The Prognostic Value of miR-302b in Patients With NSCLC

Yue Zhao (fnisve@yeah.net)
Cangzhou Central Hospital
https://orcid.org/0000-0002-8398-7546

Xiangjun Kong
Cangzhou Central Hospital

Hongbing Wang
Cangzhou Central Hospital

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Abstract

Background: Lung cancer is the most common cancer worldwide. The most frequent type of lung cancer is non-small cell lung cancer (NSCLC). MicroRNAs (miRNAs) have been reported to play important role in human cancers. Studies suggest that the aberrant expression of miRNAs could act as the diagnostic or prognostic biomarker in human cancers, including lung cancer. MicroRNA-302b (miR-302b) has ever been investigated in several human cancers. The aim of this study was to examine the prognostic value of miR-302b in patients with NSCLC.

Methods: Quantitative real-time RT-PCR (qRT-PCR) analysis were used to measure the expression level of miR-302b in NSCLC and adjacent noncancerous samples. The relationship of miR-302b with the clinicopathological data of NSCLC was analyzed by Chi-square test. The prognostic value of miR-302b was assessed by using the Kaplan-Meier survival curves and Cox regression analysis.

Results: The expression level of miR-302b was downregulated in the NSCLC samples compared with the paired adjacent noncancerous samples ($P < 0.05$). The decreased miR-302b was found correlated with the differentiation ($P = 0.019$) and lymph node metastasis ($P = 0.019$) of NSCLC. The survival curves suggested that patients with lower miR-302b expression had poor overall survival than those with high miR-302b expression. The results of Cox analysis demonstrated that the expression of miR-302b was an independent and effective prognostic factor in NSCLC patients with the $P$ of 0.002 (HR = 2.508, 95% CI = 1.410 - 4.463).

Conclusion: In one word, the expression of miR-302b was decreased in NSCLC samples, and the miR-302 expression might act as a prognostic biomarker in NSCLC patients.

Background

Lung cancer is the most common cancer worldwide with the high incidence and mortality [1]. Based on the statistics, approximately 85% − 90% of lung cancers are non-small cell lung cancer (NSCLC). NSCLC represents a great health problem for men and women around the world [2, 3]. Patient with lung cancer usually perform little obvious symptoms at the early stage time of tumor, causing the patients usually diagnosed at the advanced stage [4, 5]. So far, the mainly treatments for NSCLC patients are clinical surgery, chemotherapy and radiotherapy, but the resistances to these treatments have been on the rise [6–10]. Despite a large of improvements in the diagnosis, prognosis and treatments of NSCLC, the 5-year survival rate is still no more than 15%, and the prognosis is still not ideal [11]. Therefore, finding more effective biomarkers for prognosis of NSCLC patients is urgently needed, which might be helpful to improve the quality of life of patients suffering from this disease.

MicroRNAs (miRNAs) are a class of small (18–22 nucleotides in length) noncoding RNAs, which can downregulate the expression of mRNAs by binding the 3'-untranslated region (3'-UTR) of the targeted mRNAs [12, 13]. In recently studies, the upregulated or downregulated expression of miRNAs have been investigated in different kinds of human cancers and been reported to involved in the progression of
human cancers [14–16]. The diagnostic and prognostic value of miRNAs have also received lots of attentions in human cancers [17–20]. In the clinical area of NSCLC, there are some miRNAs have been reported to involved in the processes of NSCLC. Kim et al. have demonstrated that the miR-126 and miR-200c act as the prognostic biomarkers in patients with NSCLC [21]. MiR-675-5p, as another example, has been described correlated with the tumor progression and development in NSCLC patients [22]. MicroRNA-302b (miR-302b) is a member of these miRNAs, which has been investigated in several human cancers, such as hepatocellular carcinoma, esophageal squamous cell carcinoma and breast cancer [23–25]. However, the relationship between miR-302b with the NSCLC patients has never been reported.

In the current study, we examined the expression level of miR-302b in NSCLC samples and aimed to explore whether the miR-302b represents an crucial role in the prognosis of NSCLC.

**Methods And Materials**

**Patients and specimens collection**

Total of 142 patients were recruited in this study, who were diagnosed as NSCLC patients in Cangzhou Central Hospital. None of these patients had received any chemotherapy or radiotherapy before the clinical surgery in our study. The 142 NSCLC tissue samples and paired noncancerous samples were collected from the participants and immediately frozen in liquid nitrogen and stored at - 80 °C for the extraction of RNA. All the participants were provided the written informed consent. The application of the NSCLC samples were approved by the ethics committee of Cangzhou Central Hospital. Moreover, the clinicopathological characteristics of NSCLC patients were recorded, including age, sex, tumor size, smoking history, differentiation, TNM stage and lymph node metastasis. All the patients were followed up rang from 6 months to 60 months.

**RNA extraction and quantitative real-time RT-PCR (qRT-PCR)**

Total RNA including miRNAs were obtained from both the NSCLC samples and adjacent noncancerous samples by using the TRIzol reagent (Incitrogen, Carlsbad, CA, USA) as per the manufacturer’s instructions. The miRNA isolation was carried out from the total RNA using mirVanaTM miRNA Isolation Kit (Ambion Inc. Austin, Texas, USA) according to manufacturer’s instructions.

The RNAs were conversed transcribed into single stranded cDNAs by using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, USA). The qRT-PCR was used to detect the expression of miR-302b in cancer samples and adjacent noncancerous samples. The PCR reaction was carried out by using the Taqman MicroRNA Assay Kit (Applied Biosystems, USA) on a 7500 Real-Time PCR System detection system (Applied Biosystems, USA). The RNU6B gene was selected to be the endogenous gene to normalize the relative miR-302b expression level. The expression of miR-302b was quantitated with $2^{-\Delta\Delta Ct}$ method. All samples were carried out in triplicate.

**Statistical analysis**
All data were analyzed with the SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The difference of expression level of miR-302b in NSCLC samples compared with the matched adjacent noncancerous samples were performed by using the Student’s t test. Chi-square test was applied to investigate the association between the expression of miR-302b and the clinicopathological features of NSCLC. The relationship between miR-302b and the survival rates of NSCLC patients was examined by plotting the survival curves with the Kaplan-Meier method, and the differences were calculated with the log-rank test. In order to confirm whether the expression of miR-302b represented a prognostic factor in patients with NSCLC, the Cox regression analysis was used in this study. $P < 0.05$ was considered statistically significant.

**Results**

**The expression level of miR-302b in tissues**

In the present study, qRT-PCR was applied to measure the expression level of miR-302b in both NSCLC samples and paired adjacent noncancerous samples. The result of qRT-PCR showed that the expression of miR-302b was lower in NSCLC samples than that in the adjacent noncancerous samples ($P < 0.001$, Figure 1).

**Association of miR-302b with the clinicopathological features of NSCLC**

Total of the clinicopathological date were listed in Table 1. The median miR-302b expression level was used as a cutoff value to divide all 142 patients into two groups: high miR-302b expression group (n = 70) and low miR-302b expression group (n = 72). According to the analysis of Chi-square test, we found that the expression of miR-302b was correlated with the differentiation ($P = 0.029$) and lymph node metastasis ($P = 0.019$) of NSCLC. However, there was no significantly association between miR-302b expression and the age, sex, tumor size, smoking history and TNM stage (all $P > 0.05$).

**The prognostic value of miR-302b in patients with NSCLC**

The relationship between miR-302b expression with the survival rates of NSCLC patients was performed by constructing the Kaplan-Meier survival curves (Figure 2). The results of survival curves suggested that the patients with low miR-302b expression had worse overall survival than the cases with higher miR-302b expression (log-rank, $P = 0.000$). To confirm the prognostic value of miR-302b, we used the Cox analysis to perform the influence of miR-302b and the clinicopathological parameters on the overall survival of NSCLC patients. The results of Cox analysis in Table 2 demonstrated that the expression of miR-302b represented an independent prognostic factor for patients with NSCLC (HR = 2.508, 95%CI = 1.410 - 4.463, $P = 0.002$).

**Discussion**
As we all know, lung cancer is the most common malignancy around the world [26]. The mortality of lung cancer is rather higher than that in the colorectal, gastric and breast cancer [27]. Almost 75% patients with lung cancer are diagnosed at the advanced stage due to the absence of obvious clinical symptoms [28]. So far, the molecular mechanisms involved in the progression of lung cancer are still unclearly. Thus, the systematical and effective therapeutic strategies are difficult to improve [29]. Lung cancer can be classified into two mainly types: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC is the major one, which accounts for more than 80% in lung cancers. Although the improvements in early diagnosis, prognosis and treatments have been maken continually, the 5-year survival rates of patients with NSCLC are still less than 20% [30]. Consequently, it is utmost significance to explore more prognostic factor for patients suffering from lung cancer.

Accumulated evidences suggested that the miRNAs in human beings usually located in the cancer related genomic regions, and the aberrant expression of miRNAs have been investigated in a wide of human cancers [31, 32]. Thus, it is considered that the function of miRNAs might be very important in human cancers. Recently years, more and more miRNAs have been recognized involved in the diagnosis, prognosis and progression of different kinds of human cancers, including lung cancers [33–35]. MiR-449a, as an example, has been described as the suppressor of NSCLC by Ding and his colleagues in 2014 [36]. MiR-302b is one important member of these miRNAs, which belongs to the miR-302 family [37]. There are some studies show that the miR-302b acts as an crucial role in processes of several human cancers [38]. However, the correlation of miR-302b with NSCLC has not been elucidated in the previous studies. The aim of the current study was investigating the expression level of miR-302b in NSCLC samples, and exploring the prognostic value of miR-302b in patients with NSCLC.

In the current study, qRT-PCR was used to measure the expression level of miR-302b in both NSCLC samples and paired adjacent noncancerous samples, and suggested that the miR-302b expression was downregulated in the NSCLC samples compared with the noncancerous samples. This downregulated expression of miR-302b has ever been found in other human cancer samples yet. Khalilil and colleagues have demonstrated that the miR-302b was downregulated in gastric adenocarcinoma samples [39]. Zhang et al. have also reported that the downregulated miR-302b was detected in esophageal squamous cell cancer and may act as a tumor suppressor in patients with this disease [24].

Moreover, we focused on the relationship between miR-302b expression and the clinicopathological data of NSCLC. The downregulated expression of miR-302b was associated with the differentiation and lymph node metastasis (all \( P < 0.05 \)), suggesting that miR-302b related to the development and progression of NSCLC. The survival curves in this study suggested that the patients with lower expression of miR-302b possessed low survival rate compared with the higher expression of miR-302b cases. In order to examine the prognostic value of miR-302b in NSCLC, the Cox regression analysis was performed. The results showed that the downregulation of miR-302b was an independent and effective prognostic factor for patients with NSCLC.

**Conclusions**
In conclusion, miR-302b expression is decreased in NSCLC and is associated with tumor progression. Besides, low miR-302b expression was associated with worse overall survival in patients with NSCLC. MiR-302b could be considered as an independent prognostic factor for patients with NSCLC.

**Declarations**

**Ethics approval and consent to participate:** This study was supported by the Ethics Committee of Cangzhou Central 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.

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**Authors' contributions:** Y.Z., X.K. conceived and designed the experiments, analyzed the data, and wrote the paper. H.W. performed the experiments. All authors read and approved the final manuscript.

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**Tables**
| Features                  | No.          | miR-302b expression | P values |
|--------------------------|--------------|---------------------|----------|
|                          | n = 142      | Low (n = 72)        | High (n = 70) |   |
| Age (years)              |              |                     | 0.302    |
| ≤ 58                     | 63           | 35                  | 28       |
| > 58                     | 79           | 37                  | 42       |
| Sex                      |              |                     | 0.853    |
| Male                     | 78           | 39                  | 39       |
| Female                   | 64           | 33                  | 31       |
| Tumor size (cm)          |              |                     | 0.125    |
| ≤ 3                      | 63           | 35                  | 43       |
| > 3                      | 79           | 37                  | 27       |
| Smoking history          |              |                     | 0.093    |
| Yes                      | 71           | 41                  | 30       |
| No                       | 71           | 31                  | 40       |
| Differentiation          |              |                     | 0.029    |
| Well; Moderate           | 67           | 28                  | 40       |
| Poor                     | 70           | 44                  | 30       |
| TNM stage                |              |                     | 0.066    |
| I-II                     | 68           | 29                  | 39       |
| III-IV                   | 74           | 43                  | 31       |
| Lymph node metastasis    |              |                     | 0.019    |
| Yes                      | 71           | 43                  | 28       |
| No                       | 71           | 29                  | 42       |
Table 2
Multivariate Cox regression analyses for miR-302b in NSCLC patients

| Variables          | Multivariate analysis |       |      |
|--------------------|-----------------------|-------|------|
|                    | HR        | 95%CI  | P value |
| MiR-302b           | 2.508     | 1.410–4.463 | 0.002 |
| TNM stage          | 1.827     | 1.060–3.149 | 0.030 |
| Lymph node stage   | 4.287     | 2.220–8.279 | 0.000 |

HR: hazard ratio; CI: confidence interval.

Figures

Figure 1

The different expression of miR-302b in NSCLC samples and the matched adjacent noncancerous samples. The relative expression of miR-302b examined by qRT-PCR was lower in NSCLC samples than that in the matched adjacent noncancerous samples.
Figure 2

The survival curves based on the expression of miR-302b. Patients with low miR-302b expression lived significantly shorter than those with high miR-302b expression.