Saudi Journal of Biological Sciences 28 (2021) 1561–1568

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Dysregulation of exosomal miR-192 and miR-194 expression in lung adenocarcinoma patients

Tongfei Wang [a], Wei Li [b], Haitao Li [a], Weina Li [c,*]

[a] Department of Oncology, Xi'an No. 3 Hospital, the Affiliated Hospital of Northwest University, Xi'an, Shaanxi 710018, China
[b] Department of Orthopedics, Tangdu Hospital, Fourth Military Medical University, Xi'an, Shaanxi 710038, China
[c] Biotechnology Center, School of Pharmacy, Fourth Military Medical University, Xi'an, Shaanxi 710032, China

ARTICLE INFO

Article history:
Received 19 November 2020
Revised 6 January 2021
Accepted 7 January 2021
Available online 20 January 2021

Keywords:
Non-small cell lung cancer
miR-194
miR-192
Histopathology

ABSTRACT

Non-small cell lung cancer (NSCLC) is the main reason of cancer linked mortality and around 80% of cases diagnosed in advanced stage. Therefore current study designed to evaluate the deregulation of miRNA-194 and miRNA-192 in different body fluid of Non small cell lung cancer participants. Present study recruited newly diagnosed histopathologically confirmed. It was observed that the 40% NSCLC participants showed elevated miR-194 expression and 60% NSCLC participants showed reduced miR-194 expression in serum sample while in Bronchial wash, only 20% NSCLC participants showed elevated miR-194 expression while 80% showed reduced miR-194 expression ($p = 0.003$). It was found that the 54% NSCLC participants showed elevated miR-192 expression and 55% NSCLC participants showed reduced miR-192 expression in serum sample while In Bronchial wash sample, only 25% NSCLC participants showed high miR-192 expression while 75% showed low miR-192 expression ($P = 0.0004$). Expression of miR-194 was significantly associated with TNM stages ($p < 0.0001$, $p < 0.0001$), distant organ metastases ($p < 0.0001$, $p < 0.0001$), pathological grade ($p = 0.0009$, $p = 0.0005$) among serum sample and bronchial wash sample. Same observation was found with expression of miR-192 and it was significantly associated with TNM stages ($p < 0.0001$, $p < 0.0001$), distant organ metastases ($p < 0.0001$, $p < 0.0001$), pathological grade ($p = 0.006$, $p = 0.001$) among serum sample and bronchial wash sample. It was observed that the NSCLC participants who had high serum based miR-194 expression showed 22 months of overall median survival while low expression of serum based miR-194 expression showed 18 months of overall median survival. Present study suggests that decreased expression of miR-194 and miR-192 was significantly associated with different clinical features of NSCLC cases. However, significantly higher number of NSCLC cases showed low expression of miR-194 and miR-192 in bronchial lavage sample. Decreased poor overall survival was found to be associated with bronchial wash sample with respect to low miR-194 and miR-192 expression while NSCLC participants showed better overall survival with high miR-194 and miR-192 expression. This suggested decreased expression of miR-192 and miR-194 expression could be the potential prognostic marker among NSCLC participants.

1. Introduction

Non small cell lung cancer (NSCLC) has been one of the most important cause of cancer-related mortality and around 20% of NSCLC participants diagnosed in early stage and 80% in advanced stage of disease (Siegel et al., 2013). NSCLC patient's treatment shown to improve the 5-year survival around 5% only (Pignon et al., 2008) and it accounts for more than 80% of all lung cancers accompanied by poor prognosis (Jemal et al., 2008). Single stranded non-coding miRNAs are in the length of 18–24 nucleotides and plays significant role in multiple signalling processes through opposite regulation of the target gene expression by complementary binding to the 3 prime untranslated regions (UTRs) of specific target mRNA (Senye et al., 2012). Aberrant miRNA expression is a common feature of many cancers and its dysregulation can both promote and inhibit metastasis (Del Vescovo et al., 2014).

* Corresponding author.
E-mail address: nanavv9810@163.com (W. Li).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.01.013
1319-562X/

MicroRNAs have been involved in regulation of gene activity nearly thirty percent of all protein-coding genes (Friedman et al., 2009). Significantly, a considerable number of miRNAs targeted domains are located at genomic regions linked to carcinoma (Calin et al., 2004). Because of their capacity to regulate almost all aspects of important cellular function, such as differentiation, development, apoptosis, proliferation and increased sensitivity or chemotherapeutic resistance (Sassen et al., 2008). Decreased expression of miR-194 was connected with increased tumor size and advancement of tumor stage in gastric cancer (Song et al., 2012). MiR-194 is a vertebrate particular miRNA with a known function to be involved in the mitochondrial energy production (Siengdee et al., 2015) and it has also been concerned to number of malignancies such as oral cancer (Chi, 2015), breast carcinoma (Iizuka et al., 2013). MiR-194 expression was also associated with NSCLC metastasis as well as deregulated expression participated in cancer cells to metastasize (Wu et al., 2014).

Lagos-Quintana et al. (2003) first time cloned the miRNA-192 and was found to be situated on human chromosome number eleven and is transcribed in miR-194 cluster (Hino et al., 2008). In tissue of hepatocellular carcinoma miR-192 alterations have been identified, miR-192 targets transcriptional thymidylate synthase (TYMS) to persuade 5-fluorouracil resistance (Boni et al., 2010) and regulate cellular production through the targeting p53-microRNA circuit (Song et al., 2008).

Traditional medicinal plants have been widely used to treat cancer. These traditinal medicine play an important role in improving gastrointestinal side effects, reducing cancer-related fatigue, protecting liver function, and ameliorating bone marrow suppression (Valsalam et al., 2019; Kumaresan et al., 2018). Nanoparticles, including ZnO nanoflakes anchored carbon nanoplates were found to be effective on MCF7 cell lines (Arasu et al., 2019). Seaweeds also have been used traditionaly to treat various diseases (Ganesan et al., 2020). Anticancer, anti-tubercular and broad spectrum antibiotics were isolated from various natural sources to treat various dieases (Khusro et al., 2020; Quaik et al., 2020; Balasubramanian et al., 2020). In a study, Mani et al. (2020) characterized proanthocyanidin-chitosan nanoparticles and reported activity against colorectal carcinoma HT-29 cells. The innate immune function of threonine/ serine -protein kinase in freshwater *Macrobrachium rosenbergii* was analyzed in response to pathogen and interactions with host (Ravichandran et al., 2020). Antioxidant natural compounds have been widely used to treat types of cancers. Antioxidant regulation properties of WL15 from glycine-rich protein 2 and cysteine was reported previously (Sannasimuthu et al., 2020; Issac et al., 2020). Zebra fish model has been widely used to analyze antioxidant properties of natural sources to treat types of cancers (Guru et al., 2020). The present research study aimed to evaluate the role of miRNA-194 and miRNA-192 in NSCLC participants.

2. Materials and methods

2.1. Sample collection and blood serum separation

Present research study included histopathologically confirmed new cases of NSCLC participants as well as 100 healthy participants. NSCLC participants with any the previous report of cancer or any body organ metastasized cancer were not considered for this study. After diagnosis confirmation three millilitre of patient's venous blood sample were collected in non EDTA (plain) vials as well as from healthy individuals and centrifuged at 1500 rpm to separate the serum and collected in two millilitre of eppendorf tube and stored at −70 °C for further use.

2.2. Bronchial wash sample collection

During bronchoscope procedure, bronchial wash sample were collected in a sterile RNase free 1.5 ml tube with the help of experienced clinician and further centrifuged at 10,000 rpm for 15 min, further pellet was suspended in phosphate buffer saline and stored at −80 °C for total RNA extraction.

2.3. Total RNA extraction

Serum and bronchial wash samples were thawed and total RNA extraction was done by Trizol reagent using manufacturer protocol and preserved at −70 °C in RNase-free 1.5 ml vials. The quality and quantity of total extracted RNA were checked by the taking absorption ratio of 260/280 nm using a nanodrop.

2.4. Polyadenylation and complementary DNA synthesis

A total of 10 ng of extracted RNA was used for polyadenylation of miRNA and then cDNA synthesis was performed using advanced miRNA cDNA Synthesis Kit (TaqMan, Thermo Scientific) using manufactures instructions. Reverse transcriptase enzyme and other required reagents were subsequently added to bring poly (A) – tail to miRNAs and further cDNA was synthesised using a universal RT primer supplied with kit.

2.5. QRT-PCR for miRNA-194 and miRNA-192 expression

miRNA-194 and miRNA-192 expression level from serum was analysed by quantitative real-time PCR (QRT-PCR) method. QRT-PCR reaction was performed in machine Quant Studio 6 using taqman master mixture (4444556), Taqman probe for miR-194 (478743_mir) and miR-192 (478741_mir) for expression and quantification. Then hsa-miR-16 (477860_mir) was used as house keeping control to estimate the expression.

2.6. Statistical analysis

All the recorded data were analysed by using SPSS 20.0 and Graph Pad Prism software. Expression of results was represented as mean and standard deviation. Each sample was examined in triplicate and then expression was calculated using internal control as reference. The miRNA expression levels were obtained by relative quantification using the $2^{-(\Delta\Delta ct)}$ method. Findings < 1 fold was considered to indicative of down regulation and > 1 fold was considered to be over expression. Chi square test was performed to compare qualitative outcomes in different groups of study variables. The Kaplan–Meier curve were plotted to estimate the survival of NSCLC participants and p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Demographic characteristics of NSCLC participants

All the demographic characteristics were depicted in Table 1. In brief a total of 100 NSCLC participants and 100 healthy volunteers were taken in the present study. Participants with age group of ≤55 years and >55 years were 23% and 77% among NSCLC cases and in controls ≤55 years and >55 years were 25% and 75%. Males and females were 84% and 16% among NSCLC participants while in controls male and females were 80% and 20%, respectively (see Table 2).

Table 1
Demographic characteristic of NSCLC participants and healthy participants.

Variables	NSCLC (%)	Healthy controls (%)
Total participants	100 (100)	100 (100)
Age		
≤55 years	23	25
>55 years	77	75
Gender		
Males	84	80
Females	16	20
Fever		
Yes	54	
No	46	
Fatigue		
Yes	77	
No	23	
Loss of appetite		
Yes	69	
No	31	
Loss of weight		
Yes	75	
No	25	
Dysphasia		
Yes	47	
No	53	
Bone pain		
Yes	53	
No	47	
TNM stages		
Early stage (I & II)	31	
Advanced stage (III & IV)	69	
Distant Metastasis		
Yes	42	
No	58	
Histopathological type		
Adenocarcinoma	56	
Squamous cell carcinoma	44	
Pathological grade		
Well differentiated type	30	
Moderately differentiated type	36	
Poorly differentiated type	34	
Smoking status		
Yes	82	
No	18	
Smoking type		
Cigarette	48	
Pipe	34	

3.2. Comparison of miR-194 expression in serum and bronchial wash

It was observed that the 40% NSCLC participants represented high miR-194 expression and 60% NSCLC participants had low miR-194 expression in serum sample while in bronchial wash, only 20% NSCLC participants showed high miR-194 expression while 80% showed low miR-194 expression ($p = 0.003$) (Fig. 1).

3.3. Comparison of miR-192 expression in serum and bronchial wash

It was found that the 54% NSCLC participants had high miR-192 expression and 55% NSCLC participants had low miR-192 expression in serum while in bronchial wash, only 25% NSCLC participants showed high miR-192 expression while 75% showed low miR-192 expression ($p = 0.0004$) (Fig. 2).

3.4. Expression of miR-194 in serum and bronchial wash sample

About 40% of NSCLC participants showed high miR-194 expression while 60% showed low expression. It has been observed that NSCLC participants who were in early stage (1&II) of disease had 28% high miR-194 expression while 3% had low miR-194 expression. NSCLC participants who were in advanced stage of disease (III&IV) showed 12% high miR-194 expression while 57% low miR-194 expression ($p < 0.0001$). NSCLC participants who had distant organ metastases showed 3% high miR-194 expression and 39% showed low miR-194 expression. NSCLC participants who were negative for distant organ metastases showed 37% high miR-194 expression and 21% showed low miR-194 expression ($p < 0.0001$). NSCLC participants with different pathological grade such as well differentiated, moderately differentiated, poorly differentiated had 20%, 8%, 12% high miR-194 expression while 10%, 28%, 22% showed low miR-194 expression ($p = 0.0009$) respectively. NSCLC participants with bronchial wash sample, 20% participants had high miR-194 expression while 80% had low miR-194 expression. NSCLC participants who were in early stage (1&II) of disease had 19% high miR-194 expression and 12% had low miR-192 expression while NSCLC participants in advanced stage of disease (III&IV) showed 1% had high miR-194 expression while 68% had low miR-194 expression ($p < 0.0001$). NSCLC participants who had distant organ metastases showed 0% high miR-194 expression and 42% showed low miR-194 expression while NSCLC participants who were negative for distant organ metastases showed 20% high miR-194 expression and 38% showed low miR-194 expression ($p < 0.0001$).

NSCLC participants with different pathological grade such as well differentiated, moderately differentiated, poorly differentiated had 13%, 2%, 5% high miR-194 expression while 17%, 34%, 29% showed low miR-194 expression ($p = 0.0005$), respectively (see Table 3).

3.5. Serum miR-192 expression level in bronchial wash sample

NSCLC patient's serum based expression of miR-192 expression was analyzed, 45% of participants showed high miR-912 expression while 55% participants had low miR-192 expression. NSCLC participants who had positive reporting for bone pain showed 17% high miR-192 expression while 36% NSCLC participants had low miR192 expression, while NSCLC participants who were negative for bone pain, among them 28% had high miR192 expression while 19% showed low miR-192 expression ($p = 0.01$). NSCLC participants who were in early stage (1&II) of disease had 28% high miR-192 expression while 3% had low miR-192 expression while NSCLC participants in advanced stage of disease (III&IV) showed 17% high miR-192 high expression while 52% low miR-192 expression ($p < 0.0001$). NSCLC participants who had distant organ metastases showed 2% high miR-192 expression while 40% showed low miR-192 expression. NSCLC participants who were negative for distant organ metastases showed 43% high miR-192 expression while 15% showed low miR-192 expression ($p < 0.0001$). NSCLC participants with different pathological grade such as well differentiated, moderately differentiated, poorly differentiated had 20%, 10%, 15% high miR-192 expression while 10%, 26%, 19% showed low miR-912 expression ($p = 0.006$), respectively.

NSCLC participants with bronchial wash sample, 25% participants had high miR-192 expression while 75% had low miR-192 expression. NSCLC participants in early stage (1&II) of disease had 23% high miR-192 expression and 8% had low miR-192 expression. NSCLC participants in advanced stage of disease (III&IV) showed 2% high miR-192 expression while 67% had low miR-192 expression ($p < 0.0001$). NSCLC participants who had distant organ metastases showed 0% had high miR-192 expression while 42% showed low miR-192 expression while NSCLC participants who were negative for distant organ metastases showed 25% high serum miR-192 expression while 33% showed low miR-192expression ($p < 0.0001$). NSCLC participants with different pathological grade such as well differentiated, moderately differentiated, poorly differentiated had 14%, 3%, 8% high miR-192 expression while 16%, 33%, 26% showed low miR-912 expression ($p = 0.001$).

Table 2
Level of microRNA-194 expression in NSCLC participants' serum sample and bronchial wash sample.

Variables	Expression of miR-194 from Serum High/Low	p value	Expression of miR-194 from Bronchial wash High/Low	p value
Age (in years)				
≤55 years	14/28	0.34	7/35	0.64
>55 years	26/32		13/45	
Gender				
Males	33/51	0.99	17/67	0.84
Females	7/9		3/13	
Fever				
Yes	21/33	0.99	8/46	0.24
No	19/27		12/34	
Fatigue				
Yes	30/47	0.88	16/61	0.77
No	10/13		4/19	
Loss of appetite				
Yes	23/46	0.09	12/57	0.48
No	17/14		8/23	
Loss of weight				
Yes	26/49	0.09	14/61	0.77
No	14/11		6/19	
Dysphasia				
Yes	14/33	0.07	5/42	0.04
No	26/27		15/38	
Bone pain				
Yes	18/35	0.26	10/43	0.99
No	22/25		10/37	
TNM stages				
Stage I & stage II	28/3	<0.0001	19/12	<0.0001
Stage III & stage IV	12/57		1/68	
Distant Metastasis				
Yes	3/39	<0.0001	0/42	<0.0001
No	37/21		20/38	
Histopathological type				
Adenocarcinoma	21/35	0.70	8/48	0.17
Squamous cell carcinoma	19/25		12/32	
Pathological grade				
Well differentiated type	20/10	0.0009	13/17	0.0005
Moderately differentiated type	8/28		2/34	
Poorly differentiated type	12/22		5/29	
Smoking status				
Yes	32/50	0.86	18/64	0.35
No	8/10		2/16	
Smoking type				
Cigarette	15/33	0.13	8/40	0.26
Pipe	17/17		10/24	

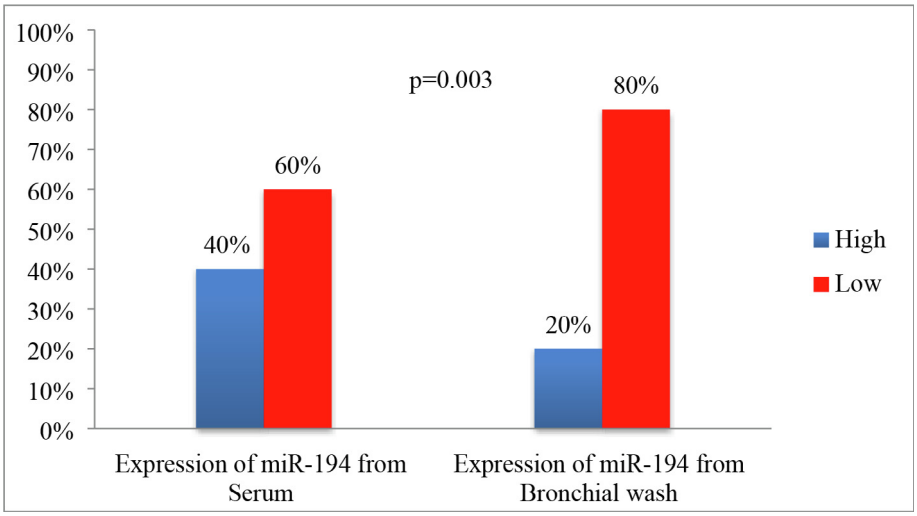

Fig. 1. Comparison of miR-194 expression in serum and bronchial wash samples.

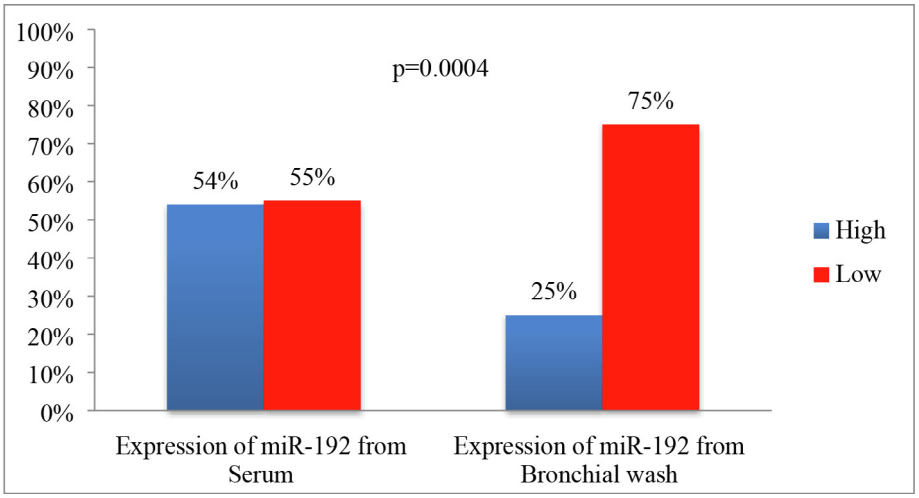

Fig. 2. Comparison of miR-192 expression in serum and bronchial wash samples.

Table 3
microRNA-192 expression in NSCLC participants' serum sample and bronchial wash sample.

Variables	Expression of miR-192 from Serum High/Low	p value	Expression of miR-192 from Bronchial wash High/Low	p value
Age				
≤55 years	17/25	0.56	7/35	0.16
>55 years	28/30		18/40	
Gender				
Males	37/47	0.86	20/64	0.76
Females	8/8		5/13	
Fever				
Yes	22/32	0.46	12/42	0.64
No	23/23		13/33	
Fatigue				
Yes	33/44	0.58	21/56	0.13
No	12/11		4/19	
Loss of appetite				
Yes	27/42	0.12	18/51	0.88
No	18/13		7/24	
Loss of weight				
Yes	33/42	0.92	20/55	0.60
No	12/13		5/20	
Dysphasia				
Yes	18/29	0.28	10/37	0.56
No	27/26		15/38	
Bone pain				
Yes	17/36	0.01	11/42	0.41
No	28/19		14/33	
TNM stages				
Stage I &stage II	28/3	<0.0001	23/8	<0.0001
Stage III & stage IV	17/52		2/67	
Distant Metastasis				
Yes	2/40	<0.0001	0/42	<0.0001
No	43/15		25/33	
Histopathological type				
Adenocarcinoma	21/35	0.13	11/45	0.24
Squamous cell carcinoma	24/20		14/30	
Pathological grade				
Well differentiated type	20/10	0.006	14/16	0.001
Moderately differentiated type	10/26		3/33	
Poorly differentiated type	15/19		8/26	
Smoking status				
Yes	39/43	0.40	21/61	0.99
No	6/12		4/14	
Smoking type				
Cigarette	19/29	0.13	10/38	0.35
Pipe	20/14		11/23	

3.6. NSCLC participants' survival with respect to miR-194

NSCLC participants' survival was calculated based on high and decreased expression of miR-194 in serum and bronchial sample (Fig. 3). It was observed that the NSCLC participants who had high serum miR-194 expression showed 22 months of overall median survival while low expression of serum based miR-194 expression showed 18 months of overall median survival. NSCLC participants who had high miR-194 expression bronchial wash sample showed 33 months of overall survival while low miR-194 expression in NSCLC participants showed 19 months of overall median survival ($p = 0.01$).

3.7. NSCLC participants' survival with respect to miR-192

NSCLC participant's survival was calculated based on high and low expression of miR-192 in serum and bronchial was sample (Fig. 4). It was observed that the NSCLC participants who had high serum based miR-192 expression showed 23 months of overall median survival while low expression of serum based miR-192 expression showed 18 months of overall median survival. NSCLC participants who had high miR-192 expression bronchial wash was showed 30 months of overall survival while low miR-192 expression in NSCLC participants showed 20 months of overall median survival ($p = 0.04$).

4. Discussion

Lung cancer has been major principal causes of death worldwide (Schottenfeld et al., 2013; Malvezzi et al., 2013) and improved survival is greatly depend on early diagnosis because of late diagnosis leads to critical stage (Del Vescovo et al., 2014). Patient's prognostic and diagnostic biomarkers should be unproblematic to detect in different body fluids. In the case of lung cancer, miRNAs have all the criteria to be the biomarker and that should be considered as non-invasive tumor prognostic and diagnostic biomarker potential molecular markers. Micro RNAs sequences have complementation to target gene transcript to regulate post-translational expression and showed impact on cellular functions such as proliferation, differentiation and cell death or apoptosis (Lewis et al., 2005). The microRNAs expression has to be particularly altered in the cancer and involved invarying the expression of multiple target genes (Kong et al., 2012). The variation in microRNAs expression has been involved in several solid tumors (Lin et al., 2010) and suggested in last few years that cell free miRNAs from blood, saliva, plasma and other body fluids has much information (Lagos-Quintana et al., 2001). Therefore present study analyzed the expression level of miR-194 and miR-192 in serum and bronchial lavage in NSCLC cancer participants. About 60% NSCLC participants had low miR-194 expression in serum sample while 80% NSCLC participants had low expression in bronchial lavage sample. It was also observed that 55% NSCLC participants showed low miR-192 expression in serum sample while 75% NSCLC participants showed low miR-192 expression in bronchial lavage sample. It was observed that the 3% NSCLC participants in early stage of disease had low serum miR-194 expression while only 57% NSCLC participants showed low serum miR-194 expression in advanced stage NSCLC participants. Similarly 12% early stage NSCLC participants showed low miR-194 expression while only 68% advanced stage NSCLC participants showed low expressionin bronchial lavage sample. About 21% of NSCLC participants without distant organ metastases showed higher miR-194 expression and 39% NSCLC participants showed high miR-194 expression with distant organ metastasis in NSCLC participants serum sample while 38% NSCLC participants without distant organ metastases showed low miR-194 expression while 42% NSCLC participants with distant organs metastasis showed low miR-194 expression in bronchial lavage sample. NSCLC participants with different pathological grade such as well and poorly differentiated showed 10%, 22% low miR-194 expression while 28% low miR-194 expression in moderately differentiated NSCLC participants serum sample respectively. In NSCLC patient's bronchial lavage sample, well differentiated and poorly differentiated showed 17%, 29% low miR-194 expression while 34% low miR-194 expression in moderately differentiated NSCLC participants. Similar pattern was observed with miR-192 expression, NSCLC participants with early stage of disease showed 3% high miR-192 expression and 52% participants in advanced stage showed low miR-192 expression in serum sample. NSCLC patient's bronchial lavage sample showed 8% low miR-92 expression in early stage of disease and 67% had low miR-192 expression in advanced stage of disease. NSCLC participants without distant organ metastases had 15% low miR-192 expression while distant organ metastatic participants had 40% low miR-192 expression in serum sample, while in bronchial lavage sample only 33% had low miR-192 expression in participants without distant

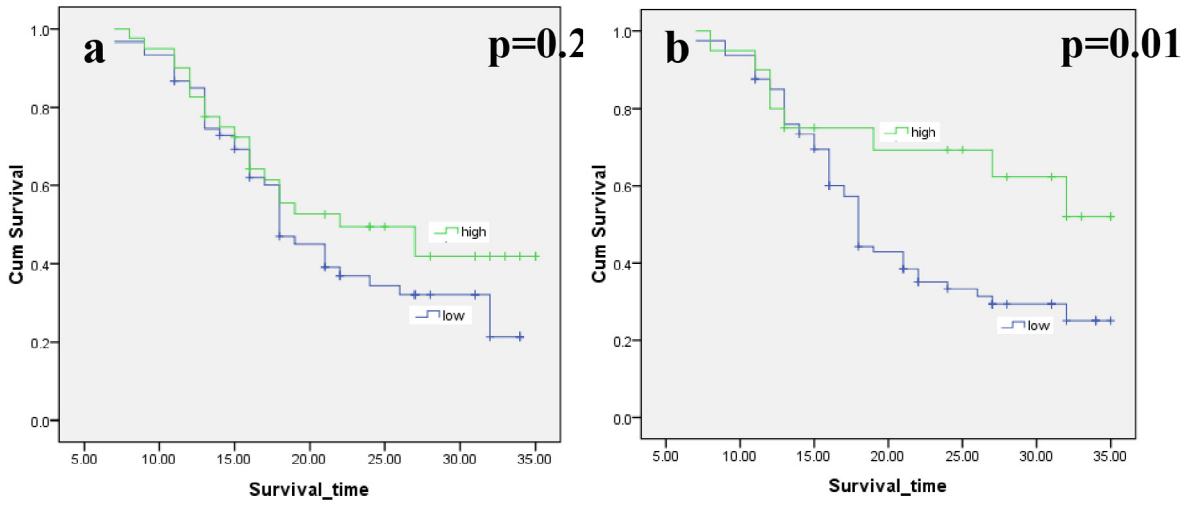

Fig. 3. Kaplan Meier survival curve with respect to miR-194 expression: (a) miR-194 expression in serum and NSCLC participants' survival, (b) miR-194 expression in bronchial wash and NSCLC participant's survival.

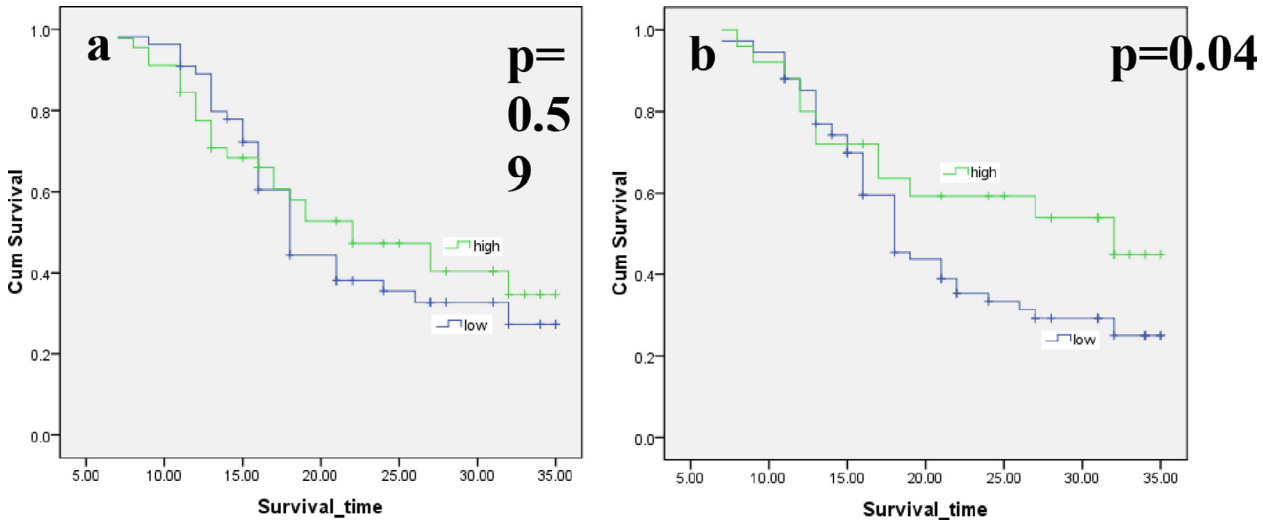

Fig. 4. Kaplan Meier survival curve with respect to miR-192 expression: (a) miR-192 expression in serum and NSCLC participants' survival, (b) miR-192 expression in bronchial wash and NSCLC participants' survival.

organ metastatic participants while 42% NSCLC participants with distant organ metastatic had low miR-192 expression. NSCLC participants with well differentiated and poorly differentiated sample showed 10%, 19% low miR-192 expression while 26% showed low miR-192 expression in moderately differentiated NSCLC participants serum sample respectively. NSCLC patient's bronchial lavage sample, well differentiated and poorly differentiated showed 16%, 26% low miR-192 expression while 33% low miR-192 expression in moderately differentiated NSCLC participants. Kaplan Meier survival analysis showed NSCLC participants with high miR-194 and miR-192 expression in bronchial lavage sample showed better NSCLC participants median survival while low expression was associated with poor overall median NSCLC participants' survival.

In current research work bronchial lavage and serum has been utilised to investigate extracellular microRNAs to receive an attention to conclude very specific for clinical utility in prognosis of NSCLC cancer. If the tumor is unobservable to the bronchoscopist bronchial washing or lavage is commonly performed and could be utilised for molecular study. It was reported that miR-194 was down-regulated and connected with higher tumor size and cellular differentiation in colorectal carcinoma, down-regulation of miR-194 involved in metastasis of tumor and disease spread. Additionally, reduced expression of miR-194 was found in primary tumor samples with metastasis and high expression participate in suppression of motility and invasion of lung cancer cells and decreased expression of miR-194 increases metastasis of NSCLC (Katoh and Katoh, 2004). It has been demonstrated that decreased miR-194 in NSCLC patients tissues in contrast to neighbouring normal lung tissues as well as miR-194 participated in NSCLC spread by regulating cell expansion and invade to other organs tissue (Wang et al., 2013; Li et al., 2014a).

It has been revealed that miR-194 has role in HG-induced development of NSCLC and has been observed that miR-194 was down-regulated in tissues of lung cancer, and expression of miR-194 was linked to clinicopathologic characteristics of NSCLC. Decreased miR-194 expression level was significantly associated to boost cell expansion, cell migration, and incursion in NSCLC cells by interplaying with NFAT5. Focusing on miR-194 alteration could be the hopeful therapeutic target for NSCLC participants with diabetes (Meng et al., 2019). A particular miRNA can transform the signalling arrangement by targeting several genes functions. Numerous miR-194 markers have been recognized in diverse cell contexts and organs, MAP4K4 is one of the spot of miR-194 and mediatesan effect on cellular expansion in hepatocellular carcinoma (Li et al., 2014b). Zhao et al. (2015) illustrated that down expression of miR-194 enhances the cell expansion and invasion in bladder cancer. In renal carcinoma cell (RCC), miR- 194 increased expression results in diminished cell migration and invasionability of RCC (Khella et al., 2013. Lian et al. (2016) suggested that the reduced level of miR-192 was associated with poor overall survival (Wu et al., 2016). Several research groups have also been reported the roles of miR-192-5p in suppressing tumor progression of myeloma, ovarian cancer and pancreatic cancer (Botla et al., 2016).

5. Conclusion

The present study revealed higher number of miR-192 and miR-194 down expression in bronchial lavage sample contrast to serum sample. Down-expression of miR-192 and miR-194 expression was linked with NSCLC clinical outcomes such as TNM stage, distant organ metastases and different histo-pathological grade of NSCLC participants. Low level expression of miR-192 and miR-194 in bronchial lavage sample showed decreased overall median survival of NSCLC participants. This suggested decreased expression of miR-192 and miR-194 could be the potential prognostic marker among NSCLC participants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Arasu, M.V., Madankumar, A., Theerthagiri, J., Salla, S., Prabu, S., Kim, H.S., Al-Dhabi, N.A., Arokiyaraj, S., Duraipandiyan, V., 2019. Synthesis and characterization of ZnO nanoflakes anchored carbon nanoplates for antioxidant and anticancer activity in MCF7 cell lines. Mat. Sci. Eng. 102, 536–540.

Balasubramanian, B., Meyyazhagan, A., Chinnappan, A.J., Alagamuthu, K.K., Shanmugam, S., Al-Dhabi, N.A., Ghilan, A.K.M., Duraipandiyan, V., Arasu, M.V., 2020. Occupational health hazards on workers exposure to lead (Pb): A genotoxicity analysis. J. Infect. Public Health 13 (4), 527–531.

Boni, V., Bitarte, N., Cristobal, I., Zarate, R., Rodriguez, J., Maiello, E., Garcia-Foncillas, J., Bandres, E., 2010. miR-192/ miR-215 influence 5-fluorouracil resistance through cell cycle-mediated mechanisms complementary to its post-

transcriptional thymidilate synthase regulation. Mol. Cancer Ther. 9, 2265–2275.
Botla, S.K., Savant, S., Jandaghi, P., Bauer, A.S., Mucke, O., Moskalev, E.A., et al., 2016. Early epigenetic downregulation of microRNA-192 expression promotes pancreatic cancer progression. Cancer Res. 76, 4149–4159.
Calin, G.A., Sevignani, C., Dumitru, C.D., et al., 2004. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proc. Natl. Acad. Sci. USA 101, 2999–3004.
Chi, H., 2015. miR-194 regulated AGK and inhibited cell proliferation of oral squamous cell carcinoma by reducing PI3K-Akt-FoxO3a signaling. Biomed. Pharmacother. 71, 53–57.
Del Vescovo, V., Grasso, M., Barbareschi, M., Denti, M.A., 2014. MicroRNAs as lung cancer biomarkers. World J. Clin. Oncol. 5, 604–620.
Friedman, R.C., Farh, K.K., Burge, C.B., Bartel, D.P., 2009. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res. 19, 92–105.
Ganesan, A.R., Subramani, K., Balasubramanian, B., Liu, W.C., Arasu, M.V., Al-Dhabi, N.A., Duraipandiyan, V., 2020. Evaluation of in vivo sub-chronic and heavy metal toxicity of under-exploited seaweeds for food application. J. King Saud Univ. Sci. 32 (1), 1088–1095.
Guru, A., Lite, C., Freddy, A.J., Issac, P.K., Pasupuleti, M., Saraswathi, N.T., Arasu, M.V., Al-Dhabi, N.A., Arshad, A., Arockiaraj, J., 2020. Intracellular ROS scavenging and antioxidant regulation of WL15 from cysteine and glycine-rich protein 2 demonstrated in zebrafish in vivo model. Develop. Comp. Immunol. 114, 103863.
Hino, K., Tsuchiya, K., Fukao, T., Kiga, K., Okamoto, R., Kanai, T., Watanabe, M., 2008. Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation. RNA 14, 1433–1442.
Iizuka, D., Imaoka, T., Nishimura, M., Kawai, H., Suzuki, F., Shimada, Y., 2013. Aberrant microRNA expression in radiation-induced rat mammary cancer: the potential role of miR- 194 overexpression in cancer cell proliferation. Radiat. Res. 179, 151–159.
Issac, P.K., Guru, A., Chandrakumar, S.S., Lite, C., Saraswathi, N.T., Arasu, M.V., Al-Dhabi, N.A., Arshad, A., Arockiaraj, J., 2020. Molecular process of glucose uptake and glycogen storage due to hamamelitannin via insulin signalling cascade in glucose metabolism. Mol. Biol. Rep. 47 (9), 6727–6740.
Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., et al., 2008. Cancer statistics. CA. Cancer J. Clin. 58, 71–96.
Katoh, M., Katoh, M., 2004. Human FOX gene family (Review). Int. J. Oncol. 25, 1495–1500.
Khella, H.W., Bakhet, M., Allo, G., Jewett, M.A., Girgis, A.H., Latif, A., Girgis, H., Von Both, I., Bjarnason, G.A., Yousef, G.M., 2013. miR-192, miR-194 and miR-215: a convergent microRNA network suppressing tumor progression in renal cell carcinoma. Carcinogenesis. 34, 2231–2239.
Khusro, A., Aarti, C., Mahizhaveni, B., Dusthackeer, A., Agastian, P., Esmail, G.A., Ghilan, A.K.M., Al-Dhabi, N.A., Arasu, M.V., 2020. Purification and characterization of anti-tubercular and anticancer protein from *Staphylococcus hominis* strain MANF2: In silico structural and functional insight of peptide. Saud. J. Biol. Sci. 27 (4), 1107–1116.
Kong, Y.W., Ferland-McCollough, D., Jackson, T.J., Bushell, M., 2012. MicroRNAs in cancer management. Lancet Oncol. 13, e249–e258.
Kumaresan, V., Pasupuleti, M., Arasu, M.V., Al-Dhabi, N.A., Arshad, A., Amin, S.N., Yusoff, F.M., Arockiaraj, J., 2018. A comparative transcriptome approach for identification of molecular changes in *Aphanomyces invadans* infected *Channa striatus*. Mol. Biol. Rep. 45 (6), 2511–2523.
Lagos-Quintana, M., Rauhut, R., Lendeckel, W., Tuschl, T., 2001. Identification of novel genes coding for small expressed RNAs. Science 294 (5543), 853–858.
Lagos-Quintana, M., Rauhut, R., Meyer, J., Borkhardt, A., Tuschl, T., 2003. New microRNAs from mouse and human. RNA 9, 175–179.
Lewis, B.P., Burge, C.B., Bartel, D.P., 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120, 15–20.
Li, X., Chen, S., Sun, T., Xu, Y., Chen, Y., Liu, Y., Xiang, R., Li, N., 2014. The transcriptional regulation of SOX2 on FOXA1 gene and its application in diagnosis of human breast and lung cancers. Clin. Laborat. 60, 909–918.
Li, Z., Ying, X., Chen, H., et al., et al. 2014. MicroRNA-194 inhibits the epithelial-mesenchymal transition in gastric cancer cells by targeting FoxM1. Dig. Dis. Sci. 59 (9), 2145–2152.
Lian, J., Jing, Y., Dong, Q., Huan, L., Chen, D., Bao, C., et al., 2016. miR-192, a prognostic indicator, targets the SLC39A6/SNAIL pathway to reduce tumor metastasis in human hepatocellular carcinoma. Oncotarget 7, 2672–2683.
Lin, P.Y., Yu, S.L., Yang, P.C., 2010. MicroRNA in lung cancer. Br. J. Cancer. 103, 1144–1148.
Malvezzi, M., Bertuccio, P., Levi, F., La Vecchia, C., Negri, E., 2013. European cancer mortality predictions for the year 2013. Ann. Oncol. 24, 792–800.
Mani, S., Balasubramanian, B., Balasubramani, R., Chang, S.W., Ponnusamy, P., Esmail, G.A., Arasu, M.V., Al-Dhabi, N.A., Duraipandiyan, V., 2020. Synthesis and characterization of proanthocyanidin-chitosan nanoparticles: An assessment on human colorectal carcinoma HT-29 cells. J. Photochem. Photobiol. B: Biol. 210, 111966.
Meng, X., Li, Z., Zhou, S., Xiao, S., Yu, P., 2019. miR-194 suppresses high glucose-induced non-small cell lung cancer cell progression by targeting NFAT5. Thorac Cancer. 10 (5), 1051–1059.
Pignon, J., Tribodet, H., Scagliotti, G., Douillard, J., Shepherd, F., Stephens, R., et al., 2008. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. J. Clin Oncol. 26 (21), 3552–3559.
Quaik, S., Embrandiri, A., Ravindran, B., Hossain, K., Al-Dhabi, N.A., Arasu, M.V., Ignacimuthu, S., Ismail, N., 2020. Veterinary antibiotics in animal manure and manure laden soil: scenario and challenges in Asian countries. J. King Saud Univ. Sci. 32 (2), 1300–1305.
Ravichandran, G., Pasupuleti, M., Arasu, M.V., Al-Dhabi, N.A., Arshad, A., Arockiaraj, J., 2020. Innate immune function of serine/threonine-protein kinase from Macrobrachium rosenbergii in response to host-pathogen interactions. Fish Shellfish Immunol. 106, 332–340.
Sannasimuthu, A., Ramani, M., Pasupuleti, M., Saraswathi, N.T., Arasu, M.V., Al-Dhabi, N.A., Arshad, A., Mala, K., Arockiaraj, J., 2020. Peroxiredoxin of Arthrospira platensis derived short molecule YT12 influences antioxidant and anticancer activity. Cell Biol. Int. 44 (11), 2231–2242.
Sassen, S., Miska, E.A., Caldas, C., 2008. MicroRNA: implications for cancer. Virchows Arch. 452, 1–10.
Schottenfeld, D., Beebe-Dimmer, J.L., Buffler, P.A., Omenn, G.S., 2013. Current perspective on the global and United States cancer burden attributable to lifestyle and environmental risk factors. Annu. Rev. Public Health 34, 97–117.
Senye, M., Mir, C.F., Morton, S., Thie, N.M., 2012. Topical nonsteroidal anti-inflammatory medications for treatment of temporomandibular joint degenerative pain: a systematic review. J. Orofacial Pain. 26, 26–32.
Siegel, R., Naishadham, D., Jemal, A., 2013. Cancer statistics, 2013. CA Cancer J. Clin. 63 (1), 11–30.
Siengdee, P., Trakooljul, N., Murani, E., Schwerin, M., Wimmers, K., Ponsuksili, S., 2015. MicroRNAs regulate cellular ATP levels by targeting mitochondrial energy metabolism genes during C2C12 myoblast differentiation. PloS One 10, e0127850.
Song, B., Wang, Y., Kudo, K., Gavin, E.J., Xi, Y., Ju, J., 2008. miR-192 Regulates dihydrofolaterEdUctase and cellular proliferation through the p53-microRNA circuit. Clin. Cancer Res. 14, 8080–8086.
Song, Y., Zhao, F., Wang, Z., Liu, Z., Chiang, Y., Xu, Y., Gao, P., Xu, H., 2012. Inverse association between miR-194 expression and tumor invasion in gastric cancer. Ann. Surgical Oncol. 19 (Suppl 3), 509–517.
Valsalam, S., Agastian, P., Arasu, M.V., Al-Dhabi, N.A., Ghilan, A.K.M., Kaviyarasu, K., Ravindran, B., Chang, S.W., Arokiyaraj, S., 2019. Rapid biosynthesis and characterization of silver nanoparticles from the leaf extract of Tropaeolum majus L. and its enhanced in-vitro antibacterial, antifungal, antioxidant and anticancer properties. J. Photochem. Photobiol. B. 191, 65–74.
Wang, L., Qin, H., Li, L., Feng, F., Ji, P., Zhang, J., Li, G., Zhao, Z., Gao, G., 2013. Forkhead-box A1 transcription factor is a novel adverse prognosis marker in human glioma. J. Clin. Neurosci. 20, 654–658.
Wu, S.Y., Rupaimoole, R., Shen, F., Pradeep, S., Pecot, C.V., Ivan, C., et al., 2016. A miR-192-EGR1-HOXB9 regulatory network controls the angiogenic switch in cancer. Nat. Commun. 7, 11169.
Wu, X., Liu, T., Fang, O., Leach, L.J., Hu, X., Luo, Z., 2014. MiR-194 suppresses metastasis of non-small cell lung cancer through regulating expression of BMP1 and $p27^{kip1}$. Oncogene 33, 1506–1514.
Zhao, Y., Li, F., Zhang, X., et al., 2015. MicroRNA-194 acts as a prognostic marker and inhibits proliferation in hepatocellular carcinoma by targeting MAP4K4. Int. J. Clin. Exp. Pathol. 8 (10), 12446–12454.