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Original Article

Reduced circulating exosomal miR-382 predicts unfavorable outcome in non-small cell lung cancer

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Abstract: Circulating microRNAs (miRNAs) have been demonstrated as robust and promising biomarkers for non-small cell lung cancer (NSCLC). Our aim was to determine the significance of serum exosomal miR-382 in NSCLC. Circulating exosomes were collected from 126 patients with NSCLC and 60 normal controls before treatment and one month after surgery. The circulating exosomal miR-382 expression was measured with quantitative RT-PCR (qRT-PCR) in all the participants. Our findings demonstrated that circulating exosomal miR-382 was very reduced in NSCLC. In addition, it showed high accuracy for discriminating NSCLC patients from healthy subjects. Interestingly, serum exosomal miR-382 improved the diagnostic accuracy of carcinoembryonic antigen (CEA). Moreover, its level increased significantly one month following surgical resection. Reduced circulating exosomal miR-382 was positively associated with poor clinical variables. NSCLC cases with lower serum exosomal miR-382 suffered worse overall survival (OS) and serum exosomal miR-382 was independently associated with OS. Taken together, circulating exosomal miR-382 is a robust biomarker for evaluating the progression of NSCLC.

Keywords: Circulating exosomal miR-382, prognosis, NSCLC

Introduction

Lung cancer is the most common cause of cancer death, and non-small cell lung cancer (NSCLC) is the major subtype [1, 2]. Despite great progress in treating NSCLC, the overall survival of NSCLC remains dismal [3]. Due to no obvious clinical symptoms and the lack of early detection methods, most NSCLC cases are first diagnosed at an advanced stage [4, 5]. Therefore, identification of robust biomarkers is of great significance to improve the outcome of NSCLC.

MicroRNAs (miRNAs) are non-coding RNAs involved in regulating many processes such as growth, differentiation and development [6]. Evidence has demonstrated that miRNAs are key players for cancer initiation and progression, and they can function as either oncogenes or tumor suppressors [7, 8]. Exosomes are membrane vesicles which contain many active molecules including miRNAs [9]. Exosomal miRNAs can be isolated from the blood and stably detected, making them attractive for the detection of cancer, including NSCLC. For instance, serum exosomal miR-126, miR-126b, and miR-17-5p showed good performance for NSCLC diagnosis and prognosis [10-12].

miR-382 is located at the14q32 cluster and involved in tumor development [13]. miR-382 is dramatically decreased in NSCLC and plays a tumor suppressive role NSCLC [14, 15]. However, the diagnostic and prognostic role of circulating exosomal miR-382 in NSCLC is unknown. The current study aimed to measure circulating exosomal miR-382 levels in NSCLC, and further analyze its value for diagnosis and prognosis prediction of NSCLC.

Materials and methods

Patients and blood samples

The study was approved by the Ethics Committee of Sichuan Cancer Hospital & Institute,

Table 1. Correlation between clinical variables and serum exosomal miR-382 expression

Variable	Number	High miR-382	Low miR-382	*P*-value
Age (years)				0.3691
<60	55	30	25	
≥60	71	33	38	
Gender				0.1349
Male	82	45	37	
Female	44	18	26	
Smoking status				0.4692
No	52	28	24	
Yes	74	35	39	
Differentiation				0.0627
Well	31	20	11	
Moderate/Poor	95	43	52	
Vascular invasion				0.0718
No	72	41	31	
Yes	54	22	32	
Tumor size (cm)				0.0121
<5	56	35	21	
≥5	70	28	42	
Lymph node metastasis				0.0065
No	75	45	30	
Yes	51	18	33	
TNM stage				<0.0001
I/II	67	46	21	
III/IV	59	17	42	

and written informed consent was obtained from all the NSCLC patients and healthy subjects. This study cohort included 126 NSCLC subjects who underwent surgery and sixty healthy volunteers. The clinical information of NSCLC cases was presented in **Table 1**. Venous blood samples were taken from the NSCLC subjects prior to any treatment. Within 1 h after sample collection, the serum was harvested by centrifugation at 3,000 r/min for 20 min, and then stored at -80°C. The exosomes were isolated and purified with ExoQuick Exosome Precipitation Solution (SBI, Mountain View, CA, USA).

RNA isolation and qRT-PCR

Total RNA was extracted from exosomes with mirVana PARIS RNA isolation kit (Ambion, Foster City, CA, USA). The TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) was used to generate cDNA. The cDNA amplification was conducted with SYBR Premix DimerEraser kit (TaKaRa, Dalian, China) on the ABI Prism 7900HT Detection System (Applied Biosystems). Cel-miR-39 was added as a control. The relative circulating exosomal miR-382 level was determined with $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

The data were analyzed by GraphPad Prism 8.0 (GraphPad Software, La Jolla, CA, USA). The differences in serum exosomal miR-382 in different groups were determined by The Mann-Whitney U-test. The correlations between clinical variables and circulating exosomal miR-382 levels were analyzed by the chi-square test. Receiver-operating characteristic (ROC) analysis was performed to determine the discriminative performance of circulating exosomal miR-382. The overall survival (OS) differences were analyzed with Kaplan-Meier plots. Univariate and multivariate analyses were performed to identify independent risk variables. $P<0.05$ was considered significant.

Results

Circulating exosomal miR-382 was reduced in NSCLC

Serum exosomal miR-382 was dramatically reduced in NSCLC cases compared to normal controls ($P<0.0001$) (**Figure 1A**). NSCLC patients with ≥5 cm tumor size ($P<0.0001$, **Figure 1B**), positive lymph node metastasis ($P<0.0001$, **Figure 1C**), or advanced TNM stage ($P<0.0001$, **Figure 1D**) had lower serum exosomal miR-382 levels. One month after surgery, serum exosomal miR-382 levels were detected. Of all patients, 108 patients showed increased serum exosomal miR-382 expression while 18 patients demonstrated decreased expression. As presented in **Figure 2**, circulat-

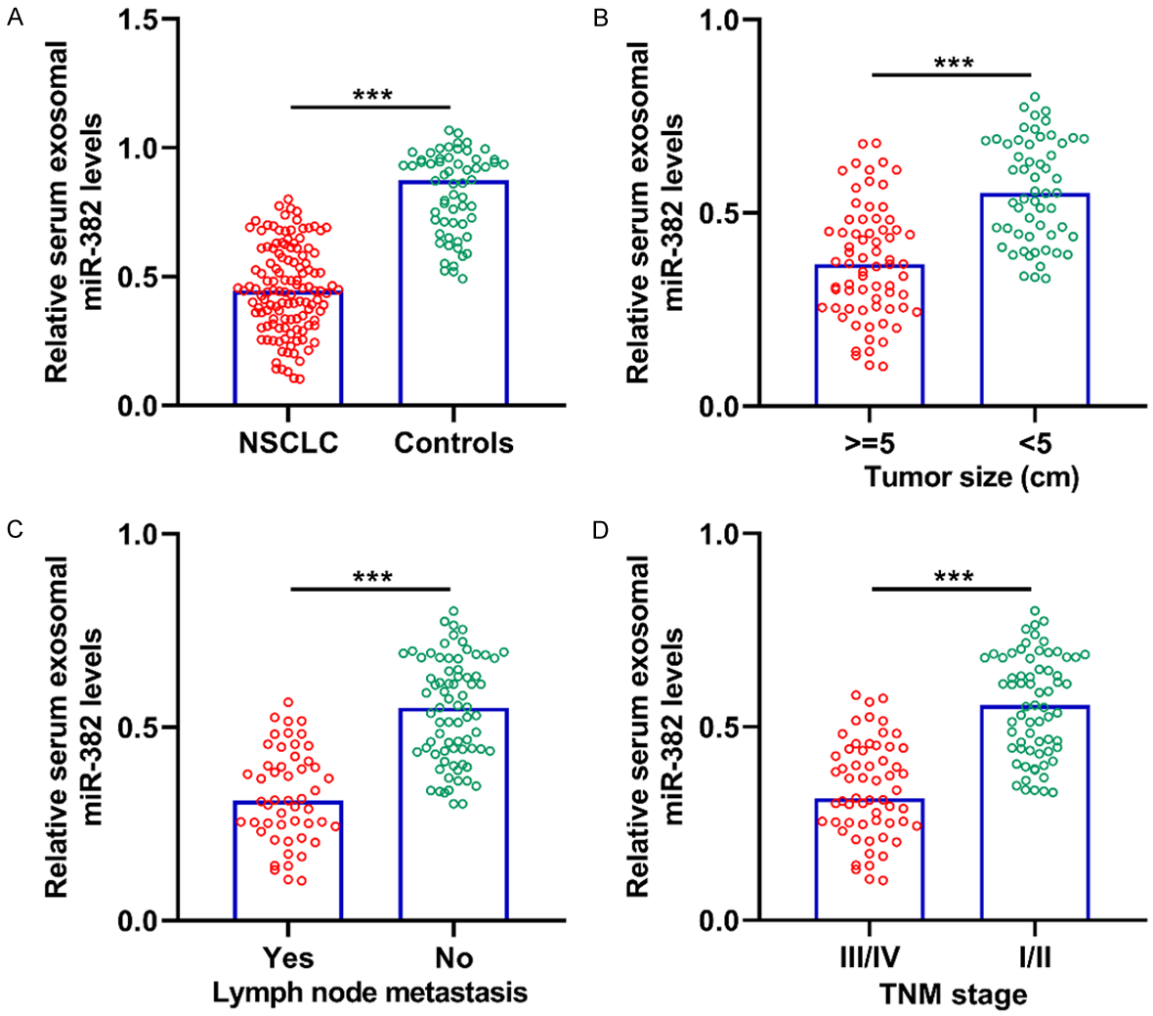

Figure 1. A. The level of circulating exosomal miR-382 was markedly lower in NSCLC patients. B-D. Circulating exosomal miR-382 was lower in NSCLC patients with ≥5 cm tumor size or those with lymph node metastasis or at an advanced TNM stage.

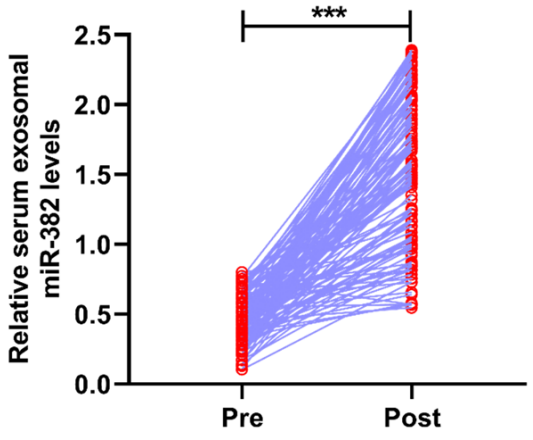

Figure 2. Circulating exosomal miR-382 level was markedly increased one month after surgery.

ing exosomal miR-382 was markedly increased after surgery (P<0.0001).

Diagnostic performance of circulating exosomal miR-382 in NSCLC

We compared the performance of circulating exosomal miR-382 and CEA for discriminating NSCLC cases from healthy subjects. For serum exosomal miR-382, the sensitivity was 76.2% and the specificity was 86.7% with an AUC of 0.902. For CEA, the sensitivity was 74.6% and the specificity was 81.7% with an AUC of 0.852. More importantly, the AUC of combined serum exosomal miR-382 and CEA was 0.953 with 92.9% sensitivity and 88.3% specificity for dif-

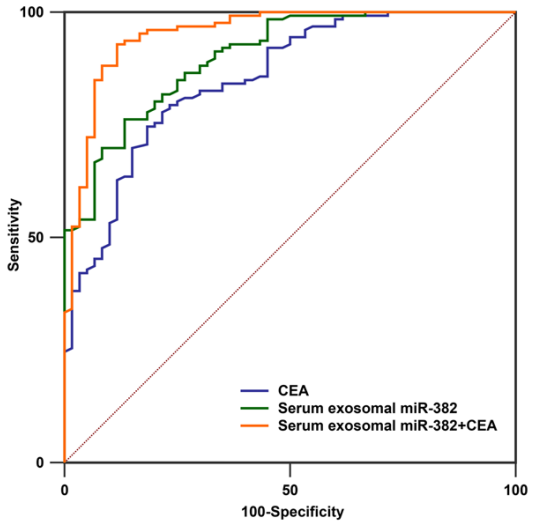

Figure 3. ROC curves were constructed to evaluate the diagnostic potential of serum exosomal miR-382, CEA, and circulating exosomal miR-382 + CEA.

ferentiating NSCLC patients from controls (**Figure 3**).

Reduced circulating exosomal miR-382 was associated with poor clinicopathologic measures in NSCLC

The NSCLC patients were split into high (n=63) and low serum exosomal miR-382 (n=63) groups using the median value. As presented in **Table 2**, reduced circulating exosomal miR-382 was positively associated with tumor size (P=0.0121), lymph node metastasis (P=0.0065), and TNM stage (P<0.0001). No significant correlation was found between circulating exosomal miR-382 and age, gender, smoking status, differentiation, or vascular invasion.

Prognostic significance of circulating exosomal miR-382 in NSCLC

As illustrated in **Figure 4A**, the NSLCC cases in the low circulating exosomal miR-382 group exhibited a significantly worse OS (P=0.0038). Based on the changed pattern of circulating exosomal miR-382 after surgery, the patients were divided into an upregulation group (n=108) and downregulation group (n=18). During the follow up, all 18 patients were diagnosed as relapsed. Patients in the downregulation group had shorter OS (P<0.0001, **Figure 4B**). Furthermore, univariate analysis revealed that circulating exosomal miR-382 (HR=3.34, 95% CI=1.98-4.96, P=0.009), lymph node metastasis, tumor size, and TNM stage were strongly associated with OS. By multivariate analysis, serum exosomal miR-382 (HR=3.47, 95% CI=2.13-5.14, P=0.007) was an independent risk variable for OS (**Table 2**).

Discussion

The role of miR-382 has been explored in NSCLC. For instance, miR-382 was dramatically reduced in NSCLC tissue specimens. miR-382 upregulation markedly suppressed the cancer cell activity through targeting LMO3 [14]. Similarly, miR-382 overexpression suppressed NSCLC tumorigenicity and displayed a significant anti-tumor activity through regulating SETD8 [15]. The findings were in line with our results. Circulating exosomal miR-382 levels were greatly reduced in NSCLC. In addition, its levels were markedly increased one month after surgery. Combining circulating exosomal miR-382 and CEA improved the discriminative power of NSCLC. Moreover, reduced circulating exosomal miR-382 was positively correlated with poor clinical variables. The patients with decreased circulating exosomal miR-382 after surgery had shorter OS. In univariate and multivariate analyses, low serum exosomal miR-382 was correlated with poor prognosis for NSCLC. The data demonstrated that circulating exosomal miR-382 may be a robust biomarker for NSCLC.

To date, the tumor-suppressive functions of serum exosomal miR-382 have been reported in other cancers. miR-382 was reduced in colorectal cancer (CRC) tissues and cell lines. Forced expression of miR-382 remarkably attenuated cancer cell proliferation, growth, and migration through directly silencing SP1 [16], NR2F2 [17] and enhanced chemosensitivity [18]. In glioma, miR-382 was inversely correlated with SETD8 expression. SETD8 overexpression highly promoted carcinogenesis *in vitro* and *in vivo* [19]. Enhanced miR-382 expression provides a tumor-suppressive effect through inhibiting cell proliferation and invasion in prostate cancer (PC) cells, and *vice versa*. COUP-TFII was its downstream target gene [20].

Interestingly, miR-382 seems to play a contradictory role in some cancer types. MiR-382 was frequently overexpressed in gastric adenocarcinoma tissues [21]. Similarly, miR-382 was sig-

Table 2. Univariate and multivariate Cox regression analyses for OS in all NSCLC patients

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*	HR (95% CI)	*P*
Age	1.23 (0.95-1.62)	0.256	-	-
Gender	1.45 (1.16-1.95)	0.108	-	-
Smoking status	1.17 (0.83-1.54)	0.312	-	-
Differentiation	1.61 (1.28-2.21)	0.074	-	-
Vascular invasion	1.56 (1.24-2.13)	0.087	-	-
Tumor size	2.27 (1.42-3.26)	0.022	2.45 (1.56-3.58)	0.019
Lymph node metastasis	2.93 (1.72-4.43)	0.013	3.12 (1.82-4.69)	0.011
TNM stage	3.82 (2.41-5.68)	0.003	4.34 (2.87-6.27)	<0.001
Serum exosomal miR-382	3.34 (1.98-4.96)	0.009	3.47 (2.13-5.14)	0.007

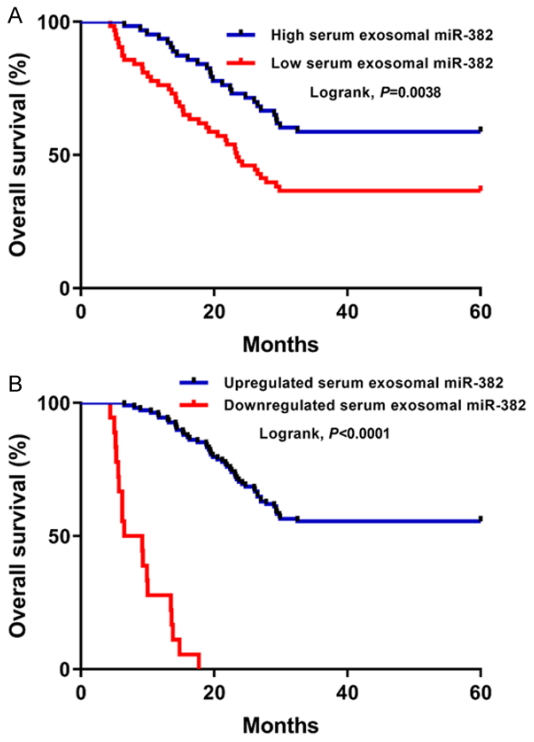

Figure 4. A. OS of all patients stratified by circulating exosomal miR-382 level. B. The OS of all patients stratified by the changed status of serum exosomal miR-382 expression one month following surgery.

nificantly increased in breast cancer (BC), and it was demonstrated as an independent factor for the poor prognosis of BC. *In vitro* analysis showed that miR-382-5p overexpression significantly promoted BC cell clonogenicity by directly targeting RERG [22]. Thus, the complex regulatory function of miR-382 in tumor warrants further exploration.

In summary, the expression level of circulating exosomal miR-382 is markedly reduced in NSCLC. In addition, decreased circulating exosomal miR-382 was correlated with aggressive clinical features and unfavorable outcome. Collectively, circulating exosomal miR-382 is a novel and robust biomarker for evaluating the prognosis of NSCLC.

Disclosure of conflict of interest

None.

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References

[1] Schwartz AG and Cote ML. Epidemiology of lung cancer. Adv Exp Med Biol 2016; 893: 21-41.
[2] Zheng M. Classification and pathology of lung cancer. Surg Oncol Clin N Am 2016; 25: 447-468.
[3] Chen Z, Fillmore CM, Hammerman PS, Kim CF and Wong KK. Non-small-cell lung cancers: a heterogeneous set of diseases. Nat Rev Cancer 2014; 14: 535-546.
[4] Sanchez de Cos J, Sojo Gonzalez MA, Montero MV, Perez Calvo MC, Vicente MJ and Valle MH. Non-small cell lung cancer and silent brain metastasis. Survival and prognostic factors. Lung Cancer 2009; 63: 140-145.
[5] Bahce I, Yaqub M, Smit EF, Lammertsma AA, van Dongen GA and Hendrikse NH. Personalizing NSCLC therapy by characterizing tumors using TKI-PET and immuno-PET. Lung Cancer 2017; 107: 1-13.
[6] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[7] Li K, Xu Y and Yuan LN. Down-regulation of miR-5702 is associated with clinical progression and poor prognosis in patients with non-small-cell lung cancer. Eur Rev Med Pharmacol Sci 2019; 23: 2047-2052.
[8] Liu R, Zhang YS, Zhang S, Cheng ZM, Yu JL, Zhou S and Song J. MiR-126-3p suppresses the growth, migration and invasion of NSCLC via targeting CCR1. Eur Rev Med Pharmacol Sci 2019; 23: 679-689.
[9] Dreyer F and Baur A. Biogenesis and functions of exosomes and extracellular vesicles. Methods Mol Biol 2016; 1448: 201-216.
[10] Zhang Y, Zhang Y, Yin Y and Li S. Detection of circulating exosomal miR-17-5p serves as a novel non-invasive diagnostic marker for non-small cell lung cancer patients. Pathol Res Pract 2019; 215: 152466.
[11] Grimolizzi F, Monaco F, Leoni F, Bracci M, Staffolani S, Bersaglieri C, Gaetani S, Valentino M, Amati M, Rubini C, Saccucci F, Neuzil J, Tomasetti M and Santarelli L. Exosomal miR-126 as a circulating biomarker in non-small-cell lung cancer regulating cancer progression. Sci Rep 2017; 7: 15277.
[12] Liu W, Liu J, Zhang Q and Wei L. Downregulation of serum exosomal miR-216b predicts unfavorable prognosis in patients with non-small cell lung cancer. Cancer Biomark 2020; 27: 113-120.
[13] Sarver AL, Thayanithy V, Scott MC, Cleton-Jansen AM, Hogendoorn PC, Modiano JF and Subramanian S. MicroRNAs at the human 14q32 locus have prognostic significance in osteosarcoma. Orphanet J Rare Dis 2013; 8: 7.
[14] Chen D, Zhang Y, Lin Y, Shen F, Zhang Z and Zhou J. MicroRNA-382 inhibits cancer cell growth and metastasis in NSCLC via targeting LMO3. Exp Ther Med 2019; 17: 2417-2424.
[15] Chen T, Ren H, Thakur A, Yang T, Li Y, Zhang S, Wang T and Chen M. miR-382 inhibits tumor progression by targeting SETD8 in non-small cell lung cancer. Biomed Pharmacother 2017; 86: 248-253.
[16] Ren Y, Zhang H and Jiang P. MicroRNA-382 inhibits cell growth and migration in colorectal cancer by targeting SP1. Biol Res 2018; 51: 51.
[17] Zhou B, Song J, Han T, Huang M, Jiang H, Qiao H, Shi J and Wang Y. MiR-382 inhibits cell growth and invasion by targeting NR2F2 in colorectal cancer. Mol Carcinog 2016; 55: 2260-2267.
[18] Yao H, Xia D, Li ZL, Ren L, Wang MM, Chen WS, Hu ZC, Yi GP and Xu L. MiR-382 functions as tumor suppressor and chemosensitizer in colorectal cancer. Biosci Rep 2019; 39: BSR20180441.
[19] Ma Z. Downregulation of SETD8 by miR-382 is involved in glioma progression. Pathol Res Pract 2018; 214: 356-360.
[20] Zhang W, Liu J, Qiu J, Fu X, Tang Q, Yang F, Zhao Z and Wang H. MicroRNA-382 inhibits prostate cancer cell proliferation and metastasis through targeting COUP-TFII. Oncol Rep 2016; 36: 3707-3715.
[21] Seo AN, Jung Y, Jang H, Lee E, Bae HI, Son T, Kwon O, Chung HY, Yu W and Lee YM. Clinical significance and prognostic role of hypoxia-induced microRNA 382 in gastric adenocarcinoma. PLoS One 2019; 14: e0223608.
[22] Ho JY, Hsu RJ, Liu JM, Chen SC, Liao GS, Gao HW and Yu CP. MicroRNA-382-5p aggravates breast cancer progression by regulating the RERG/Ras/ERK signaling axis. Oncotarget 2017; 8: 22443-22459.