The Relationship Between microRNA-195 and the Prognosis of Cervical Cancer

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Abstract

Background: MicroRNA-195 (miR-195), a tumor suppressor, had reported to be involved in carcinogenesis and the progression of some cancers. However, the prognostic value of miR-195 in cervical cancer remained unclear. The purpose of this study was to detect the expression of miR-195 in cervical cancer tissues and to investigate its correlation with tumor progression and prognosis.

Methods: Quantitative real-time polymerase chain reaction (qRT-PCR) was used to detect the relative mRNA expression of miR-195 in cervical cancer tissues and corresponding adjacent normal tissues. The relationship between miR-195 expression and clinical characteristics of patients was analyzed by chi-square test. Kaplan-Meier method was applied to compare the overall survival, and the prognostic value of miR-195 was estimated via cox regression analysis.

Results: Compared with normal tissues, miR-195 expression was significantly down-regulated in cervical cancer tissues ($P < 0.001$). Importantly, decreased expression of miR-195 was closely associated with FIGO stage, lymph node metastasis and vascular invasion ($P < 0.05$). Additionally, Kaplan-Meier analysis indicated that patients with high miR-195 expression had obviously longer overall survival than those with low miR-195 expression (log rank test, $P = 0.001$). And miR-195 was an independent prognostic factor of cervical cancer patients via univariate and multivariate cox regression analyses.

Conclusions: Decreased expression of miR-195 is associated with the progression of cervical cancer. And miR-195 may have potency to predict the prognosis of cervical cancer.

Background

As a most frequent malignant tumor among women, cervical cancer is one of the causes of cancer-related deaths in the world [1]. A large amount of cases are diagnosed with cervical cancer every year [2]. It is a big problem that damages the health of females. Although the screening methods and therapies have been improved, the clinical outcome is still poor [3]. Accumulated evidence has indicated that the International Federation of Gynaecology and Obstetrics (FIGO) stage, tumor size and lymph node metastasis are the independent prognostic factors of cervical cancer, but these clinical factors have some limitations for predicting the prognosis of patients [4, 5]. Therefore, identification of novel bio-markers is urgently needed to improve the diagnosis, treatment and prognosis of cervical cancer.

MicroRNAs (MiRNAs) are small endogenous non-coding RNAs that can regulate the expression of genes at the post-transcriptional level [6]. It had been reported that miRNAs could be involved in several physiological processes, such as cell proliferation, differentiation and cell cycle [7]. Besides, the aberrant expression of miRNAs had been shown to play important roles in the pathogenesis and progression of a variety types of human cancers [8]. Among these miRNAs, miR-195, belonging to the miR-15/16/195/424/497 family, is located on human chromosome 17p13.1 which is a frequently deleted region in tumors. A large number of studies had demonstrated that miR-195 was abnormally expressed in various tumors [9, 10]. Moreover, miR-195 had been proven to be a tumor suppressor of cancers and
inhibit tumor cell growth, invasion, migration and colony formation [11, 12]. It also revealed that the expression of miR-195 was found to be down-regulated in cell lines, serum and tissue samples of cervical cancer, and serum miR-195 could be a potential bio-marker for the diagnosis of this tumor [13, 14]. However, little is known about the molecular mechanism and the prognostic value of miR-195 in cervical cancer.

In this study, we detected the relative miR-195 expression in cervical cancer tissues and corresponding adjacent normal tissues. And the correlation between miR-195 expression and clinicopathological features of patients was analyzed. We also estimated the prognostic value of miR-195 in cervical cancer.

Methods

Study subjects and samples

In this study, a total of 123 cervical cancer patients who underwent surgery were enrolled from Harrison International Peace Hospital. Histopathogical diagnosis was confirmed by the Department of Oncology. None of patients had received radiotherapy, chemotherapy or any other treatments before surgery. This study was approved by the Ethical Committee of the hospital and the written informed consents were obtained from all patients in advance.

The paired tumor tissues and corresponding adjacent normal tissues were collected from patients with cervical cancer. These specimens were immediately frozen in liquid nitrogen and stored at −80°C for RNA extraction. The detail clinicopathological characteristics of patients were shown in Table 1. A 5 years’ follow-up was conducted via a telephone or questionnaires. Patients who died from other diseases or unexpected events should be excluded from this study.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs were isolated from tissue samples using Trizol reagent (Invitrogen, USA). TaqMan microRNA Reverse Transcription Kit (Applied Biosystems, USA) was used to synthesize the first strand cDNA. The relative mRNA expression of miR-195 was detected by miScript SYBR Green PCR kit (Qiagen, USA). U6 was utilized as internal control and 2^(-ΔΔCt) method was used to calculate the relative quantification of miR-195 expression. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were performed with SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) or GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA, USA). The data were expressed as mean ± standard deviation (SD). Student’s t-test was used to evaluate the difference between two groups. And
chi-square test was applied to analyze the correlations between miR-195 expression and clinical characteristics of patients. The overall survival of patients with different miR-195 expression was compared via Kaplan-Meier analysis. Univariate and multivariate cox regression analyses were used to estimate the prognostic value of miR-195 in cervical cancer. \( P < 0.05 \) was considered as statistically significant.

**Results**

**The expression of miR-195 was down-regulated in cervical cancer**

The relative mRNA expression levels of miR-195 in cervical cancer tissues and adjacent normal tissues were detected by qRT-PCR. It showed that miR-195 expression was obviously lower in cervical cancer tissues than that in normal tissues at mRNA level \( (P < 0.001, \text{Figure 1}) \).

**Relationship between miR-195 expression and clinical features of patients with cervical cancer**

To explore the involvement of miR-195 in the development of cervical cancer, we analyzed the correlation between miR-195 and clinical characteristics of patients. The detail clinical characteristics of cervical cancer patients were shown in Table 1. 123 female patients had a mean age of 55.15 ± 14.54 years (range, 35 - 73 years). The median miR-195 expression level was used as a cutoff value to divide all 123 patients into two groups: high miR-195 expression group \( (n = 61) \) and low miR-195 expression group \( (n = 62) \). As shown in Table 1, the low expression of miR-195 was significantly associated with FIGO stage \( (P = 0.009) \), lymph node metastasis \( (P = 0.009) \), and vascular invasion \( (P = 0.002) \). However, no obvious correlation was found between miR-195 expression and age, tumor size or histological grade \( (P > 0.05, \text{Table 1}) \).
### Table 1
Relationship between miR-195 expression and clinical features of patients with cervical cancer

| Clinical Features          | Cases (n = 123) | MiR-195 expression | 2   | P    |
|---------------------------|-----------------|--------------------|-----|------|
|                           |                 | Low (n = 62)       |     |      |
|                           |                 | High (n = 61)      |     |      |
| Age (years)               |                 |                    | 1.821 | 0.177 |
| ≤ 55                      | 57              | 25                 | 32  |      |
| > 55                      | 66              | 37                 | 29  |      |
| Tumor size (cm)           |                 |                    | 3.280 | 0.07  |
| ≤ 4                       | 62              | 26                 | 36  |      |
| > 4                       | 60              | 35                 | 25  |      |
| Histological differention|                 |                    | 2.933 | 0.087 |
| Well/Moderate             | 61              | 26                 | 35  |      |
| Poor                      | 62              | 36                 | 26  |      |
| FIGO stage                |                 |                    | 6.909 | 0.009 |
| b⁻a                       | 66              | 26                 | 40  |      |
| b⁻a                       | 57              | 36                 | 21  |      |
| Lymph node metastasis     |                 |                    | 6.868 | 0.009 |
| Negative                  | 64              | 25                 | 39  |      |
| Positive                  | 59              | 37                 | 22  |      |
| Vascular invasion         |                 |                    | 8.846 | 0.003 |
| Absent                    | 60              | 22                 | 38  |      |
| Present                   | 63              | 40                 | 23  |      |

Decreased expression of miR-195 was associated with poor prognosis of cervical cancer patients

To investigate the prognostic value of miR-195 in cervical cancer patients, a follow-up was performed with 5 years. The result of Kaplan-Meier analysis revealed that low expression of miR-195 was strongly...
correlated with shorter overall survival of patients (log rank test, $P = 0.001$, Figure 2). In addition, miR-195 expression was found to be linked with the prognosis and it might be an independent prognostic factors of cervical cancer patients according to univariate and multivariate cox regression analyses (HR = 2.148, 95% CI = 1.082 - 4.266, $P = 0.029$, Table 2).

Table 2
Univariate and multivariate cox regression analyses of prognostic factors in patients with cervical cancer

| Factors                              | Univariate analysis | Multivariate analysis |
|--------------------------------------|---------------------|-----------------------|
|                                      | HR                  | 95% CI                | $P$       | HR      | 95% CI    | $P$ |
| MiR-195 (Low vs High)                | 3.043               | 1.562 - 5.929         | 0.029     | 2.148   | 1.082 - 4.266 | 0.029 |
| Age (years)                          | 1.795               | 0.957 - 3.368         | 0.068     | -       | -         | -   |
| (> 55 vs ≤ 55)                       |                     |                       |           |         |           |     |
| Tumor size (cm)                      | 2.706               | 1.410 - 5.193         | 0.003     | -       | -         | -   |
| (> 4 vs ≤ 4)                         |                     |                       |           |         |           |     |
| Histological grade                  | 0.300               | 0.154 - 0.586         | 0.000     | -       | -         | -   |
| (Poor vs Well/Moderate)              |                     |                       |           |         |           |     |
| FIGO stage                           | 4.015               | 2.057 - 7.835         | 0.000     | 2.260   | 1.119 - 4.564 | 0.023 |
| (³b-¹a vs ³b-¹a)                     |                     |                       |           |         |           |     |
| Lymph node metastasis               | 0.179               | 0.086 - 0.374         | 0.000     | 0.256   | 0.120 - 0.548 | 0.000 |
| (Positive vs Negative)               |                     |                       |           |         |           |     |
| Vascular invasion                   | 0.357               | 0.186 - 0.685         | 0.002     | -       | -         | -   |
| (Present vs Absent)                  |                     |                       |           |         |           |     |

Note: - indicated no data.

Discussion

In the present study, the relative mRNA expression levels of miR-195 in cervical cancer tissues and corresponding adjacent normal tissues were detected. The result showed that miR-195 expression was significantly reduced in cervical cancer tissues compared with that in normal tissues at mRNA level. And its low expression was correlated with the development of cervical cancer. Moreover, we found that miR-195 expression was closely related to clinical outcome of this tumor.
Previous studies had demonstrated that some miRNAs were associated with tumorigenesis and development of various cancers [7, 15]. Due to the differences of target genes, these miRNAs could act as oncogenes or tumor suppressors in a various types of human tumors [16]. As a member of miRNAs, the abnormal expression of miR-195 was more commonly occurred in many cancers. Serum miR-195 was considered as a potential bio-marker for the diagnosis, therapy and prognosis of cancers [17, 18]. Moreover, Deng et al. mentioned that miR-195 expression was reduced in cell lines and tissues of gastric cancer, and inhibited tumor growth through epigenetical regulation in this tumor [19]. Decreased expression of miR-195 in cervical cancer cells and tissues was indicated to promote cell invasion and migration via targeting Smad3 [20]. These studies were consistent with our result, suggesting that miR-195 played a tumor suppressive role and exhibited tissue-specific in human tumors, including cervical cancer.

A large number of researches had indicated that over-expression of miR-195 could inhibit tumor cell proliferation, invasion and migration in vitro, implying that miR-195 was involved in tumor growth and metastasis, including cervical cancer [20-22]. However, the relationship between miR-195 and the progression of cervical cancer in clinical practice needs much further studies. In this study, a notable correlation was found between low miR-195 expression and advanced FIGO stage, positive lymph node metastasis and present vascular invasion which were some aggressive clinical characteristics representing advanced progression and metastasis of cancers. Our finding suggested that miR-195 expression was associated with the progression of cervical cancer. Similar results were observed in gastric cancer and osteosarcoma [23, 24]. Nevertheless, a study of Song et al. reported that miR-195 expression was strongly correlated with tumor size and histological grade, but not linked with tumor stage of colorectal cancer patients, which was inconsistent with our study [25]. The functions of miR-195 in the development of different cancers might be varied. Thus, given these limitations of sample size and its source, further studies are needed to explore the role of miR-195 in the progression of human tumors.

The prediction of clinical outcome of cervical cancer is very important to provide better therapeutic strategies for patients. FIGO stage and lymph node metastasis had been proven to be independent prognostic factors of cervical cancer [26]. They were indicated to be correlated with miR-195 expression in this study, suggesting that miR-195 might be related to the prognosis of cervical cancer. We found that patients with high expression of miR-195 tended to have longer overall survival than those with low miR-195 expression via Kaplan-Meier analysis. Univariate and multivariate analyses revealed miR-195 might have potency to be an independent factor for the prognosis of cervical cancer. A growing body of evidence had demonstrated that miR-195 expression was closely associated with the overall survival of human tumors, which was similar to our result [27, 28].

**Conclusion**

In conclusion, the decreased expression of miR-195 is associated with the progression of cervical cancer. What's more, miR-195 may have potential application for the prognosis of breast cancer. However, the
exact molecular mechanism by which miR-195 regulated the progression of cervical cancer remains poorly known.

**Declarations**

**Ethics approval and consent to participate:**

This study was supported by the Ethics Committee of Harrison International Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

**Consent for publication:**

The patients provided written informed consent for the publication of any associated data and accompanying images

**Availability of data and materials:**

All data generated or analysed during this study are included in this article.

**Competing interests:**

The authors declare that they have no competing interests.

**Funding:**

Not applicable.

**Authors' contributions:**

S.C. conceived and designed the experiments, analyzed the data, and wrote the paper. L.Z. performed the experiments. All authors read and approved the final manuscript.

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Figures

Figure 1

The relative expression of miR-195 in cervical cancer tissues and adjacent normal tissues. Compared with normal tissues, miR-195 expression was significantly reduced in tumor tissues (*** indicated P less than 0.001).
Figure 2

Kaplan-Meier curve analysis for the overall survival of patients with cervical cancer. The overall survival of patients with low expression of miR-195 was markedly shorter than those with high miR-195 expression (log rank test, P = 0.002).