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Research article

Identification of miRNAs as prognostic factors for esophageal squamous cell carcinoma

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Abstract: *Objective:* The prognostic value of microRNAs for esophageal squamous cell carcinoma (ESCC) is still not be well identified. *Methods:* The microRNA expression profiles of 119 paired ESCC tissue samples and para-carcinoma tissues from GEO database under accession number of GSE43732. A mutation information based feature selection method was applied to identify the discriminative microRNAs between paired ESCC tissues and para-carcinoma tissues. *Results:* In para-carcinoma tissues, patients had better survival with higher has-miR-410 (log-rank p = 0.0123), has-miR-411-5p (log-rank p = 0.0152), has-miR-193b-5p (log-rank p = 0.0188) and has-miR-4486 (log-rank p = 0.0307) expression levels. When compared with para-carcinoma tissues, there has more correlations between miRNA expression levels and survival in tumor tissues. We identified 20 potential miRNAs associated with prognosis. Besides, a heatmap was draw to explore miRNA expression levels in tumor tissues and survival. *Conclusions:* The present study identified 24 miRNAs in 119 paired ESCC tissue samples and para-carcinoma tissues, including 4 miRNAs in para-carcinoma tissues and 20 in tumor tissues, respectively. The dysregulation of these miRNAs were associated with different outcomes. We thought this study could provide novel noninvasive early biomarkers for ESCC patients.

Keywords: esophageal squamous cell carcinoma; microRNAs; prognosis; genes; tumor

Abbreviations: ESCC: esophageal squamous cell carcinoma

1. Introduction

Esophageal squamous cell carcinoma (ESCC) is the major histological type (60–70%) of esophageal cancer, which acts as the fourth most common type of cancer diagnosed in China [1]. However, there remains the geographic and population barriers in China: More than half of ESCC patients occur in China while male patients comprise 80% of cases [2]. Squamous-cell carcinoma arises from the epithelial cells that line the esophagus. Causes of the squamous-cell type include tobacco, alcohol, very hot drinks, poor diet, and chewing betel nut [3]. Clinically, the TNM staging system, based mainly on the anatomical characteristics of tumors, has been applied for predict outcomes of postoperative ESCC patients while patients with the same stage and similar therapeutic strategies have different prognosis [3,4]. The above results could be explained by the heterogeneous nature of cancers. Thus the molecular and genetic features harbored in tumors may differ and act as important prognostic factors for ESCC patients.

MicroRNAs are a set of small RNA molecules, ranging from 19 to 22 nucleotides in length, that often act as negative regulators in gene expression process through degrading the target mRNA or inhibiting translation [5,6]. MicroRNAs are stably expressed in serum, plasma and other body fluids, which could be used as a clinical biomarker for diagnose and prognosis. Hu et al. [7] found miR-1285-3p is a potential prognostic marker in human osteosarcoma and acts as a tumor suppressor by targeting YAP1. MiR-492 was found regulating metastatic properties of hepatoblastoma via CD44 [8]. MiR-129 was demonstrated as a novel therapeutic target and biomarker in gastrointestinal cancer [9].

Currently, microRNA has been identified as a molecular tool for disease diagnose, including lung cancer [10], colorectal cancer [11], heart failure [12] and so on. However, the prognostic value of microRNAs for ESCC is still not be well identified. Therefore, we aimed to explore the prognostic value of a number of microRNAs for ESCC patients. In this study, we extracted and analyzed microRNA expression profiles of 119 paired ESCC tissue samples and para-carcinoma tissues from Transcript Expression Omnibus (GEO) database.

2. Methods

2.1. The microRNA expression files of ESCC tissues and para--carcinoma tissues

We downloaded the microRNA expression profiles of 119 paired ESCC tissue samples and para-carcinoma tissues from GEO database under accession number of GSE43732 [2]. All the patients had a primary ESCC with no distant metastasis and underwent a complete resection. The expression levels of microRNAs were measured with Agilent Human miRNA V19.0 Microarray. The normalized data provided by Chen et al. [2] was used to identify the differentially expressed microRNAs among ESCC tissue samples and para-carcinoma tissues.

2.2. The discriminative microRNAs between paired ESCC tissues and para-carcinoma tissues

In the process of data preprocessing, we preserved microRNAs existed at least a half of samples. For the missing data, the k-nearest neighbors (k-NN) algorithm was used for filling the missing values [13].

A mutation information based feature selection method (mRMR, minimal Redundancy Maximal Relevance, http://home.penglab.com/proj/mRMR/) was applied to identify the discriminative microRNAs between paired ESCC tissues and para-carcinoma tissues [14]. The miRNAs were then

divided into two groups according to the median expression level, and the survival status were further explored between the two groups.

3. Results

In para-carcinoma tissues, there are significant differences between miRNA expression levels and patients survival. Patients had better survival with higher has-miR-410 (log-rank p = 0.0123), has-miR-411-5p (log-rank p = 0.0152), has-miR-193b-5p (log-rank p = 0.0188) and has-miR-4486 (log-rank p = 0.0307) expression levels (Figure 1).

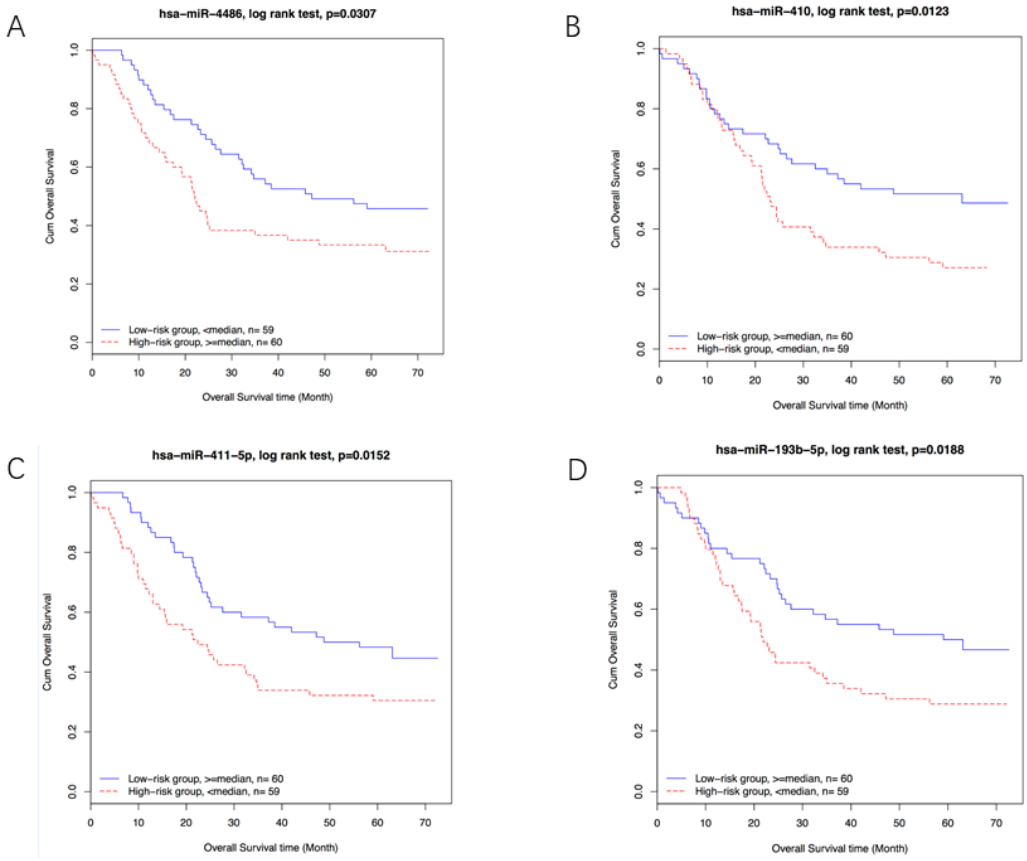

Figure 1. Survival curves for patients with ESCC according to different microRNAs.

When compared with para-carcinoma tissues, there has more correlations between miRNA expression levels and survival in tumor tissues. We found patients owned promising prognosis with higher has-miR-26b-5p (log-rank p = 0.00796), has-miR-181a-2-3p (log-rank p = 0.0103), has-miR-145-3p (log-rank p = 0.0155), has-let-7e-5p (log-rank p = 0.0184), has-miR-195-5p (log-rank p = 0.0229), has-miR-125b-5p (log-rank p = 0.0246), has-miR-151a-5p (log-rank p = 0.0249), has-miR-324-3p (log-rank p = 0.0294), has-miR-146a-5p (log-rank p = 0.032), has-miR-15a-5p (log-rank p = 0.0327), has-miR-1290 (log-rank p = 0.0355), has-miR-205-5p (log-rank p = 0.0393), has-miR-4763-3p (log-rank p = 0.0411), has-miR-198 (log-rank p = 0.0418), has-miR-23b-3p (log-rank p = 0.0437), has-miR-218-5p (log-rank p = 0.0445), has-miR-4324 (log-rank p = 0.045), has-miR-378g (log-rank p = 0.0452), has-miR-143-3p (log-rank p = 0.0464), has-miR-650 (log-rank p = 0.0496) expression levels (Figure S1,

multipage form). Besides, a heatmap was draw to explore miRNA expression levels in tumor tissues and survival. Low risk group was defined as longer follow-up time and alive patients while the high risk group was defined as shorter follow-up time and dead patients (Figure 2).

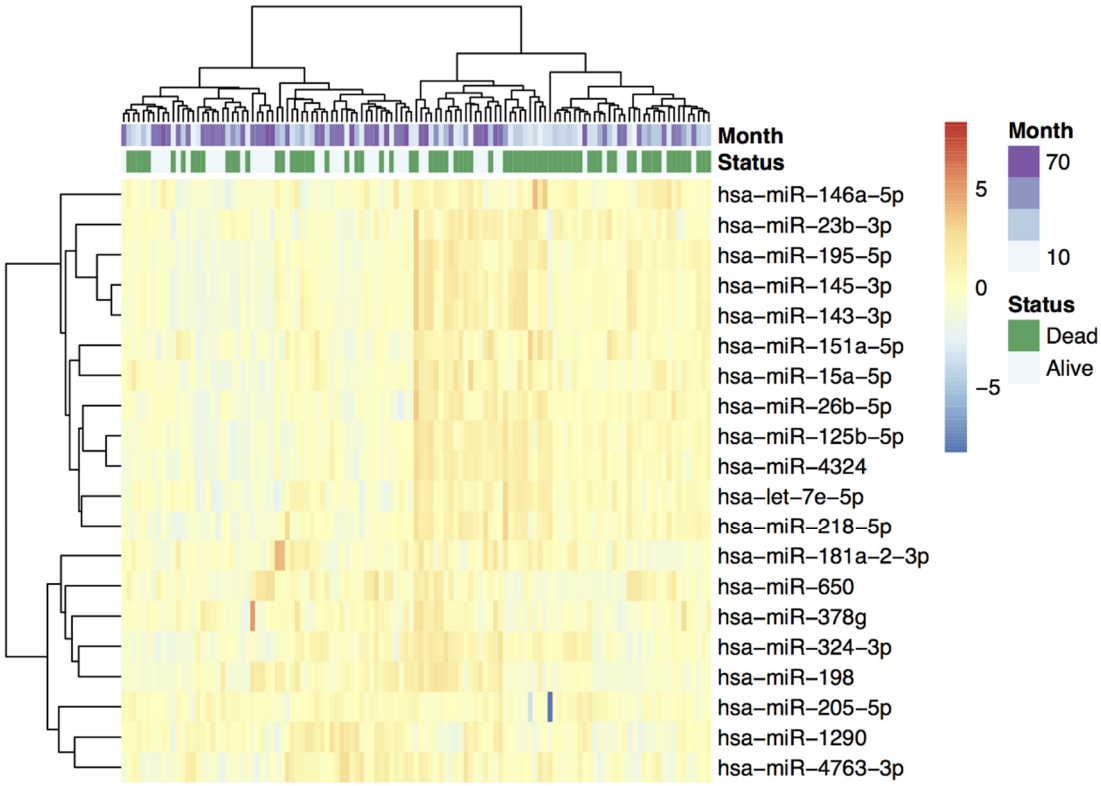

Figure 2. A heatmap of tumor miRNAs and survival status.

The potential target genes of miRNAs in para-carcinoma and tumor tissues, which expression levels were associated with survival, were listed in Tables S1–3, respectively. Interestingly, TEA domain transcription factor 4 (TEAD4) was identified as a potential target gene for hsa-miR-324-3p.

4. Discussion

Esophageal squamous cell carcinoma accounts for about 90% of cases of esophageal cancer worldwide [15]. Although the incidence and mortality rates related to esophageal adenocarcinoma are rising, esophageal squamous cell carcinoma still remains the predominant esophageal cancer subtype in China [1]. As the development of new technology like whole-genome and whole-exome sequencing, eight mutated genes (ADAM29, FAM135B, NFE2L2, PIK3CA, CDKN2A, TP53, RB1 and NOTCH1) were detected in Chinese esophageal squamous cell carcinoma patients [16]. In this article, we explore the potential prognostic factors according to transcriptome data.

TEAD4, a TEA domain transcription factor family member, shares nearly identical DNA-binding domains and that bind the same cis-acting sequence [17]. Besides, the Hippo pathway downstream transcription coactivators YAP and TAZ occupy target gene loci largely through their interactions with TEAD4 [18]. TEAD4 also has critical functions in the process of embryonic development [19,20] and

expresses in various cancers, such as colorectal cancer, lung cancer, oral squamous cell carcinoma and so on [21–23]. Besides, it has been proved TEAD4 promotes cellular growth, metastasis, and poor survival outcomes, suggesting TEAD4 could act as a potential key molecule in cancer therapy [21]. In our study, TEAD4 was identified as a potential target gene for hsa-miR-324-3p. Hsa-miR-324-3p was reported had relationship with acute lymphoblastic leukemia [24] and acted as a novel biomarker for detection of early stage breast cancer [25].

MiRNA-26b-5p was reported associated with breast cancer and radiation exposure [26,27]. Has-miR-26b-5p was also recognized as a tumor suppressor through promotion of apoptosis and suppression of cell growth [28]. In sporadic breast cancer, decreased hsa-miR-26b-5p expression was reported [29]. Has-miR-181a-2-3p was identified as a potential prognostic factor for papillary thyroid cancer using TCGA database [30]. Slattery et al. [31] demonstrated that microRNAs have been implicated in colorectal cancer (CRC) development and associated with prognostic indicators such as disease stage and survival. They also identified 13 kinds of miRNAs associated with CRC mortality among patients with colon cancer. Has-miR-145-3p (hazard ratio (HR) 2.94, 95% confidence interval (CI) 1.54, 5.61) has the most notable association [31].

Falzone et al. [32] analyzed the expression of miRNAs and further compared the miRNAs expression levels of CRC tumor tissues and normal tissues. They found has-miR-195-5p could act as a new molecule able to identify precancerous lesions in CRC. As for has-miR-125b-5p, it has been identified in different diseases. In human osteoarthritic chondrocytes, has-miR-125b-5p regulates IL-1β induced inflammatory genes via targeting TRAF6-mediated MAPKs and NF-κB signaling [33]. In lung adenocarcinoma, the expression of hsa-miR-125b-5p was significantly up-regulated in stage I lung adenocarcinoma tissues compared with that in adjacent tissues and expression of hsa-miR-125b-5p could be used to distinguish lung adenocarcinoma from adjacent tissues [34]. Besides, miRNAs play a crucial role in controlling intestinal epithelial barrier function partly by modulating the expression of tight junction proteins. MiR-125b was involved in barrier function dysregulation through the modulation of claudin-2 and cingulin expression in the jejunum in irritable bowel syndrome with diarrhea [35].

As for ESCC, many studies have clarified some miRNAs can act as biomarkers. Yang et al. [36] demonstrated that induced ectopic overexpression of miR-760 dramatically inhibited ESCC cells proliferation, attenuated migration, and invasion facilitated apoptosis in vitro. Tang et al. [37] found miR-204-5p could affect ESCC proliferation, invasion, apoptosis, and cell cycle in cell and mouse models. Besides, they also found miR-204-5p expression was negatively correlated with IL-11 expression and IL-11 overexpression reversed the suppressive effects of miR-204-5p in the cell lines, which indicated that miR-204-5p functions as a tumor suppressor by directly targeting IL-11 in ESCC.

There are some limitations in this study. First, all the data was downloaded from GSE43732, and we do not verify the results in our own patients. Second, we only select some miRNAs as prognostic factors, but we did not combine several miRNAs to predict the prognosis of ESCC, which we will explore in the future.

In conclusion, the present study identified 24 miRNAs in 119 paired ESCC tissue samples and para-carcinoma tissues, including 4 miRNAs in para-carcinoma tissues and 20 in tumor tissues, respectively. The dysregulation of these miRNAs were associated with different outcomes. We thought this study could provide novel noninvasive early biomarkers for ESCC patients.

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Conflict of interest

The authors declare no conflict of interest in this paper.

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