (21). In this paper, the gene anti-letimide monoclonal/polyclonal gene antibody is initially applied to the treatment and early clinical diagnosis of malignant hepatocellular carcinoma, and a specific and sensitive system of early diagnosis and treatment of malignant tumor hepatocellular carcinoma is
established for it provides an experimental theoretical basis (22). Taking CC cell line Hela as the research object, the effect of miR-494 on the biological characteristics of CC cell proliferation, apoptosis and chemotherapy sensitivity was confirmed (23). The correlation analysis of miR-494 and LETMD1 expression was carried out to clarify that miR-494 targeted and regulated the expression of LETMD1, and the small fragment interfering RNA (siRNA) technology was used to inhibit the expression of the LETMD1 gene. As shown in Figure 5, by studying the proliferation of PANC-1 cells after small fragment interference, cycle, apoptosis, invasion and metastasis and other biological characteristics, further clarify the mechanism of action of miR-494 by regulating LETMD1 (24).

Figure 5. Diagram of the mechanism of action of miR-494 in regulating LETMD1

In this paper, clinical CC tissues and normal marginal cervical tissues were collected, and the monoclonal antibodies prepared using Western blot technology and immunohistochemistry technology were used to detect the specific expression of LETMD1 expression products in the collected tissue samples (25). The results of the study predict that LETMD1 is abnormally expressed in CC tumor tissues of healthy people. The number of normal expressions of LETMD1 in cervical tissues of healthy people with sclerosis is significantly less than that of CC tumors organization (26). However, this still needs to be verified by studying a large number of clinical specimens or animal models of different processes of cervical cell carcinoma (27-29). The monoclonal antibody prepared in this study has a high specificity of anti-LETMDI, and the threshold of use is low, so it can be successfully applied to actual clinical research (30).

Through the analysis of the relationship between the expression of miR-494 tumor cells and the clinicopathological characteristics of patients with pancreatic cancer malignancies and the correlation of clinicopathology, it was found that the level of miR-494 expression was related to the age, tumor tissue size, and distance of pancreatic cancer malignant tumor patients. Lymphatic metastasis is closely related, but there is no obvious correlation with the degree of pancreatic cancer tumor tissue cell differentiation, tumor location, and nerve invasion.

According to the research results, it was found that restoring the expression of miR-494 in pancreatic cancer cells can significantly inhibit the proliferation of CC cells, promote aging and apoptosis, and induce G1 phase arrest. Depending on gender, age, tumor size, and tumor site, the expression level of miR-494 increased by 15%, and the postoperative survival probability increased by 20%. The vascular invasion of CC cells was reduced by 33%, and distant metastasis was reduced by 11%, and the survival time of patients was significantly prolonged. When miR-494 regulated the expression of LETMD1, the proliferation, differentiation and migration rate of the Hela cell line on day 1 was 0.22, and by day 5 it only increased by 0.44. After the expression of LETMD1, the proportion of cancer cells decreased by 5%, the proportion of macrophages increased by 2%, and the dendritic cells increased by 3%.

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None.

Interest conflict
The authors declare no conflict of interest.

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