JBUON 2021; 26(4): 1440-1445
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Clinical significance of detecting HDL and miR-103 levels in lung carcinoma patients

Yan Zhang[1], Xinhong Zhang[2], Zhihai Han[1]

[1]Pulmonary and Critical Care Medicine Department, the Sixth Medical Center of PLA General Hospital, Beijing, China.
[2]Department of Medical Oncology, the Sixth Medical Center of PLA General Hospital, Beijing, China.

Summary

Purpose: *To elucidate the clinical significance of microRNA (miR)-103 and HDL in influencing pathological features in lung carcinoma, and to predict chemotherapy efficacy of lung carcinoma according to the expression changes of miR-103 and HDL before and after chemotherapy.*

Methods: *Serum levels of miR-103 and HDL were detected in lung carcinoma patients (n=60) and healthy subjects (n=60) by qRT-PCR. The correlation between miR-103 and HDL in serum samples of lung carcinoma patients was assessed. In addition, their influence on pathological features in lung carcinoma were analyzed. Changes in HDL and miR-103 levels in lung carcinoma patients based on their therapeutic efficacy were evaluated and analyzed.*

Results: *Serum levels of miR-103 and HDL were lower in lung carcinoma patients than those of healthy subjects. MiR-103 level was correlated to that of HDL in serum samples of lung carcinoma patients. HDL level was correlated to smoking, TNM staging and presence of lymph node metastasis of lung carcinoma, while miR-103 level was correlated to TNM staging and presence of lymph node metastasis of lung carcinoma. Serum levels of miR-103 and HDL were significantly enhanced in lung carcinoma patients achieving PR after chemotherapy ($p<0.05$). No significant differences in miR-103 and HDL levels before and after chemotherapy were observed in lung carcinoma patients achieving stable disease (SD) or progressive disease (PD).*

Conclusion: *MiR-103 and HDL are involved in the progression of lung carcinoma. Their expression changes after chemotherapy can be utilized for predicting therapeutic efficacy and prognosis in lung carcinoma patients.*

Key words: *lung carcinoma, miR-103, HDL, prognosis*

Introduction

According to the data released by GLOBOCAN, it is estimated that there were globally 18.1 million new cases of cancers and 9.6 million cancer deaths in 2018. Lung carcinoma is a highly prevalent tumor, accounting for 11.6% of total cancer cases and 18.4% of cancer deaths [1]. With the progression of therapeutic strategies, and comprehensive application of targeted drugs and immune therapies, the prognosis of lung carcinoma has been largely improved. However, unbalanced development of medical conditions in different regions of China results in low 5-year survival (16.1%) of lung carcinoma in our country [2].

MicroRNAs (miRs) are non-coding RNAs with 18-22 nt long and they are able to regulate diverse life activities [3]. MiRNAs are involved in tumor progression by mediating oncogenes or tumor suppressors [4,5]. Hetta et al [6] proposed that miRNA-21 and miRNA-23a can be served as biomarkers for diagnosing early-stage lung carcinoma. In addition, miRNA expression varies in lung carcinoma cases with different histological subtypes [7].

Corresponding author: Zhihai Han, MD. Pulmonary and Critical Care Medicine Department, the Sixth Medical Center of PLA General Hospital, 6 Fucheng Road, Haidian District, Beijing, China.
Tel: +86 018600310135; Email: hanzhihai@sohu.com
Received: 25/04/2021; Accepted: 27/05/2021

It is reported that overexpression of miR-103 can inhibit proliferative, invasive and apoptotic abilities in lung carcinoma cells [8].

Currently, abnormal lipid metabolism has been confirmed to be vital in the pathological processes of atherosclerosis and cardiovascular diseases [9-11]. Ahn J et al [12] suggested that high level of high density lipoprotein (HDL) can reduce cancer risks, especially lung carcinoma, prostate cancer, liver cancer and hematopoietic system tumors. Their susceptibilities are negatively correlated to HDL level. For every 1 mg / dL increase in serum level of HDL in the body, the risk of cancer is reduced by 2.3% [13]. Kucharska-Newton et al [14] detected an obvious decline of HDL-C level in lung carcinoma patients, which is closely linked to the incidence of lung carcinoma. Nevertheless, the relationship between HDL and cancer development remains controversial in other studies [15]. Previous studies have shown that HDL level can be regulated by miRs [16,17]. In this paper, we retrospectively analyzed lung carcinoma cases for assessing the clinical significance of miR-103 and HDL in influencing therapeutic efficacy of chemotherapy.

Methods

Subjects

Initially diagnosed lung carcinoma patients (n=60) in our hospital were included. They did not have history of anti-cancer treatments. TNM staging was defined according to the previously reported criteria [18]. Patients with coronary atherosclerotic heart disease, cerebrovascular disease, liver and kidney dysfunction, thyroid dysfunction, diabetes, hyperlipidemia, medications that could affect blood lipid metabolism, obesity (>15% of standard body mass index/BMI), other primary, metastatic or recurrent malignancies and incomplete medical records were excluded. Included patients were treated by cisplatin combined bevacizumab or cisplatin-based chemotherapy for at least 2 cycles. Patients had no history of blood transfusion within 4 weeks prior to chemotherapy. During the same period, healthy subjects (n=60) having physical examinations in our hospital were recruited as control group. This study was approved by the ethics committee of the Sixth Medical Center of PLA General Hospital (015-CN-PLA-87421). Signed written informed consent forms were obtained from all participants before the study entry.

Serum sample detection

After overnight fasting, blood samples of elbow vein were collected from each subject in the morning. Serum level of HDL was detected in the laboratory using an automatic biochemical instrument (Beckman, Franklin Lakes, NJ, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNAs in tissues were collected using the RNAiso Plus (TaKaRa, Dalian, China) and reversely transcribed into complementary (c)DNAs. A reaction mixture (20 μL) containing 400 ng cDNA, 10 μL of TB Green series (TaKaRa, Dalian, China), 0.6 μL of forward sequence, 0.6 μL of reverse sequence and 6.8 μL of RNase-free ddH_2O was subjected to qRT-PCR at 95°C for 15 min, and 40 cycles at 95°C for 10 s, 56°C for 20 s and 72°C for 30 s. MiR-103 sequences were F: 5'-GAGAGAGCAGCATTGTACAG-3', R: 5'-CAGTGCGTGTCGTGGA-3'; U6 sequences were F: 5'-CTCGCTTCGGCAGCACA-3', R: 5'-AACGCTTCACGAATTTGCGT-3'.

Effectiveness evaluation

Using RECIST method, chemotherapy efficacy of lung carcinoma was evaluated [19]. Response criteria were listed as follows: CR (complete remission): Disappearance of tumor lesions for four weeks or more; PR (partial response): At least a 30% decrease in tumor lesions for four weeks or more; SD (stable disease): Conditions between PR and PD; PD (progressive disease): At least a 20% increase in tumor lesions or appearance of new lesions.

Statistics

SPSS 19.0 (IBM, Armonk, NY, USA) was used for statistical analyses. Data were expressed as mean ± SD (standard deviation). Differences between two groups were compared using the Student's t-test. The correlation between HDL and miR-103 levels in serum samples of lung carcinoma was explored by Spearman correlation test. P<0.05 was statistically significant.

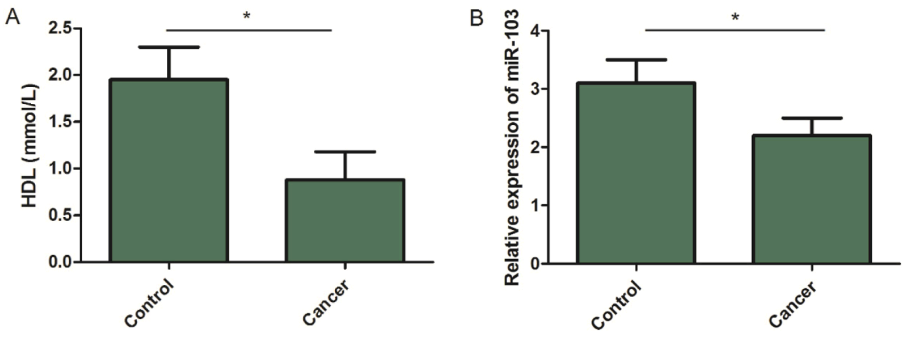

Figure 1. Serum levels of HDL **A** and miR-103 **B** were remarkably lower in lung carcinoma patients, compared with those in healthy subjects. *p<0.05.

Results

Low serum levels of HDL and miR-103 in lung carcinoma cases

Compared with healthy subjects, serum levels of HDL and miR-103 were much lower in lung carcinoma patients (Figure 1A, 1B).

Differences in HDL level in lung carcinoma patients based on pathological features

By analyzing clinical data of the included patients, no significant difference in serum level of HDL was identified in lung carcinoma patients classified by sex, age and histological subtypes ($p>0.05$). Higher level of HDL was detected in non-smoking patients than in smoking people. Stage I+II lung carcinoma patients had higher level of HDL than stage III+IV patients. In addition, non-metastatic patients expressed higher level of HDL compared with patients with lymph node metastasis (Table 1). It is indicated that smoking, TNM staging and presence of lymph node metastasis could affect HDL level in lung carcinoma patients.

Differences in miR-103 expression in lung carcinoma patients based on pathological features

No significant difference in serum level of miR-103 was identified in lung carcinoma patients classified by sex, age, smoking and histological subtypes ($p>0.05$). Stage I+II lung carcinoma patients had higher level of miR-103 than stage III+IV patients. Besides, non-metastatic patients expressed higher level of miR-103 compared with patients with lymph node metastasis (Table 2). We believed that miR-103 level may be related to TNM staging and presence of nodal metastasis in lung carcinoma.

HDL level changes before and after chemotherapy

All lung carcinoma patients finished 2-cycle chemotherapy and the therapeutic effectiveness was evaluated using RECIST criteria. There were 32 cases achieving PR, 18 achieving SD and 10 achieving PD. No patient achieved CR. Serum level of HDL was significantly enhanced in lung carcinoma patients achieving PR after chemotherapy ($p<0.05$). No significant difference in HDL level before and after chemotherapy was observed in lung carcinoma patients achieving SD or PD ($p>0.05$) (Table 3). It is suggested that high level of HDL was conductive to the remission of lung carcinoma.

MiR-103 expression changes before and after chemotherapy

MiR-103 expression changes before and after chemotherapy were similarly analyzed. Lung carcinoma patients achieving PR had elevated level of miR-103 compared with pre-treatment level ($p<0.05$). No significant difference in miR-103 expression before and after chemotherapy was observed in lung carcinoma patients achieving SD or PD ($p>0.05$) (Table 4).

Table 1. Differences in HDL level in lung carcinoma patients based on pathological features

Variables	*n*	*HDL (mmol/L)*	*t*	*p*
Sex				
Male	43	1.16±0.52	-0.13	0.897
Female	17	1.18±0.58		
Age (years)				
<55	22	1.18±0.35	-0.107	0.915
≥55	38	1.19±0.35		
Smoking				
Yes	39	1.13±0.28	-2.446	0.017
No	21	1.33±0.34		
Histological subtype				
Squamous cell carcinoma	23	1.11±0.48	-0.076	0.939
Adenocarcinoma	37	1.12±0.5		
TNM staging				
I+II	22	1.32±0.64	2.8	0.007
III+IV	38	0.97±0.33		
Lymph node metastasis				
No	33	1.25±0.45	2.155	0.035
Yes	27	1.03±0.31		

Correlation between HDL and miR-103

The above data have demonstrated that HDL and miR-103 probably influenced chemotherapy efficacy in lung carcinoma patients. Furthermore, we assessed their correlation in serum samples. Before chemotherapy, serum level of HDL was positively correlated to that of miR-103 in lung carcinoma patients (r=0.4809, p<0.001) (Figure 2A). After chemotherapy, their positively correlation still existed in lung carcinoma patients (r=0.6127, p<0.001) (Figure 2B).

Discussion

Lung carcinoma remains the leading cause of cancer-related deaths worldwide. For patients with advanced lung carcinoma, the overall 5-year survival is only about 15% [20], which may be attributed to high rates of lymph node invasiveness and distant metastases [21]. Multidrug resistance is also a vital reason for the prognosis [22]. Therefore, it is of significance to seek for novel diagnostic targets for lung carcinoma.

Table 2. Differences in miR-103 expression in lung carcinoma patients based on pathological features

Variables	*n*	*miR-103*	*t*	*p*
Sex				
Male	43	2.04±0.72	0.765	0.447
Female	17	1.89±0.58		
Age (years)				
<55	22	1.95±0.85	0.56	0.578
≥55	38	1.83±0.77		
Smoking				
Yes	39	2.14±1.03	0.476	0.636
No	21	2.01±0.97		
Histological subtype				
Squamous cell carcinoma	23	1.91±0.96	0.164	0.87
Adenocarcinoma	37	1.87±0.89		
TNM staging				
I+II	22	2.13±0.83	2.374	0.021
III+IV	38	1.72±0.51		
Lymphh node metastasis				
No	33	2.28±1.04	2.039	0.046
Yes	27	1.84±0.46		

Table 3. HDL level changes before and after chemotherapy in lung carcinoma patients

Group	*n*	*HDL (mmol/L)*	*t*	*p*
PR	32			
Before		1.13±0.35	-2.483	0.016
After		1.37±0.42		
SD	18			
Before		1.09±0.38	-1.542	0.132
After		1.33±0.54		
PD	10			
Before		1.17±0.32	-0.639	0.531
After		1.32±0.67		

PR: partial response; SD: stable disease; PD: progressive disease

Table 4. MiR-103 expression changes before and after chemotherapy in lung carcinoma patients

Group	*n*	*MiR-103 level*	*t*	*p*
PR	32			
Before		2.03±1.01	-5.259	<0.001
After		3.75±1.55		
SD	18			
Before		1.97±0.96	-0.396	0.694
After		2.11±1.15		
PD	10			
Before		2.26±1.24	-0.451	0.657
After		2.53±1.43		

PR: partial response, SD: stable disease, PD: progressive disease.

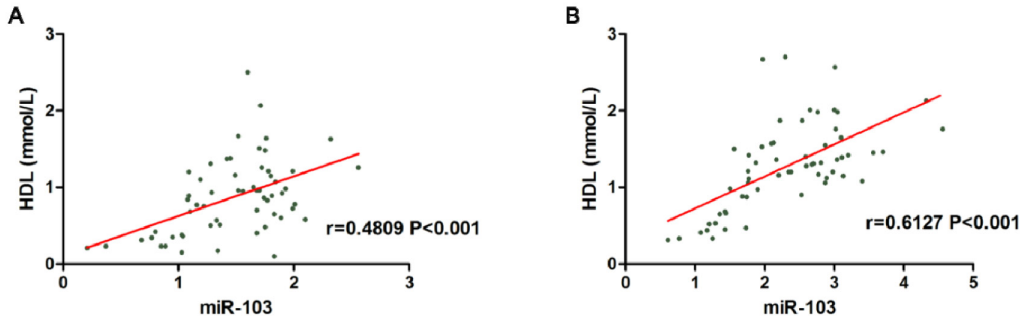

Figure 2. A positive correlation between HDL and miR-103 levels in lung carcinoma before **(A)** and after chemotherapy **(B)**.

MiRs are extensively involved in cellular functions by specifically binding target mRNAs, thereby serving either a carcinogenic or anti-cancer role [23]. It is estimated that more than 100,000 mRNAs can be regulated by miRs [24]. Some miRNAs are capable of predicting the effectiveness of anti-cancer treatment. Cui et al [25] pointed out that miR-125b is markedly upregulated in advanced NSCLC patients with a poor respond to cisplatin-based chemotherapy. MiR-103 is a cancer-associated miR. Through regulating DICER and PTEN levels, miR-103 is able to attenuate the proliferative ability in colorectal cancer cells [26]. The miR-103/KLF4 axis triggers metastasis and malignant growth of gastric cancer [27]. The inhibitory effect of miR-103 is much pronounced on hematological tumors, which can accelerate tumor cell apoptosis [28]. This study consistently found the involvement of miR-103 in lung carcinoma progression. Serum level of miR-103 was reduced in lung carcinoma patients, which was closely linked to TNM staging and lymph node metastasis. Besides, high level of miR-103 was conductive to the first-line chemotherapy in lung carcinoma patients.

HDL is a complex lipoprotein composed of various proteins and lipids showing anti-inflammatory and antioxidant effects [29]. Meanwhile, HDL neutralizes carcinogens [30]. Kucharska-Newton et al [14] found a correlation between the prevalence of lung carcinoma and HDL-C level in either smoking or non-smoking patients. Identically, we detected a higher level of HDL in non-smoking lung carcinoma patients. Moreover, HDL level was associated with TNM staging and lymph node metastasis. An effective chemotherapy could greatly increase HDL level in lung carcinoma patients. Liposomes are a promising delivery route for miR-targeted drugs. These artificial, small spherical vesicles prepared from lipid bilayers are used to deliver drugs, miRs and siRNAs. An animal experiment demonstrated that both neutral fat emulsion and cationic lipid complex can effectively transport miR-34a and let-7 into lung carcinoma cells, yielding a 60% decline of tumor lesion areas [31]. In this article, a positive correlation between serum levels of miR-103 and HDL was identified in lung carcinoma patients either before or after chemotherapy. The potential mechanism, however, should be further explored.

To sum up, serum levels of miR-103 and HDL were affected by lung carcinoma. Their expression changes could be utilized as a symbol for assessing chemotherapy effectiveness of lung carcinoma.

Conclusion

MiR-103 and HDL are two factors that can influence the progression of lung carcinoma. Their expression changes after chemotherapy can be utilized for predicting therapeutic efficacy and prognosis in lung carcinoma patients.

Conflict of interests

The authors declare no conflict of interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Huang Y, Shi Y, Yin X, Zhang Q. Analysis of efficacy and prognosis of Osimertinib combined with docetaxel for non-small cell lung cancer. JBUON 2020;25:805-10.
3. Jiang W, Zheng L, Yan Q, Chen L, Wang X. MiR-532-3p inhibits metastasis and proliferation of non-small cell

lung cancer by targeting FOXP3. JBUON 2019;24:2287-93.

4. Michael MZ, O' CS, van Holst PN, Young GP, James RJ. Reduced accumulation of specific microRNAs in colorectal neoplasia. Mol Cancer Res 2003;1:882-91.
5. Esquela-Kerscher A, Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer 2006;6:259-69.
6. Hetta HF, Zahran AM, Shafik EA et al. Circulating miRNA-21 and miRNA-23a Expression Signature as Potential Biomarkers for Early Detection of Non-Small-Cell Lung Cancer. Microrna 2019;8:206-15.
7. He WJ, Li WH, Jiang B, Wang YF, Xia YX, Wang L. MicroRNAs level as an initial screening method for early-stage lung cancer: a bivariate diagnostic random-effects meta-analysis. Int J Clin Exp Med 2015;8:12317-26.
8. Zhuan B, Lu Y, Chen Q et al. Overexpression of the long noncoding RNA TRHDE-AS1 inhibits the progression of lung cancer via the miRNA-103/KLF4 axis. J Cell Biochem 2019;120:17616-24.
9. Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000;86:943-9.
10. Garber AM, Avins AL. Triglyceride concentration and coronary heart disease. BMJ 1995;310:259-60.
11. Gotto AJ. High-density lipoprotein cholesterol and triglycerides as therapeutic targets for preventing and treating coronary artery disease. Am Heart J 2002;144:S33-42.
12. Ahn J, Lim U, Weinstein SJ et al. Prediagnostic total and high-density lipoprotein cholesterol and risk of cancer. Cancer Epidemiol Biomarkers Prev 2009;18:2814-21.
13. Shor R, Wainstein J, Oz D et al. Low HDL levels and the risk of death, sepsis and malignancy. Clin Res Cardiol 2008;97:227-33.
14. Kucharska-Newton AM, Rosamond WD, Schroeder JC, McNeill AM, Coresh J, Folsom AR. HDL-cholesterol and the incidence of lung cancer in the Atherosclerosis Risk in Communities (ARIC) study. Lung Cancer 2008;61:292-300.
15. Dilman VM, Berstein LM, Ostroumova MN, Tsyrlina YV, Golubev AG. Peculiarities of hyperlipidaemia in tumour patients. Br J Cancer 1981;43:637-43.
16. Witwer KW. XenomiRs and miRNA homeostasis in health and disease: evidence that diet and dietary miRNAs directly and indirectly influence circulating miRNA profiles. Rna Biol 2012;9:1147-54.
17. Ye Q, Zhao X, Xu K et al. Polymorphisms in lipid metabolism related miRNA binding sites and risk of metabolic syndrome. Gene 2013;528:132-8.
18. Osarogiagbon RU, Smeltzer MP, Faris N, Rami-Porta R, Goldstraw P, Asamura H. Comment on the Proposals for the Revision of the N Descriptors in the Forthcoming Eighth Edition of the TNM Classification for Lung Cancer. J Thorac Oncol 2016;11:1612-4.
19. Lucidarme O, Wagner M, Gillard P et al. RECIST and CHOI criteria in the evaluation of tumor response in patients with metastatic colorectal cancer treated with regorafenib, a prospective multicenter study. Cancer Imaging 2019;19:85.
20. Moreira AL, Eng J. Personalized therapy for lung cancer. Chest 2014;146:1649-57.
21. Tsao AS, Jolly S, Lee JM. Updates in Local-Regionally Advanced Non-Small Cell Lung Cancer. Am Soc Clin Oncol Educ Book 2019;39:553-62.
22. Olaussen KA, Postel-Vinay S. Predictors of chemotherapy efficacy in non-small-cell lung cancer: a challenging landscape. Ann Oncol 2016;27:2004-16.
23. Orellana EA, Kasinski AL. MicroRNAs in Cancer: A Historical Perspective on the Path from Discovery to Therapy. Cancers (Basel) 2015;7:1388-405.
24. Dalgard CL, Polston KF, Sukumar G, Mallon CT, Wilkerson MD, Pollard HB. MicroRNA Expression Profiling of the Armed Forces Health Surveillance Branch Cohort for Identification of "Enviro-miRs" Associated With Deployment-Based Environmental Exposure. J Occup Environ Med 2016;58:S97-103.
25. Cui EH, Li HJ, Hua F et al. Serum microRNA 125b as a diagnostic or prognostic biomarker for advanced NSCLC patients receiving cisplatin-based chemotherapy. Acta Pharmacol Sin 2013;34:309-13.
26. Geng L, Sun B, Gao B et al. MicroRNA-103 promotes colorectal cancer by targeting tumor suppressor DICER and PTEN. Int J Mol Sci 2014;15:8458-72.
27. Chou CK, Chang YT, Korinek M et al. The Regulations of Deubiquitinase USP15 and Its Pathophysiological Mechanisms in Diseases. Int J Mol Sci 2017;18:902.
28. Kfir-Erenfeld S, Haggiag N, Biton M, Stepensky P, Assayag-Asherie N, Yefenof E. miR-103 inhibits proliferation and sensitizes hemopoietic tumor cells for glucocorticoid-induced apoptosis. Oncotarget 2017;8:472-89.
29. Barter P, Gotto AM, LaRosa JC et al. HDL cholesterol, very low levels of LDL cholesterol, and cardiovascular events. N Engl J Med 2007;357:1301-10.
30. Regnstrom J, Nilsson J, Tornvall P, Landou C, Hamsten A. Susceptibility to low-density lipoprotein oxidation and coronary atherosclerosis in man. Lancet 1992;339:1183-6.
31. Trang P, Wiggins JF, Daige CL et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. Mol Ther 2011;19:1116-22.