Contents lists available at ScienceDirect IF: 0.926

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60323-0

MicroRNA-126 inhibits the proliferation of lung cancer cell line A549

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ARTICLE INFO

Article history:
Received15 December 2014
Received in revised form 20 January 2015
Accepted 15 February 2015
Available online 20 March 2015

Keywords:
microRNA-126
Proliferation
VEGF

ABSTRACT

Objective: To study the role of microRNA-126 in the development of lung cancer. **Methods:** The biological function of microRNA-126 was detected using EdU assay and CCK-8 assay; the target gene of microRNA-126 was analyzed using real time RT-PCR and Western blot assay. **Results:** In A549 cell line, overexpression of microRNA-126 inhibits the proliferation rate; VEGF is the target gene of microRNA-126; microRNA-126 exerts its function via regulating VEGF protein level. **Conclusions:** microRNA-126 inhibits the proliferation in A549 cell line.

1. Introduction

In malignant tumors, lung cancer incidence and mortality are at the forefront, and its occurrence and development are a complicated process related with many signal pathways. With environmental deterioration, lung cancer incidence and mortality are increasing year by year. In many classifications of lung cancers, non-small cell lung cancer (NSCLC) accounts for about above 80%, and it is difficult to diagnose early, so most of NSCLC are diagnosed at late stages. It is very important and urgent that treatment strategies are determined for effective treatment.

MicroRNAs are a class of highly conservative microRAN segments with length of about 22 nucleotide fragments, and regulate gene expressions mainly by binding with target gene mRNA 3'UTR at post-transcriptional level[1], and then participates in cell proliferation, apoptosis, differentiation, senescence and other physiological progresses. Abnormal expression of microRNA is closely related with occurrence and development of many tumors.

MicroRNA-126 is localized at 9q34.3 zone, and maintained high expression in lungs, hearts and kidneys of adults[2]. During lung development process in human and mouse lung, expression level of microRNA-126 showed time dependent, microRNA-126 expression gradually increases during lung development and maintains high expression level when lung matures. In addition, microRNA-126 is also closely related with occurrence and development of tumors. Tavazoie *et al* have found that microRNA-126 could inhibit mammary cancer proliferation and metastasis, and play the roles of tumor suppressor genes[3]. Musiyenko *et al* found that microRNA-126 could inhibit colon cancer cell proliferation by inhibiting PI3K signal transduction pathway[4]. In addition, microRNA-126 expression level is also closely related with five-year survival rate of NSCLC patients[5]. Gene chip results indicated that microRNA-126 expression level was significantly decreased in lung cancer patients compared with peficancerous tissues.

2. Materials and methods

2.1.Materials

VEGF overexpression plasmid and control plasmid, Vegf-3' UTR luci were constructed by GeneChem (GeneChem, China) Company; Vegf-3' UTR luci microRNA-126 binding site mutation was finished by TransGen (TransGen, China) Company.

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Foundation project: It is supported by National Natural Science Foundation of China (81073036).

Both microRNA-126 reverse transcription primers and qRT-PCR primers were bought from RiboBio Company, and operations were strictly followed by product instructions.

Anti-VEGF(sc-7269) was bought from Santa Cruz, and secondary antibody was bought from Beijing Zhongshan Biotechnology Company.

2.2. Small segments of microRNA-126 expression

Both Scramble and Mimics of microRNA-126 were bought from RiboBio (RiboBio, China) Company. Mimics are chemically synthesized segments, and have the same sequences as microRNA-126, and they are transfected to the cells to enhance endogenous microRNA-126 functions; Scramble is a segment with random arrangement of 22 nucleotides.

2.3. MicroRNA extraction

Small molecule RNA (≤200 nt) was extracted from the cell line by mir Vana™ miRNA Isolation extraction kit strictly according to product instructions.

2.4. CCK-8 assay, EdU assay and Luciferase report assay

Cell Counting Kit-8 (CCK-8) was bought from Dojindo Laboratories, Kumamoto, Japan, and operations were strictly followed by product instructions. EdU cell proliferation test kit was bought from RiboBio (RiboBio, China) Company, and operations were strictly followed by product instructions. Luciferase dual report system kit was bought from Vigorous Company, and luciferase activity was detected according to the kit instructions.

2.5. Statistics analysis

Each experiment was repeated at least three times, and the data were represented by mean SD, and the results were analyzed by SPSS statistics software One-Way ANOVA or student-t test; $P<0.05$ represented significant difference.

3. Results

3.1. Inhibitive effect of microRNA-126 overexpression on the proliferation of A549 cells

To investigate the roles of microRNA-126 in lung cancer occurrence and development, A549 cells, a human lung cancer cell line was selected. Small segment mimics of microRNA-126 were transfected into A549 cells, and detected by qRT-PCR. The result showed that the overexpression efficiency was very significant (Figure 1). CCK-8 experiment and EdU experiment are commonly methods used for detecting cell proliferation ability. CCK-8 experiment showed that cell proliferation ability of microRNA-126 overexpression group was significantly decreased compared with that of Scramble-transfected control group in 24 h and 48 h (Figure 2). EdU experiment results also showed that positive rate of EdU in microRNA-126 overexpression group was significantly decreased compared with control group (Figure 3). Consequently, microRNA-126 could inhibit A549 cells proliferation.

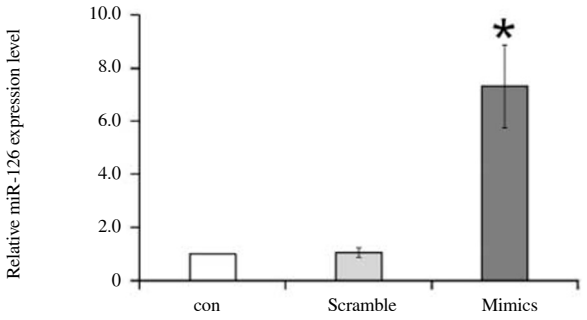

Figure 1. Expression level of miR-126 by qRT-PCR assay.
*, $P<0.05$ compared with the control.

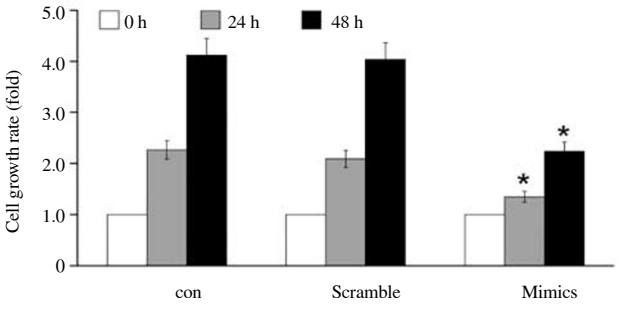

Figure 2. Cell growth rate by CCK-8 assay.
*, $P<0.05$ compared with the control.

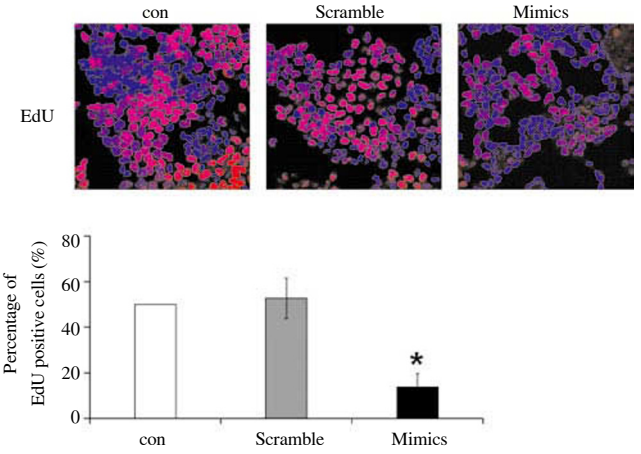

Figure 3. EdU incorporation rate by EdU assay.
*, $P<0.05$ compared with the control.

3.2. Vegf was a target gene of microRNA-126

Three authoritative miRNAs target genes-predicting databases were employed: miRanda, TargetScan, microRNA. org, and potential target genes of microRNA-126 in three informatics websites were gathered, and GO cluster analysis was processed using "proliferation" as a keyword. We found that *Vegf* was a target gene with the highest score, and bioinformatics analysis showed that 3'UTR region of *Vegf* gene mRNA had microRNA-126 binding sites, inidicating that *Vegf* might be a target gene of microRNA-126 (Figure 4). Western blot assay results showed that VEGF protein expression level was downregulated when microRNA-126 was overexpressed in A549 cells (Figure 5).

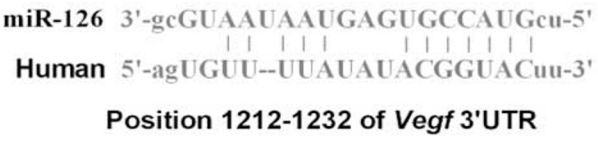

Figure 4. Posotion 1212-1232 of *Vegf* 3'UTR.

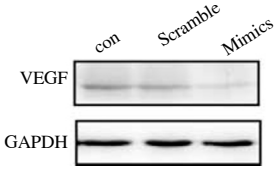

Figure 5. Protein expression level by Western blot assay.

Vegf-3'UTR segment was inserted into luciferase reporter plasmid to construct Vegf-3' UTR luci plasmid and then the plasmid was transfected to cells together with microRNA-126Mimics. It was found that overexpression of microRNA-126 decreased luciferase activity compared with control group. When binding site binding with microRNA-126 was mutated on Vegf-3'UTR luci plasmid (ACGGUAC->ACGGUGU), overexpression of microRNA-126 could not lead to decrease of luciferase activity again (Figure 6). The experiment above could determine that *Vegf* is a target gene of microRNA-126.

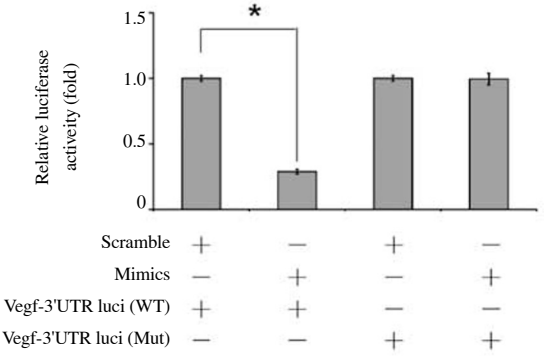

Figure 6. Luciferase activity by luciferase reporter assay.
*, *P*<0.05 compared with the control.

3.3.Reverse effect of VEGF on proliferation and migration-promoting effect of microRNA-126

VEGF overexpression plasmid was successfully constructed (Figure 7). MicroRNA-126 Mimics and VEGF overexpression plasmid (VEGF OE) were co-transfected to A549 cells, and compared with empty vector, microRNA-126-induced cell proliferation effect was reversed (Figure 8, 9).

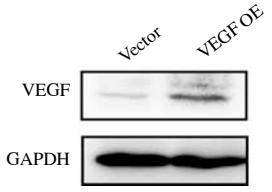

Figure 7. Effect of VEGF overexpression in A549 cells.

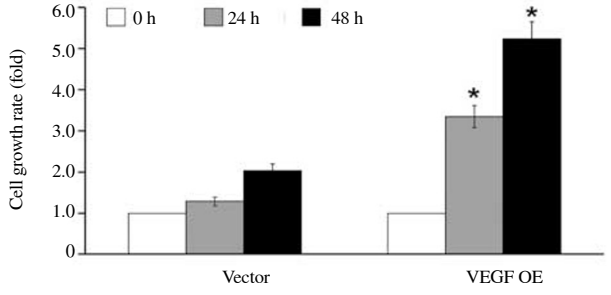

Figure 8. Cell growth rate bu CCK-8 assay.y
*, *P*<0.05 compared with the control.

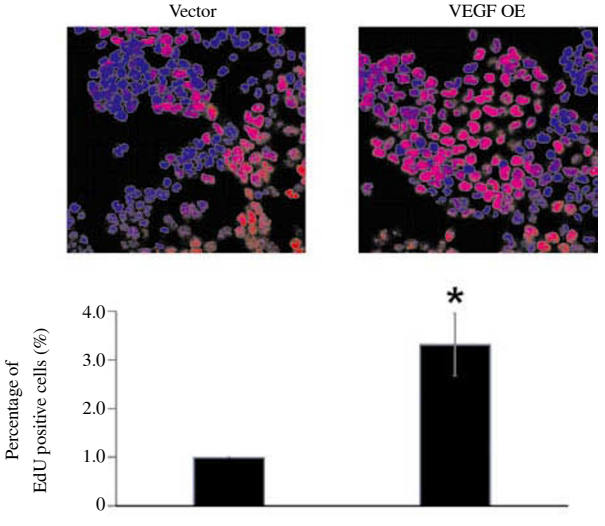

Figure 9. EdU incorporation rate by EdU assay.
*, *P*<0.05 compared with the control.

4. Discussion

Until now, studies on microRNA-126 mainly focuse on aspects of effects that it was a tumor suppressor gene[8]. In recent years, the roles of microRNA-126 in inflammation aspects also attract people's attention, but its detailed mechanism is still not clear[9]. In addition, in development process of human lung, heart and kidney, the roles of microRNA-126 also need further investigation.

At present, people's attention focuses on the functions of microRNA in tumor occurrence and development, but molecular mechanisms how microRNAs playing their roles are still not understood, and there are many conceptive and experimental problems. Up to 2014, Sanger miRBase database (miRBase 21 released) has 28645 approved microRNAs, in which only dozens of microRNAs have been analyzed in lung cancer occurrence and development up to now. It's a new arduous task to seek other lung cancer-associated microRNAs, identify its downstream target molecule and determine the mechanisms of microRNAs playing roles in lung cancer occurrence and development.

Conflict of interest statement

We declare that we have no conflict of interest.

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Document heading doi: 10.1016/S1995-7645(14)60324-2

Afebrile presentation of 2014 Western Africa Ebolavirus infection: the thing that should not be forgotten

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The 2014 Western Ebolavirus infectionis the serious emerging disease at present[1-3]. This disease result in high fatality and rapidly widespread in Africa, hence, it becomes the present concern for medical society[1-3]. The knowledge on clinical presentation on this new Ebola virus infection is extremely limited. It is known only that Ebola virus infection can present as an acute febrile illness with hemorrhagic complication. Here, the authors try to summarize on the magnitude of "fever" as presentation in the 2014 Western Ebolavirus infection. Based on available data from 15 confirmed casesreported from Guinea[1-4], not all cases showed fever at presentation. The rate of afebrile case at presentation is equal to 9.3%. Hence, it should be noted that the afebrile 014 Western Ebolavirus infection is possible and this challenge the immigration system that mainly use fever screening as tool for screening traveler from outbreak area.

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Conflict of interest statement

We declare that we have no conflict of interest.

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