European Review for Medical and Pharmacological Sciences 2017; 21 (3 Suppl): 38-42

Expression of miR-210 in senile COPD complicating primary lung cancer

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Abstract. – OBJECTIVE: To analyze the expression and clinical significance of miR-210 in patients with senile chronic obstructive pulmonary disease (COPD) complicating primary lung cancer. 30 cases of COPD

PATIENTS AND METHODS: 30 cases of primary lung cancer and 30 cases of COPD complicating lung cancer were selected. MiR-210 can function as the cancer suppressor gene in progress from COPD to primary lung cancer and may be used as an important auxiliary diagnosis index. Analysis of correlations between MiR-210 expression with clinical features such as age, sex, disease type, COPD disease classification, pathological pattern of neoplasia and TNM staging.

RESULTS: The expression of miR-210 of COPD Group was significantly higher than the Lung Cancer Group. The level of COPD Complicating Lung Cancer was the lowest ($p < 0.05$). There was no remarkable difference among sex, age, smoking status, hypertension, diabetes mellitus and pathological type in each group ($p > 0.05$). Expression of miR-210 was lower with the aggravation of COPD and an increase of TNM staging ($p < 0.05$).

CONCLUSIONS: MiR-210 expression level in the diagnosis of COPD complicating lung cancer had the sensitivity of 76.8%, specificity of 72.3%, accuracy of 0.73, 95% CI of 0.63-0.85 and critical value of 0.1825.

Key Words:
miR-210, Chronic obstructive pulmonary disease, Primary lung cancer, Cancer suppressor gene, Receiver operating curve.

Introduction

Chronic obstructive pulmonary disease (COPD) is a common and frequently-occurring respiration disease[1]. Chronic bronchitis or emphysema featured by irreversible airflow obstruction and is often correlated with abnormal inflammatory reaction caused by harmful gas or particles[2]. COPD causes changes in tissue and organ functions with concomitant extra-pulmonary effects (e.g. systemic inflammation response, loss of weight, skeletal muscle dysfunction)[3]. The morbidity and mortality of lung cancer are both relatively high among the cancers[4], and the clinical detection rate of COPD complicating primary lung cancer is about 30% of all COPD and 20% of all lung cancers[5]. Presently, there is no consensus on whether COPD leads to primary lung cancer[6,7].

The miRNA is an endogenous, non-coding small RNA, with the length of 18-25 nucleotides. Presently, 2,000 types of miRNA have been discovered. In the COPD rat pulmonary tissue, the expressions of 20 types of miRNAs such as miR-30c-2, miR-199a-5p, miR-30a and miR-145 are up-regulated, and the expression of miR-376b-3p is down-regulated[8,9]. The miR-210 is a type of hypoxia activating factor, which has shown abnormal expression in the occurrence and progress of lung cancer[10,11]. This research aims to analyze the expression and significance of miR-210 in senile COPD complicating primary lung cancer.

Patients and Methods

Patients

Patients who were admitted to hospital for diagnosis of COPD and primary lung cancer from 2014 to 2016, were continuously selected for this study. Cases included 30 cases of COPD, 30 cases of primary lung cancer and 30 cases of COPD

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complicating lung cancer. COPD was diagnosed by chest CT, pulmonary function, and medical history. Lung cancer was diagnosed definitely by pathology. Cases involving secondary lung cancer, phthisis, pulmonary infection, respiratory failure, etc. were excluded. The baseline information was similar for the three groups of patients. See Table I for details.

This study was approved by the Ethics Committee of The First Affiliated Hospital of Medical School of Zhejiang University. Signed written informed consents were obtained from all participants before the study.

Methods

Fluorogenic quantitative PCR method was used to detect the expression level of peripheral blood miR-210. Correlation of expression level with clinical features such as age, sex, disease type, COPD disease classification, pathological pattern of neoplasia and staging were analyzed.

Blood Specimen Collection

3 ml elbow venous blood was sampled within 48h after the admission; sodium citrate anticoagulant (Approval Number of the State Drug Administration: H20058914; Manufacturer: Sichuan Nigale BioTech Co., Ltd. Specification: 160 ml: 6.4 g) was used for anticoagulation treatment; supernatant was taken after 3,000 g centrifugation for 20 min; then the supernatant was stored in -70°C liquid nitrogen for 24h before testing.

RT-PCR Detection

Total RNA in the serum was extracted with RNA kit (Invitrogen, Carlsbad, CA, USA); the concentration and purification were measured with ultraviolet spectrophotometer (BioTek, Biotek Winooski, Vermont, USA); the integrity was detected by agarose gel electrophoresis, and then the total RNA in the serum was stored at -80°C for use. Stem-loop primer PCR method was used to make analysis; the reaction system was small fragment RNA 2 μL or mmu-miR-210 (100 pmol/μL) 1 μL+DEPC water 10 μL. Degeneration was made at 65°C for 10 min and 25°C for 5 min; the ice-bath treatment was made for 5 min; reagent loading buffer (2.5 μL) and double distilled water for RNA inhibitory enzyme, reverse transcriptase and non-RNA-enzyme (1 μL respectively) were replenished; extension was made at 42°C for 30 min and anneal was made at 70°C for 10 min. The fluorescence intensity was detected and the target gene expression quantity was calculated. Results were expressed by $2^{-\Delta\Delta}$Ct method.

Statistical Analysis

SPSS20.0 software (Version X; IBM, Armonk, NY, USA) was used for statistical analysis. The measurement data was expressed by mean ± standard deviation. The *t*-test or single factor AVONA analysis was used for comparison. The enumeration data was expressed by cases or (%). (Correction) the $\chi 2$-test was used for comparison. $p < 0.05$ indicated that differences have statistical significance.

Results

Relationship Between miR-210 Expression Level and Clinical Features

The expression of miR-210 in the COPD Group was higher than the lung cancer group

Table I. Comparison of baseline information of patients of three groups.

Group	COPD group (n = 30)	Lung cancer group (n = 30)	COPD complicating lung cancer group (n = 30)	F/χ^2	*p*
Male/Female	18/12	17/13	16/14	0.271	0.873
Age (years old)	66.7 ± 7.3	67.2 ± 7.4	67.3 ± 7.2	0.069	0.912
Smoking [cases (%)]	8 (26.7)	9 (30.0)	9 (30.0)	0.108	0.947
Hypertension [cases (%)]	5 (16.7)	6 (20.0)	6 (20.0)	0.145	0.930
Diabetes mellitus [cases (%)]	3 (10.0)	4 (13.3)	3 (10.0)	0.219	0.896
COPD Mild	7 (23.3)		8 (26.7)		
Moderate	17 (56.7)		15 (50.0)	0.269	0.874
Severe	6 (20.0)		7 (23.3)		
Pathological classification adenocarcinoma		12 (40.0)	13 (43.3)	0.069	0.793
Squamous carcinoma		18 (60.0)	17 (56.7)		
TNM staging I-II		10 (33.3)	12 (40.0)	0.287	0.592
III-IV		20 (66.7)	18 (60.0)		

Table II. Relationship between miR-210 expression level and clinical feature.

	COPD group	Lung cancer group	COPD complicating lung cancer group
miR-210	0.3624 ± 0.0629	0.2157 ± 0.0348	0.1632 ± 0.0257
Male	0.3562 ± 0.0725	0.2251 ± 0.0527	0.1528 ± 0.0326
Female	0.3626 ± 0.0639	0.2036 ± 0.0632	0.1724 ± 0.0421
< 67 years	0.3636 ± 0.0852	0.2253 ± 0.0638	0.1762 ± 0.0637
≥ 67 years	0.3621 ± 0.0329	0.2025 ± 0.0375	0.1624 ± 0.0532
Smoking	0.3527 ± 0.0439	0.2252 ± 0.0528	0.1637 ± 0.0428
Non-smoking	0.3696 ± 0.0527	0.2132 ± 0.0438	0.1624 ± 0.0352
Hypertension	0.3627 ± 0.0428	0.2163 ± 0.0529	0.1618 ± 0.0429
Non-hypertension	0.3618 ± 0.0527	0.2147 ± 0.0427	0.1652 ± 0.0637
Diabetes mellitus	0.3529 ± 0.0428	0.2252 ± 0.0638	0.1529 ± 0.0538
Non-diabetes	0.3728 ± 0.0537	0.2037 ± 0.0537	0.1732 ± 0.0635
COPD Mild	0.3852 ± 0.0827		0.1935 ± 0.0632
Moderate	0.3626 ± 0.0635		0.1632 ± 0.0527
Severe	0.3528 ± 0.0732		0.1437 ± 0.0433
Pathological classification adenocarcinoma		0.2234 ± 0.0537	0.1534 ± 0.0537
Squamous carcinoma		0.2016 ± 0.0432	0.1685 ± 0.0428
TNM staging I-II		0.2425 ± 0.0527	0.1832 ± 0.0527
III-IV		0.2031 ± 0.0342	0.1534 ± 0.0329

while the COPD complicating lung cancer group was the lowest ($p < 0.05$). There was no difference in sex, age, smoking status, hypertension, diabetes and pathological type in each group ($p > 0.05$). The miR-210 level was reduced with aggravation of COPD and an increase of TNM staging ($p < 0.05$). See Table II for results.

ROC Analysis on miR-210 Diagnosis of COPD Complicating Lung Cancer

By taking the miR-210 expression levels of the three groups as diagnosis indicator, COPD complicating lung cancer as diagnosis results and including the results into ROC model, results were obtained with sensitivity of 76.8%, specificity of 72.3%, accuracy (AUC value of the area under the curve) of 0.73, 95% CI of 0.63-0.85 and critical value of 0.1825 (Figure 1).

Discussion

Based on genetic analyses, the pathogenic mechanism of lung cancer is correlated with changes in DNA sequence. The morbidity of non-small cell lung cancer (NSCLC) is relatively high, which mainly involves DNA methylation, histone modification, non-coding RNA regulation, etc. and covers function changes of multiple aspects such as repair of DNA damage, cell cycle control, histone modification after interpretation and growth factor regulation[12]. COPD is closely related with occurrence of lung cancer and miRNAs may play an important role[13].

miRNA is a small fragment non-coding RNA with regulation functions found in eucaryotes. Though miRNA exists in low quantity, it has relatively strong gene regulation function(s). It mainly has negative gene expression effects and regulates about 60% of overall gene expression.

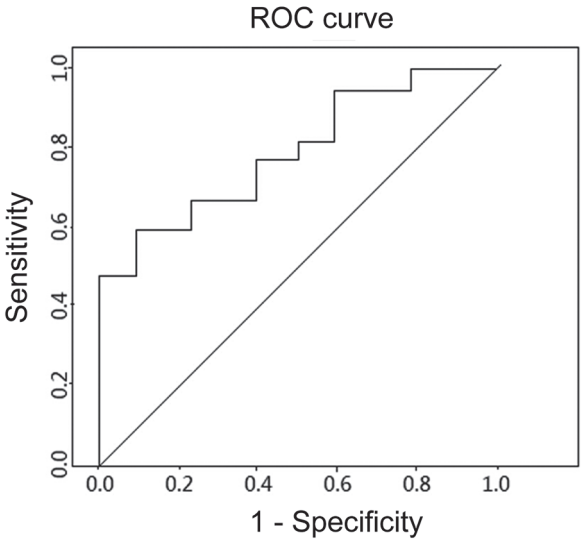

Figure 1. Receiver operating characteristic curve (ROC) of miR-210 in diagnosis of COPD complicated with lung cancer.

Furthermore, it participates in the occurrence and development of multiple diseases and plays an important part in cell function regulation[14]. It has been shown previously that the mutation status of human epidermal growth factor receptor (EGFR) is closely related to the generation of NSCLC. The impact of carrying EGFR gene mutation(s) on the treatment and prognosis has become a key and sensitive target of NSCLC mediation[15]. The abnormal expression of multiple miRNAs may participate in the regulation of the EGFR gene and the state of activation of signal pathways. One miRNA may simultaneously act on several types of target genes, and one gene may also be regulated simultaneously by multiple miRNAs. They collectively participate in the generation, development, infiltration and metastasis of the tumors and induce the apoptosis of tumor cells[16]. miRNA plays an important role in the occurrence, epithelial-mesenchymal transition and therapeutic reaction of lung cancer. It may be used as an important biomarker for tumor diagnosis and prognosis to provide a favourable basis for the accurate diagnosis of lung cancer. It may also be used as potential therapeutic target. The application of high-throughput sequencing further facilitates miRNA research[17].

Research has shown that miRNA-145 may suppress the generation of initiating cells of adenocarcinoma of lung by regulating the EMT pathway[18]. The expression of miR-100 decreases in lung cancer tissue, the expression of miR-148a increases, and both of them may participate in the generation and development of lung cancer[19]. miR-210 participates in various types of lung diseases such as COPD and lung cancer. It may affect DNA repair, disturb mitochondria metabolism, adjust biological activities such as cell cycle and is expected to be a new indicator for lung cancer intervention treatment success[20].

Expression of miR-210 in the COPD group was remarkably higher than the lung cancer group while level of COPD complicating lung cancer was the lowest. Our study indicates that miR-210 may participate in the generation of COPD and lung cancer and serve as the cancer suppressor gene in the process of mediating COPD to lung cancer. It bears close relationship with the disease condition development and tumor staging of COPD. ROC analysis also indicates that it may be used as an important auxiliary diagnosis indicator for COPD complicating lung cancer.

Conclusions

This research only analyzes the relationship with COPD and lung cancer from the abnormal expression of miR-210 in clinical circulating blood. Further research will use cell levels and animal models to investigate the relationship of COPD and lung cancer as well as its internal mechanism of action. Future studies will also focus on the possible role of the abnormal expression of miR-210 in the relationship of COPD and lung cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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