RESEARCH ARTICLE

# Involvement of MicroRNA-198 Overexpression in the Poor Prognosis of Esophageal Cancer

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## Abstract

**Objective: This study aimed to investigate whether the miR-198 expression level is related to clinicopathological factors and prognosis of esophageal cancer. Methods: MicroRNA was extracted from esophageal cancer patients who underwent surgery for assessment using the Taqman@ MicroRNA assay. The correlation between miR-198 expression and clinicopathological features was analyzed, and the significance of miR-198 as a prognostic factor and its relationship with survival was determined. Results: MicroRNA-198 (miR-198) expression was higher in patients with poor prognosis than those with good prognosis ($P < 0.05$). Kaplan-Meier analysis results showed that the miR-198 expression level had a significant correlation with survival time ($P = 0.030$) and that patients with a higher expression of miR-198 had a shorter survival time. Cox multi-factor model analysis showed that patient prognosis ($P = 0.014$), tumor length ($P = 0.040$) and expression ($P = 0.012$), and survival time had a significant correlation; the corresponding risks were 7.268, 1.246, and 3.524, respectively. Conclusion: miR-198 overexpression is involved in the poor prognosis of esophageal cancer and can be used as a biomarker for selection of cases requiring especial attention.**

**Keywords:** Esophageal cancer - microRNA-198 - prognosis

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## Introduction

Esophageal cancer (EC) is one of the eight most common tumors in the world. The incidence of EC is high in China, and mortality due to EC is also high in the world, with an average case fatality rate of 15.59/10,0000 (Parkin et al., 2005). The disease is usually determined by clinical diagnosis in the middle-late period, and the five-year survival rate is lower than 20%. Currently, its clinical treatments mainly include surgery, radiation therapy, and chemotherapy. However, the disease has a low survival rate. To determine a specific biomarker at the gene level and establish effective prevention, obtaining an early diagnosis method has become one of the research focuses in the field of tumor and EC prevention (Matsushima et al., 2010).

MicroRNA (miRNA) is a kind of non-coding, single-strand RNA molecule with a nucleotide length of 2 l to 25 and is widely found in plants, animals, and viruses (Lee et al., 1993). miRNA can regulate approximately 60% of human genes (Hobert, 2008; Liang et al., 2009). As much knowledge about the formation and function of biological characteristics of the miRNA has been accumulated, miRNA has been found to be closely related to the formation of tumors. miRNA is usually located in cancer-related genome areas, that is, the genome instability regions, which express abnormality in almost all tumors, participate in the basic signal transduction pathways, and regulate many important cancer-related gene expressions by regulating mRNA translation (Zhou et al., 2009; He et al., 2012; Yang et al., 2013). Previous studies show that 46 miRNAs have abnormal expressions, seven of which exhibit significant differences. Moreover, the high expression of hsa-miR-103/107 is closely correlated with a low survival rate. To date, hsa-miR-335, hsa-miR-181d, hsa-miR-25, hsa-miR-7, and hsa-miR-495 have been confirmed to be correlated with the histopathological stages of EC. Moreover, abnormal miRNA expression spectra are different between esophageal squamous cell carcinoma (ESCC) tissues and esophageal adenocarcinoma tissues (Lin et al., 2012; Xu et al., 2012).

In clinical practice, we found that EC patients at the same pathological stage who received the same surgical therapy by the same surgeon had distinct prognoses. However, whether the different miRNA expression among patients is what leads to the different postoperative prognoses remains to be explored.

The current study was performed to understand the

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biological role of miR-198 expression in EC by examining the relationship between the miR-198 expression and the clinicopathological characteristics and survival in EC patients.

## Materials and Methods

*Tissue samples*

Forty-six patients in the same pathological stage but with different prognoses in stages I to III and who received the same treatment at the First Affiliated Hospital of Xinxiang Medical University between September 2006 and December 2009 were enrolled in this study. Among the 46 patients, 18 had good prognoses, whereas the other 28 had poor prognoses. Paracancerous normal esophageal mucous membranes, 8 cm distant to the verge of the tumor tissue, were taken as controls. This study was conducted in accordance with the declaration of Helsinki and with approval from the Ethics Committee of Xinxiang Medical University. Written informed consent was obtained from all participants.

*Sample treatment and RNA extraction*

Total RNA was extracted using TRI Reagent® (Applied Biosystems, Foster City, USA) according to the kit instructions. Briefly, 0.75 mL of TRI Reagent® was added and incubated at room temperature for 5 min. After adding 0.5 mL of isopropanol and mixing well, the samples were stored for 2 h at -20 °C and centrifuged at 14,000 rpm for 15 min (4 °C). The supernatant was removed, and then 1 mL of 70% ice-cold ethanol was added to dissolve the pellet. The mixture was subsequently re-centrifuged, and the supernatant was discarded again. The final pellet was dissolved in 50 $\mu$L of DEPC H2O. The RNA yield was determined using an ultraviolet spectrophotometer.

*Real-time quantitative reverse transcription polymerase chain reaction*

Amounts of 0.5 $\mu$g were used in a reverse transcriptase reaction. After reverse transcription, a TaqMan® MicroRNA Assay (Applied Biosystems) was performed according to the protocol of the manufacturer to detect the miR-198 expression with Applied Biosystems using miR-198 as the primer and small nRNA, RNU6 (Assay ID: 001093) as the control. The relative quantitative method was used. Gene expression was quantified based on the following formula: $F = 2^{-\triangle\triangle ct}$, where $\triangle\triangle ct$ = (ct mean of the target gene in the test sample – that of the housekeeping gene in the test sample) – (ct mean of the target gene in the control sample – that of the housekeeping gene in the control sample). A higher F value indicated higher expression.

*Statistical analysis*

The relationship between the miR-198 expression level and the clinicopathological features of the patients was analyzed using the chi-square test. SPSS17.0 was used to establish and analyze the database. Kaplan-Meier analysis was used to determine the relationship between microRNA expression and survival time. COX regression model was used to analyze the influence of related factors on the survival time of patients with EC. $P < 0.05$ was considered statistically significant.

**Table 1. Patient Characteristics**

| Characters | No.of patients (%) |
|---|---|
| All Patients | 46(100.0) |
| Sex | |
| Male | 23(50.0) |
| Female | 23(50.0) |
| Age | |
| ≥60years | 21(45.7) |
| <60years | 25(54.3) |
| Site of tumor | |
| Upper thoracic | 4(8.7) |
| Middle thoracic | 35(76.1) |
| Lower thoracic | 7(15.2) |
| Size of tumor | |
| ≤5cm | 27(58.7) |
| >5cm | 19(41.3) |
| Differentiation | |
| Well | 11(16.3) |
| Moderate | 19(41.3) |
| Poor | 16(34.8) |
| Lymph node metastasis | |
| Negative | 27(58.7) |
| Positive | 19(41.3) |
| Depth of invasion | |
| Tis, T1 | 8(17.4) |
| T2 | 6(13.0) |
| T3 | 32(69.6) |
| Stage (TNM) | |
| 0-I | 7(15.2) |
| IIa-IIb | 21(45.7) |
| III | 18(39.1) |
| Recurence | |
| Early recurence | 28(60.9) |
| Non recurence | 18(39.1) |

## Results

*Patient characteristics*

Forty-six patients with ESCC (without preoperative radiotherapy and chemotherapy) were enrolled in this study. All patients underwent surgical resection at the Department of Thoracic Surgery of the First Affiliated Hospital of Xinxiang Medical University. All postoperative samples were confirmed for ESCC through histopathological examination. Tumor was staged according to the sixth edition of the International Union Against Cancer staging criteria for EC (2002). The study patients consisted of 23 males and 23 females, with ages ranging from 45 to 71 years (median, 59 years). Nineteen cases had tumor size >5 cm, and 27 cases had ≤5 cm. Eleven cases had good tumor differentiation, 19 cases had middle differentiation, and 16 cases had poor differentiation. Nineteen cases exhibited tumor lymph node metastasis, and 27 cases had no metastasis. One case was in stage 0, 6 cases were in stage I, 20 cases were in stage IIa, 1 case was in stage IIb, and 18 cases were in stage III. There were 28 early recurrence patients (tumor recurrence within 1 year after operation) and 18 non-recurrence patients (patients who survived more than

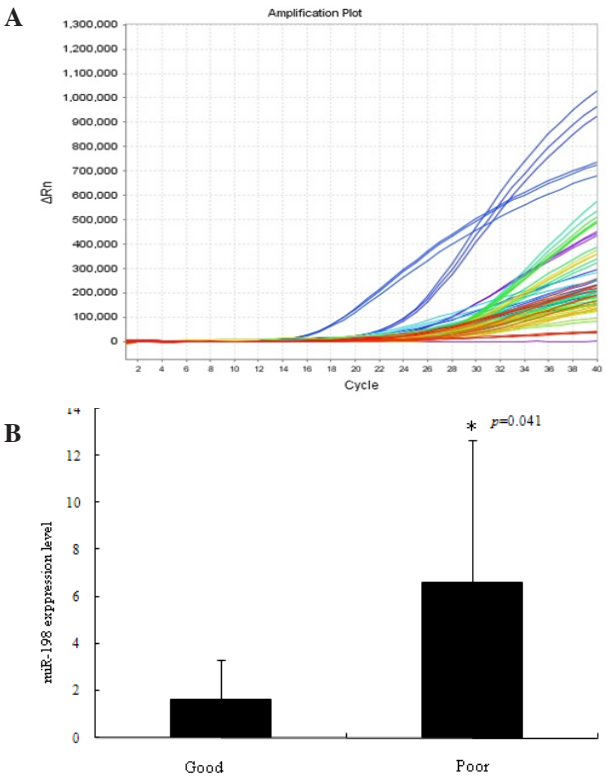


**Figure 1.** A) Amplification plot with △ct values of miR-198 in EC with good and poor prognoses; B) Comparison of miR-198 expression in EC between the sample with good prognosis and that with poor prognosis

5 years without recurrence). The patients' characteristics are outlined in Table 1.

*miR-198 expression in EC*

The results showed that the hsa-miR-198 expression level increased by 5.905-fold in the sample with bad prognosis compared with the sample with good prognosis. The expression levels in the sample with good prognosis were 1.595 ± 1.718, and those in the sample with poor prognosis were 6.584 ± 6.109. The difference between the samples was statistically significant (Figure 1, $P < 0.05$).

*miR-198 expression and prognosis and survival of EC*

The Kaplan-Meier analysis results showed that the miR-198 expression level had a significant correlation with survival time ($P = 0.030$) and that patients with a higher expression of mir-198 had a longer survival time. The Cox single factor-related risk analysis results showed that patient prognosis ($P = 0.000$), postoperation T ($P = 0.023$), tumor size ($P = 0.020$), miR-198 expression ($P = 0.007$), and patient survival time had a significant correlation. The Cox multi-factor model analysis showed that patient prognosis ($P = 0.014$), tumor length ($P = 0.040$) and expression ($P = 0.012$), and its survival time had a significant correlation, with the corresponding risks of 7.268, 1.246, and 3.524, respectively (Figure 2).

## Discussion

EC patients in the same pathological stage who received the same surgical therapy by the same surgeon could have distinct prognoses. However, whether the

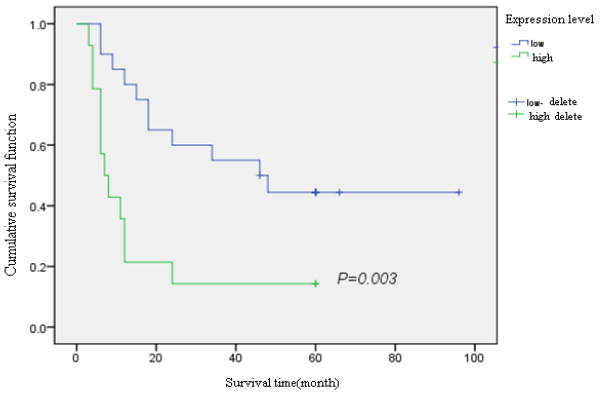


**Figure 2. Relationship Between miR-198 Expression and Survival Time**

different miRNA expressions among patients are what lead to the different postoperative prognoses remains to be explored.

The potential of miRNAs as novel biomarkers is growing, as more studies report the relationship between miRNAs and cancers (Zoon et al., 2009; Yu et al., 2010). However, there are no reports yet on miR-198 in EC. In this study, we found that the miR-198 expressions among EC patients who were in the same pathological stage but with different prognoses and who received treatment were same. miR-198 expression was higher in the sample with poor prognosis and lower in the sample with good prognosis. Moreover, the statistical analysis showed that miR-198 expression had a significant correlation with survival time.

Consistent with our results, previous studies found that miR-198 expression is higher in cancer, including retinoblastoma (Zhao et al., 2009), squamous cell carcinoma of the tongue (Wong et al., 2008), pancreatic adenocarcinoma and ampullary adenocarcinoma (Schultz et al., 2012), and hepatocellular carcinoma (Varnholt et al., 2008). This condition is explained by miR-198 that regulates the Livin expression in cancer cells. Livin has been recently considered an inhibitor of apoptosis, which has been established to be associated with a variety of cancers. Ye et al. found that miR-198 expression is negatively correlated with the Livin expression level in some prostate cancer cell lines. A study reveals that miR-198-mediates the repression of Livin expression (Ye et al., 2013). Tan et al. (2011) showed that miR-198 directly targets c-MET through its 3'UTR. The forced expression of miR-198 decreases the c-MET expression at both mRNA and protein levels, consequently diminishing the HGF-induced phosphorylation of p44/42 MAPK in HCC cells. The forced expression of miR-198 inhibits the HGF promotion of HCC cell migration and invasion in a c-MET dependent manner. miR-198 suppresses HCC cell invasion by negatively regulating the HGF/c-MET pathway. As mentioned previously, studies show that miR-198 expression is significantly elevated compared with normal tissue controls. However, there are no reports about miR-198 and EC yet, especially miR-198 expression in the EC tissue of patients who have the same pathological stage but with different prognoses in stages I to III and who received the same treatment.

In this study, we confirmed the clinical importance

of miR-198 expression by showing its association with unfavorable clinicopathological features. We found that miR-198 is overexpressed in EC with prognosis and that high expression is associated with poor prognostic factors and poor survival. However, this study has several limitations. Although patient selection was consecutive from 2006 to 2009, the lack of materials resulted in a small population for analysis, and selection bias could have occurred. Given the small number of population and short follow-up period, the number of deaths was small, which could have influenced the analysis. Moreover, there is currently no international standardized definition of the methods of analysis and expression levels for miRNAs. Some studies use the mean value of RQ to define the high and low expressions, and other studies have different ways of grouping the expression level. This discordant analysis can lead to disagreement among studies.

In conclusion, our results show that miR-198 is overexpressed in EC with prognosis, and high expression indicates an association with poor prognostic factors and poor survival. To clarify the role of miR-198 as well as its use as a biomarker and in targeting therapy, large worldwide population-based studies with a standard definition of miR-198 expression level are necessary.

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