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Original Article

Analysis of the expression of ARNTL2 and miR-204-5p and their correlation with clinical pathological features in NSCLC patients

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Abstract: Objective: To analyze the expression of Aryl Hydrocarbon Receptor Nuclear Translocator-Like 2 (ARNTL2) and miR-204-5p in non-small cell lung cancer (NSCLC) patients and their correlation with clinicopathological characteristics. Methods: Respiratory department cases from April 2020 to April 2022 were selected, and patients were divided into an NSCLC group (80 cases) and a non-NSCLC group (60 cases). The expression levels of ARNTL2 and miR-204-5p and the survival status were compared between the two groups. The predictive value of ARNTL2 and miR-204-5p for mortality in NSCLC patients was analyzed. Results: TCGA data showed ARNTL2 expression was significantly higher and hsa-miR-204-5p significantly lower in cancer versus normal tissues ($P<0.05$). Patients with high ARNTL2 or miR-204-5p expression had shorter survival than those with low expression ($P<0.05$). In NSCLC patients, ARNTL2 was elevated and miR-204-5p reduced compared to non-NSCLC ($P<0.05$). High ARNTL2 or low miR-204-5p expression correlated with older age, larger tumor size, higher malignancy, lymph node metastasis, advanced stage, and smoking history ($P<0.05$). Over 36 months, survival was lower with high ARNTL2 but higher with high miR-204-5p ($P<0.05$). Pearson analysis showed ARNTL2 positively and miR-204-5p negatively correlated with mortality ($P<0.05$). ROC analysis yielded AUCs, sensitivities, and specificities of 0.914/86.7%/86.2% for ARNTL2, 0.934/81.7%/96.2% for miR-204-5p, and 0.920/89.8%/97.7% for combined detection. Conclusion: The expression levels of ARNTL2 and miR-204-5p in NSCLC are closely associated with patient age, tumor differentiation, and lymph node metastasis, and they have high predictive value for NSCLC-related mortality.

Keywords: ARNTL2, miR-204-5p, NSCLC, pathological characteristics, lymphatic metastasis, AUC

Introduction

Non-small cell lung cancer (NSCLC) is a malignant tumor characterized by lower cellular activity compared to small cell lung cancer, later metastasis, poor survival rates, and a high risk of infection [1]. Treatments such as programmed death-ligand 1 (PD-L1) inhibitors and novel chemotherapeutic agents have been shown to effectively improve both the survival rate and safety profile in patients with NSCLC [2]. However, recent studies have revealed that the 5-year survival rate remains as low as 29.6%, with a marked decline in survival often occurring within the first year after treatment [3, 4]. These findings highlight the urgent need for novel therapeutic targets to enhance clinical outcomes in NSCLC. Aryl hydrocarbon receptor nuclear translocator-like 2 (ARNTL2), a circadian rhythm gene, has been found to promote tumor development through multiple mechanisms, including regulating the circadian expression of vascular endothelial growth factor A (VEGFA), influencing cellular activity, repair functions, and apoptosis pathways [5, 6]. According to Kinouchi K et al. [7], circadian rhythms govern a wide range of complex physiological and metabolic functions in the body and are closely associated with cancer risk, particularly cancer progression and tumor aggressiveness. However, clinical research on the role of ARNTL2 in NSCLC remains limited.

miR-204-5p, as a regulatory factor, can inhibit the expression of corresponding protein-coding genes and also enter mitochondria to regulate

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gene expression, thereby influencing the development and function of various tissues and organ systems [8, 9]. As a regulatory factor, miR-204-5p plays a role in cardiovascular diseases by transfecting and inducing cytoskeletal changes in megakaryocytes, thereby modulating platelet reactivity and affecting thrombosis and cardiovascular function [10]. In gliomas, it mediates urothelial carcinoma-associated 1 (UCA1), promoting tumor cell proliferation, metastasis, and epithelial-mesenchymal transition [11]. Additionally, changes in miR-204-5p expression are closely related to the progression of multiple tumors, including colorectal cancer, bladder cancer, breast cancer, and lung adenocarcinoma, making it a potential prognostic marker for evaluating treatment efficacy [12]. Based on these findings, this study aims to analyze the expression of ARNTL2 and miR-204-5p in NSCLC patients and their association with clinicopathological characteristics. The results may provide insights for identifying novel therapeutic targets and biological markers for NSCLC in clinical practice.

Materials and methods

Clinical data

A total of 80 NSCLC specimens collected from the Department of Pathology of our hospital between April 2020 and April 2022 were designated as the NSCLC group. Meanwhile, 60 samples of normal tissue located more than 5 cm away from the tumor were designated as the non-NSCLC group. This study was designed as an observational study. The NSCLC group included 46 male and 34 female patients, aged 36-74 years, with an average age of (56.78± 6.29) years. The non-NSCLC group consisted of 33 male and 27 female patients, aged 38-75 years, with an average age of (57.21± 6.35) years. There were no statistically significant differences in general characteristics between the two groups (P>0.05), indicating comparability.

(1) Inclusion criteria: ① Approved by the hospital's ethics committee, with informed consent obtained from all patients or their families. ② Patients aged >18 years, diagnosed with NSCLC through blood biochemistry, bronchoscopy, and sputum culture examinations [13], with available tumor and adjacent normal tissue specimens. ③ All cases involved primary tumors without other malignancies or severe underlying diseases. ④ No significant genetic defects, family history, or hereditary conditions. ⑤ ARNTL2 and miR-204-5p gene expression microarray data obtained from human samples in the database.

(2) Exclusion criteria: ① History of bronchial or lung surgery. ② Recurrent NSCLC after treatment or extensive metastasis to bones, muscles, chest wall, etc. ③ Presence of congenital immune disorders, hematologic diseases, neurological dysfunction, or drug/alcohol dependency that could significantly affect ARNTL2 and miR-204-5p expression. ④ Diagnosis of gastrointestinal polyposis or incomplete clinical data.

Database analysis

Original data on ARNTL2 and miR-204-5p expression in NSCLC were obtained from the TCGA database (https://portal.gdc.cancer.gov/) and the GEO Accession Viewer database (https://www.ncbi.nlm.nih.gov/). A total of 1,153 specimens were collected, including 110 from the normal group and 1,043 from the tumor group. Additionally, miRNA data were downloaded, comprising 1,073 specimens, with 90 in the normal group and 983 in the tumor group. Version 4.3.1 of the R programming language was used to normalize, log2-transform, and extract relevant data on ARNTL2 and miR-204-5p expression. Survival analysis was conducted using the "survival" package in R, while the "survminer" package was utilized to generate survival curves comparing NSCLC patients with high and low expression levels of ARNTL2 and miR-204-5p. The analysis was performed using RStudio Desktop (Posit|The Open-Source Data Science Company, https://posit.co/).

Methods

Source of materials: The Trizol reagent kit was purchased from Shanghai Shangbao Biotechnology Co., Ltd. (model 11131-100). The UV3000 ultraviolet spectrophotometer was obtained from Shanghai Jingke Industrial Co., Ltd. Real-time quantitative polymerase chain reaction (PCR) amplification and primer design were performed using the Primer-BLAST software, with primers synthesized by Beijing HuanZhong Ruichi Technology Co., Ltd. Kits for

miRNA probe-based reverse transcription, PCR sequencing, and fluorescence in situ hybridization (FISH) were all purchased from Hubei Aipute Biotechnology Engineering Co., Ltd.

Total RNA extraction: After collection, pathological specimens were immediately placed in RNA preservation solution, stored overnight at 4°C, and then long-term at -20°C. After thawing, samples were transferred to centrifuge tubes and homogenized in Trizol (50-100 mg/mL). After standing for 5 min, 200 μL chloroform substitute was added, shaken until milky, and left for another 5 min. Samples were centrifuged at 12,000 g for 15 min at 4°C to separate the RNA aqueous phase. The upper phase (200 μL) was transferred to a new tube, mixed with equal volume isopropanol, incubated 10 min at room temperature, and centrifuged again (12,000 g, 10 min, 4°C). The RNA pellet was washed with 1 mL 75% ethanol, centrifuged (7,500 g, 5 min, 4°C), and air-dried for 30-60 min. RNA was dissolved in 30 μL RNase-free water, gently mixed, briefly centrifuged, and diluted to 200 ng/μL. RNA concentration and purity were assessed by UV spectrophotometry. Samples with a D260/D280 ratio of 1.8-2.0 were deemed qualified and stored at -80°C.

Reverse Transcription Quantitative PCR (qRT-PCR) detection: A total of 1 μg of total RNA was used to synthesize cDNA following the instructions of the miRNA probe-based reverse transcription kit. Then, 2 μL of the synthesized cDNA was used for PCR detection, with GAPDH serving as the internal control for ARNTL2 and U6 as the internal control for miR-204-5p. The stability of both reference genes was evaluated using NormFinder, with M-values less than 0.5. The total reaction volume was 10 μL. The PCR conditions for ARNTL2 were as follows: pre-denaturation at 95°C for 3 minutes, followed by 40 cycles of 94°C for 30 seconds, 62°C for 40 seconds, and 70°C for 60 seconds. For miR-204-5p, the conditions were: pre-denaturation at 95°C for 3 minutes, followed by 35 cycles of 95°C for 12 seconds, 60°C for 60 seconds, and 72°C for 80 seconds. The primer sequences were as follows: ARNTL2 forward 5'-GGGTCT-TTAAGGCCACACCG-3', reverse 5'-CTTCCTCATTAACGACACACGA-3'; miR-204-5p forward 5'-ATTCCATCGTGAGACAGCACC-3', reverse 5'-CCTGATAAGTACTGATTCGAT-3'; GAPDH forward 5'-TGGAAATCTTGTAATCCGAA-3', reverse 5'-TTCGGTTACCGTGGTAGTG-3'; and U6 forward 5'-CCTGTGGAAGGTGGAACC-3', reverse 5'-AATCCTGGCCTTAGGTACAGCA-3'.

Gene amplification and product sequencing: PCR products were amplified using a FISH kit, and results were evaluated based on the Ratio value, calculated as the total number of red signals in 100 nuclei divided by the total number of green signals in 100 nuclei. Amplification was considered present under any of the following three conditions: a Ratio value >2, the presence of ≥15 red signals or ≥4 clustered red signals in ≥10% of cells, or ≥4 clustered red signals in ≥40% of cells. Following amplification, PCR products were identified by electrophoresis, purified using SAP, and then subjected to bidirectional sequencing using a sequencing kit. The sequencing results were compared with the ARNTL2 and miR-204-5p sequences in the GenBank database using Alignment software. Specificity was confirmed if the sequence met three criteria: similarity ≥95%, E-value ≤1e-5, and a unique match to the target gene. This confirmed specificity enables further studies of gene or protein expression. The relative expression level of miRNA was calculated using the 2^-ΔΔCt method.

Observation indicators

The expression levels of ARNTL2 and miR-204-5p in normal tissues versus cancer tissues were compared using the database. Additionally, the survival outcomes of patients with different expression levels of ARNTL2 and miR-204-5p were analyzed. The expression of ARNTL2 and miR-204-5p in the NSCLC group was compared with that in the non-NSCLC group. Furthermore, the expression levels of ARNTL2 and miR-204-5p in NSCLC patients with different pathological characteristics were compared. Finally, the 3-year survival rate of patients with different expression levels of ARNTL2 and miR-204-5p was evaluated.

Statistical analysis

Basic graphs were created using R version 4.5.1 and the "ggplot2" package in RStudio. The "survminer" and "survival" packages were used for detailed settings of time-to-event and survival curves, while "boxplot" was used to plot gene expression boxplots. Data correction and processing for other analyses were per-

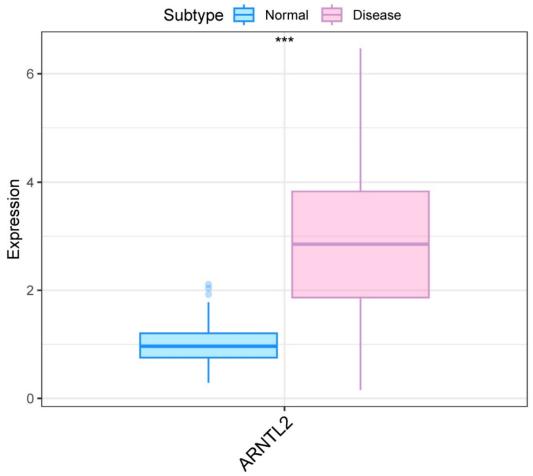

Figure 1. Comparison of ARNTL2 expression between normal tissue and cancer tissue.

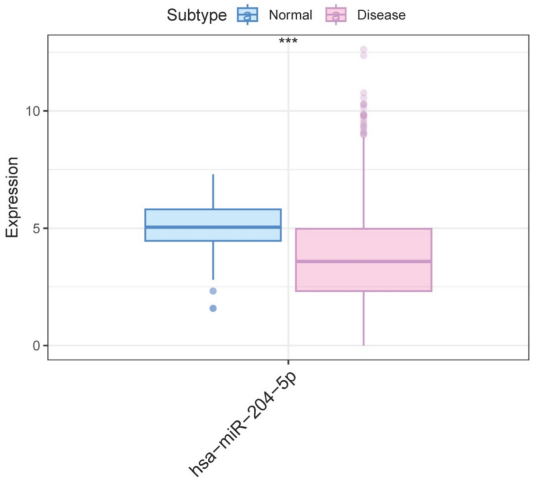

Figure 2. Comparison of miR-204-5p expression between normal tissue and cancer tissue.

formed using SPSS version 27.0 statistical software. Count data were entered as "n (%)", and the chi-square test was used to compare independent sample rates or proportions. Ordinal data were analyzed using the rank-sum test. Quantitative data were presented as "$\bar{x}\pm s$", and group comparisons were performed using the independent samples t-test. Paired t-tests were used for within-group comparisons, and one-way analysis of variance (ANOVA) and the F-test were used for comparing multiple groups. Pearson correlation analysis was used to examine the relationship between ARNTL2, miR-204-5p, and mortality in NSCLC patients. The predictive value of ARNTL2 and miR-204-5p for mortality in NSCLC patients was analyzed using

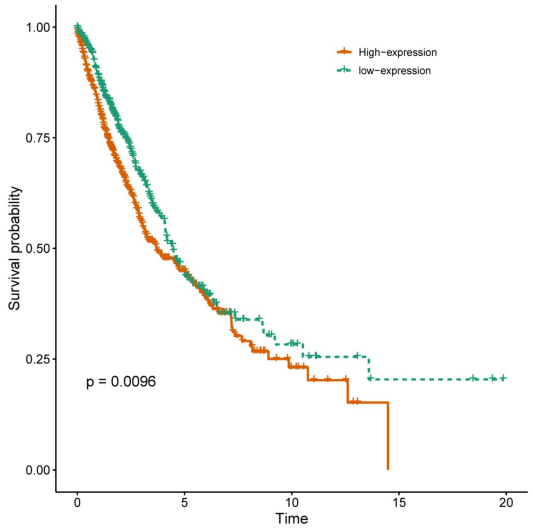

Figure 3. Comparison of survival time in different ARNTL2 expression groups.

receiver operating characteristic (ROC) curve analysis. A *P*-value of <0.05 was considered statistically significant.

Results

Database analysis results

Database analysis results showed that the expression of ARNTL2 in normal tissues was significantly lower than that in cancer tissues, whereas the expression of hsa-miR-204-5p was significantly higher in normal tissues compared to cancer tissues, with both differences being statistically significant (P<0.05) (**Figures 1** and **2**).

Comparison of different ARNTL2 and miR-204-5p expression in NSCLC

The survival times of patients with high expression of ARNTL2 and miR-204-5p were both significantly shorter than those with low expression, with the differences being statistically significant (P<0.05) (**Figures 3** and **4**).

Comparison of ARNTL2 and miR-204-5p expression between the two groups

The expression of ARNTL2 in the NSCLC group was significantly higher than that in the non-NSCLC group, whereas the expression of miR-204-5p was significantly lower in the NSCLC group compared to the non-NSCLC group, with

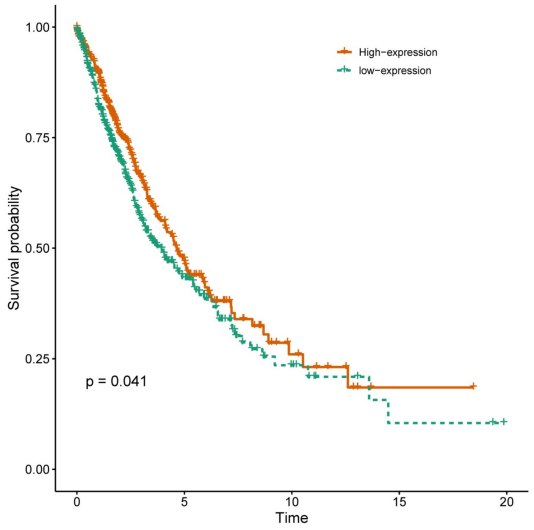

Figure 4. Comparison of survival time in different miR-204-5p expression groups.

Table 1. Comparison of ARNTL2 and miR-204-5p expression between the two groups ($\bar{x}\pm s$)

Group	ARNTL2	miR-204-5p
NSCLC group (n=80)	1.78±0.56	2.17±0.94
Non-nsclc group (n=60)	0.83±0.34	4.57±1.26
t	11.626	12.912
P	<0.001	<0.001

both differences being statistically significant (P<0.05) (**Table 1**).

Comparison of ARNTL2 and miR-204-5p expression in NSCLC patients with different pathological characteristics

The comparison of ARNTL2 and miR-204-5p expression between different genders and histological types showed no statistically significant differences (P>0.05). However, ARNTL2 expression was significantly higher in patients aged ≥60 years, with tumor diameter ≥5 cm, poorly differentiated tumors, lymph node metastasis, stage III-IV tumors, and a history of smoking, compared to those aged <60 years, with tumor diameter <5 cm, moderately/well-differentiated tumors, no lymph node metastasis, stage I-II tumors, and no history of smoking, with statistically significant differences (P<0.05). Conversely, miR-204-5p expression was significantly lower in patients with the same high-risk characteristics compared to their counterparts, also showing statistically significant differences (P<0.05) (**Tables 2** and **3**).

Comparison of 3-year survival rates in patients with different expressions of ARNTL2 and miR-204-5p

At follow-up periods of 12 months, 18 months, 24 months, 30 months, and 36 months, the survival rates of patients with high ARNTL2 expression were significantly lower than those with low ARNTL2 expression, whereas the survival rates of patients with high miR-204-5p expression were significantly higher than those with low miR-204-5p expression. These differences were all statistically significant (P<0.05) (**Table 4**).

Correlation analysis of ARNTL2, miR-204-5p, and mortality in NSCLC patients

Pearson correlation analysis showed that ARNTL2 expression was positively correlated with NSCLC patient mortality (P<0.05), while miR-204-5p expression was negatively correlated with NSCLC patient mortality (P<0.05) (**Table 5**).

The ROC curve analysis of ARNTL2 and miR-204-5p in predicting mortality in NSCLC patients

ROC curve analysis showed that the AUC, sensitivity, and specificity for predicting mortality in NSCLC patients were 0.914, 86.70%, and 86.20% for ARNTL2; 0.934, 81.70%, and 96.20% for miR-204-5p; and 0.920, 89.80%, and 97.70% for the combined diagnosis, respectively (**Figure 5** and **Table 6**).

Discussion

The role and significance of ARNTL2

NSCLC accounts for 80-85% of lung cancers, with histological type closely linked to tumor location [14]. Early treatment mainly involves surgery, but postoperative infections, reduced lung function, and early recurrence often worsen patient quality of life [15]. With the rise of targeted therapies, NSCLC molecular mechanisms have become key to inhibiting tumor metastasis and invasion. ARNTL2 (bmal2 gene) transmits circadian signals to the neuroendo-

Table 2. Comparison of ARNTL2 expression in NSCLC patients with different pathological characteristics ($\bar{x}\pm s$)

Clinical pathological features		n	ARNTL2	*t/F*	*P*
Sex	male	46	1.81±0.62	0.763	0.448
	female	34	1.70±0.66		
Age (year)	<60	21	1.13±0.52	3.918	<0.001
	≥60	59	1.77±0.68		
Histological type	adenocarcinoma	32	1.67±0.43	0.46	0.631
	Squamous carcinoma	28	1.74±0.58		
	other	20	1.59±0.61		
Tumor diameter (cm)	<5	47	1.24±0.47	5.094	<0.001
	≥5	33	1.85±0.60		
Degree of differentiation	Poorly differentiation	49	1.97±0.65	5.737	<0.001
	Medium/High differentiation	31	1.18±0.51		
Lymphatic metastasis	yes	28	1.93±0.59	6.023	<0.001
	no	52	1.22±0.45		
TNM classification	I-II	48	1.34±0.56	4.563	<0.001
	III-IV	32	1.87±0.42		
Smoking history	yes	46	1.65±0.57	4.216	<0.001
	no	34	1.16±0.41		

Table 3. Comparison of miR-204-5p expression in NSCLC patients with different pathological characteristics ($\bar{x}\pm s$)

Clinical pathological features		n	miR-204-5p	t/*F*	*P*
Sex	male	46	2.23±1.02	0.272	0.786
	female	34	2.17±0.91		
Age (year)	<60	21	2.76±1.15	3.605	0.001
	≥60	59	1.89±0.87		
Histological type	adenocarcinoma	32	2.10±0.85	0.23	0.794
	Squamous carcinoma	28	1.96±0.93		
	other	20	2.12±1.05		
Tumor diameter (cm)	<5	47	2.69±0.97	4.615	<0.001
	≥5	33	1.75±0.78		
Degree of differentiation	Poorly differentiation	49	1.68±0.82	5.391	<0.001
	Medium/High differentiation	31	2.84±1.10		
Lymphatic metastasis	yes	28	1.70±0.86	3.773	<0.001
	no	52	2.63±1.14		
TNM classification	I-II	48	1.59±0.80	5.092	<0.001
	III-IV	32	2.65±1.06		
Smoking history	yes	46	1.71±0.87	3.908	<0.001
	no	34	2.55±1.05		

crine and autonomic nervous systems, working with other clock genes to regulate peripheral tissue rhythms [16, 17]. These clock genes help maintain system stability and adjust physiological activities in response to stimuli [18, 19]. Abnormal clock gene expression disrupts cell growth, hormone secretion, and protein synthesis, increasing risks of metabolic, cardiovascular, cognitive diseases, and cancer [20]. Abnormal ARNTL2 further affects tissue rhythms via negative feedback. Sun S et al. [21] found high ARNTL2 expression linked to poorer prognosis, indicating ARNTL2 as a potential biomarker and therapeutic target for NSCLC.

Table 4. Comparison of 3-year survival rates in patients with different expressions of ARNTL2 and miR-204-5p [cases (n%)]

Follow-up time	High expression of ARNTL2 (n=51)	Low expression of ARNTL2 (n=29)	High expression of miR-204-5p (n=39)	Low expression of miR-204-5p (n=41)
6 month Follow-up	48 (94.12)	29 (100.00)	39 (100.00)	38 (92.69)
12 month Follow-up	41 (80.39)	29 (100.00)#	39 (100.00)	31 (75.61)*
18 month Follow-up	32 (62.75)	28 (96.55)#	37 (94.87)	24 (58.54)*
24 month Follow-up	25 (49.02)	25 (86.21)#	32 (82.05)	19 (46.34)*
30 month Follow-up	22 (43.14)	23 (79.31)#	29 (74.36)	16 (39.02)*
36 month Follow-up	22 (43.14)	22 (75.86)#	28 (71.79)	16 (39.02)*

Note: Comparison of high expression and low expression of ARNTL2, #P<0.05; Comparison of high expression and low expression of miR-204-5p, *P<0.05.

Table 5. Correlation Analysis of ARNTL2, miR-204-5p, and Mortality in NSCLC Patients

Variable	*r*	*P*
ARNTL2	0.431	<0.001
miR-204-5p	-0.426	<0.001

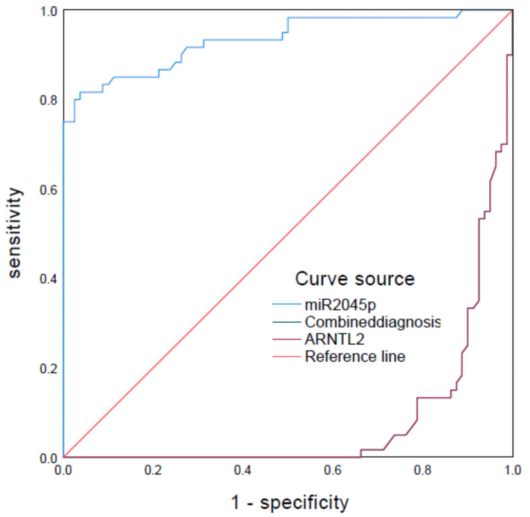

Figure 5. ROC curve analysis of ARNTL2 and miR-204-5p for predicting mortality in NSCLC patients.

The role and significance of miR-204-5p

miRNAs regulate cell differentiation and gene expression by affecting mRNA stability, transcription, translation, and splicing, influencing homeobox genes, neuronal polarity, and brain and heart cell proliferation [22, 23]. miR-204-5p is downregulated in colorectal cancer and reduces tumor invasion and chemotherapy resistance [24]. In a rat model of acute pancreatitis, miR-204-5p decreased pancreatic cell injury via tryptophan 5-monooxygenase activation protein γ, PI3K, and hippocampal pathways, alleviating inflammation [25]. Thus, upregulating miR-204-5p can reduce cell damage and inflammation, slowing disease progression.

However, different studies have suggested that in the treatment of melanoma, upregulation of miR-204-5p expression may increase resistance to therapeutic drugs, while silencing its expression can inhibit cell growth [26]. Liu H et al. [27] also showed that after isoflurane induction, miR-204-5p expression in the hippocampal tissue of rats exposed to isoflurane significantly increased, and the levels of inflammatory factors also rose. Moreover, compared to the control group, the rats experienced more severe neurological dysfunction, while downregulating miR-204-5p expression could effectively alleviate isoflurane-induced cognitive impairment. At the same time, miR-204-5p is not only closely related to tumorigenesis but also interacts with the host genes of the mir-99a-let-7c cluster, reducing the sensitivity of glioblastoma to temozolomide treatment [28]. These findings suggest that miR-204-5p's function and expression significantly differ between tumor tissues and normal tissues or in different diseases. Therefore, in clinical practice, miR-204-5p targeted therapy should be applied rationally according to the patient's specific condition.

The expression of ARNTL2 and miR-204-5p in NSCLC

This study found that in NSCLC, ARNTL2 expression is upregulated while miR-204-5p expression is downregulated. Clock genes show rhythmic expression in bronchial epithelial cells, lung segmental epithelial cells, alveolar walls,

Table 6. ROC curve analysis of ARNTL2 and miR-204-5p for predicting mortality in NSCLC patients

Variable	AUC	95% IC	Sensitivity (%)	Specificity (%)	significance
ARNTL2	0.914	0.843-0.916	86.70	86.20	<0.001
miR-204-5p	0.934	0.891-0.977	81.70	96.20	<0.001
Combined diagnosis	0.920	0.885-0.955	89.80	97.70	<0.001

and capillary walls. Upregulation of ARNTL2 reduces vasodilators and increases inflammatory factors, prostaglandins, and thromboxane release, worsening lung tissue and vascular endothelial inflammation while enhancing tumor cell infiltration and activity [29, 30]. Additionally, elevated ARNTL2 disrupts circadian rhythms in myocardial, neural, and liver/kidney cells, impairing normal blood flow homeostasis and further damaging non-diseased lung tissue, potentially leading to pneumonia and bronchitis [31]. High miR-204-5p expression lowers chemokines like IL-1β, IL-6, and TNF-α, alleviating neurogenic and tumor-related pain and inflammation [32]. Conversely, low miR-204-5p levels accelerate NSCLC progression and worsen symptoms such as hemoptysis, low-grade fever, and irritative cough caused by capillary damage and tumor bronchial obstruction [33].

Therefore, the expression of ARNTL2 in the non-NSCLC group was significantly lower than that in the NSCLC group, while the expression of miR-204-5p was significantly higher in the non-NSCLC group compared to the NSCLC group. In esophageal squamous cell carcinoma, miR-204-5p also shows reduced expression, and animal model evaluations suggest that miR-204-5p can inhibit tumor cell proliferation and induce apoptosis by regulating nestin levels [34]. This could potentially be used as a complementary strategy for chemotherapy, to reduce tumor cell viability, enhance the body's tolerance, and reduce chemotherapy-related toxic side effects and drug dosages. However, it is important to note that miR-204-5p can be regulated by various signaling pathways. For example, TFA p2a can transcribe and inhibit miR-204-5p in cervical cancer, lncRNA TUG1 and COX2 can downregulate miR-204-5p expression in focal cerebral ischemia-reperfusion injury, and CXCL12 and CXCR4 can act as functional targets of miR-204-5p, delaying lymphatic metastasis in gastric cancer [35, 36]. How to precisely and quickly utilize miRNA formulations (such as miR-204-5p) to control the occurrence and development of NSCLC, and whether it can be combined with ARNTL2-targeted therapy, are questions that still need to be confirmed.

The relationship between ARNTL2, miR-204-5p, and different pathological features in NSCLC patients

NSCLC primarily affects individuals over 50, with risk increasing with age, family history, genetics, lung diseases, or smoking [37]. In NSCLC patients aged ≥60, ARNTL2 expression is higher and miR-204-5p lower than in younger patients, highlighting age's impact. Most cases are diagnosed late, with poor postoperative survival due to limited lung function and complications like infections [38]. Tumors ≥5 cm predict worse prognosis, suggesting preoperative pathology comparing ARNTL2 and miR-204-5p in tumor and adjacent tissues can guide precise resection to preserve healthy lung and aid recovery [39]. Poorly differentiated tumors are more aggressive, stem cell-like, less sensitive to therapy, and require close follow-up to prevent recurrence or metastasis [40].

ARNTL2 and miR-204-5p expression levels differ significantly across tumor differentiation, indicating their role in predicting NSCLC malignancy and staging. Lymphatic metastasis, a main pathway for tumor spread and recurrence, can quickly form multiple lesions [41]. Significant changes in ARNTL2 and miR-204-5p warrant vigilance for metastasis and boosting patient immunity to reduce inflammation. Smoking over one year raises lung and liver cancer risks [42], with smokers showing higher inflammation markers than non-smokers [43]. In this study, 36 of 65 patients died over 3 years (55% survival). High ARNTL2 expression linked to lower survival; high miR-204-5p to higher survival. A phase III NSCLC study reported 32.1% 5-year survival after cetuximab treatment [44]. Deng H et al. found 71% of minimally invasive surgery patients had lymph node metastasis [45]. Controlling ARNTL2 and increasing miR-204-5p are crucial for better prognosis.

Summary

ARNTL2 is highly expressed and miR-204-5p is lowly expressed in NSCLC; their levels closely relate to patient age, tumor differentiation, and lymphatic metastasis. Both show strong predictive value for NSCLC patient survival and mortality and can be flexibly applied in clinical diagnosis. This study's limitation lies in the lack of detailed analysis of database data and an incomplete understanding of ARNTL2 and miR-204-5p mechanisms. Future research should integrate clinical practice to further explore their clinical mechanisms and pathological features.

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Disclosure of conflict of interest

None.

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References

[1] Kang J, Zhang C and Zhong WZ. Neoadjuvant immunotherapy for non-small cell lung cancer: state of the art. Cancer Commun (Lond) 2021; 41: 287-302.

[2] Chaft JE, Rimner A, Weder W, Azzoli CG, Kris MG and Cascone T. Evolution of systemic therapy for stages I-III non-metastatic non-small-cell lung cancer. Nat Rev Clin Oncol 2021; 18: 547-557.

[3] Sezer A, Kilickap S, Gümüş M, Bondarenko I, Özgüroğlu M, Gogishvili M, Turk HM, Cicin I, Bentsion D, Gladkov O, Clingan P, Sriuranpong V, Rizvi N, Gao B, Li S, Lee S, McGuire K, Chen CI, Makharadze T, Paydas S, Nechaeva M, Seebach F, Weinreich DM, Yancopoulos GD, Gullo G, Lowy I and Rietschel P. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. Lancet 2021; 397: 592-604.

[4] Garon EB, Hellmann MD, Rizvi NA, Carcereny E, Leighl NB, Ahn MJ, Eder JP, Balmanoukian AS, Aggarwal C, Horn L, Patnaik A, Gubens M, Ramalingam SS, Felip E, Goldman JW, Scalzo C, Jensen E, Kush DA and Hui R. Five-year overall survival for patients with advanced non-small-cell lung cancer treated with pembrolizumab: results from the phase I KEYNOTE-001 study. J Clin Oncol 2019; 37: 2518-2527.

[5] Grillault Laroche D, Curis E, Bellivier F, Nepost C, Gross G, Etain B and Marie-Claire C. Network of co-expressed circadian genes, childhood maltreatment and sleep quality in bipolar disorders. Chronobiol Int 2021; 38: 986-993.

[6] Zhao W, Yuan T, Fu Y, Niu D, Chen W, Chen L and Lu L. Seasonal differences in the transcriptome profile of the Zhedong white goose (Anser cygnoides) pituitary gland. Poult Sci 2021; 100: 1154-1166.

[7] Kinouchi K and Sassone-Corsi P. Metabolic rivalry: circadian homeostasis and tumorigenesis. Nat Rev Cancer 2020; 20: 645-661.

[8] He X and Deng L. miR-204-5p inhibits inflammation of synovial fibroblasts in osteoarthritis by suppressing FOXC1. J Orthop Sci 2022; 27: 921-928.

[9] Song N, Luo J, Huang L, Tian H, Chen Y and He Q. miR-204-5p and miR-211 synergistically downregulate the αS1-casein content and contribute to the lower allergy of goat milk. J Agric Food Chem 2021; 69: 5353-5362.

[10] Garcia A, Dunoyer-Geindre S, Nolli S, Strassel C, Reny JL and Fontana P. miR-204-5p and platelet function regulation: insight into a mechanism mediated by CDC42 and GPIIbIIIa. Thromb Haemost 2021; 121: 1206-1219.

[11] Liang C, Yang Y, Guan J, Lv T, Qu S, Fu Q and Zhao H. LncRNA UCA1 sponges miR-204-5p to promote migration, invasion and epithelial-mesenchymal transition of glioma cells via up-regulation of ZEB1. Pathol Res Pract 2018; 214: 1474-1481.

[12] Toda H, Kurozumi S, Kijima Y, Idichi T, Shinden Y, Yamada Y, Arai T, Maemura K, Fujii T, Horiguchi J, Natsugoe S and Seki N. Molecular pathogenesis of triple-negative breast cancer based on microRNA expression signatures: antitumor miR-204-5p targets AP1S3. J Hum Genet 2018; 63: 1197-1210.

[13] Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, Bruno DS, Chang JY,

Chirieac LR, D'Amico TA, Dilling TJ, Dowell J, Gettinger S, Gubens MA, Hegde A, Hennon M, Lackner RP, Lanuti M, Leal TA, Lin J, Loo BW Jr, Lovly CM, Martins RG, Massarelli E, Morgenszтern D, Ng T, Otterson GA, Patel SP, Riely GJ, Schild SE, Shapiro TA, Singh AP, Stevenson J, Tam A, Yanagawa J, Yang SC, Gregory KM and Hughes M. NCCN guidelines insights: non-small cell lung cancer, version 2.2021. J Natl Compr Canc Netw 2021; 19: 254-266.

[14] Theelen WSME, Chen D, Verma V, Hobbs BP, Peulen HMU, Aerts JGJV, Bahce I, Niemeijer ALN, Chang JY, de Groot PM, Nguyen QN, Comeaux NI, Simon GR, Skoulidis F, Lin SH, He K, Patel R, Heymach J, Baas P and Welsh JW. Pembrolizumab with or without radiotherapy for metastatic non-small-cell lung cancer: a pooled analysis of two randomised trials. Lancet Respir Med 2021; 9: 467-475.

[15] Hu D, Shen Z and Yuan L. MIR-320a/b inhibits cell viability and cell cycle progression by targeting aryl hydrocarbon receptor nuclear translocator-like in acute promyelocyte leukaemia. Pol J Pathol 2022; 73: 99-110.

[16] Medar ML, Andric SA and Kostic TS. Stress alters the transcriptional activity of Leydig cells dependently on the diurnal time. Am J Physiol Cell Physiol 2022; 323: C322-C332.

[17] Lidington D, Wan H, Dinh DD, Ng C and Bolz SS. Circadian rhythmicity in cerebral microvascular tone influences subarachnoid hemorrhage-induced injury. Stroke 2022; 53: 249-259.

[18] Cox KH and Takahashi JS. Circadian clock genes and the transcriptional architecture of the clock mechanism. J Mol Endocrinol 2019; 63: R93-R102.

[19] Costello HM and Gumz ML. Circadian rhythm, clock genes, and hypertension: recent advances in hypertension. Hypertension 2021; 78: 1185-1196.

[20] Sulli G, Lam MTY and Panda S. Interplay between circadian clock and cancer: new frontiers for cancer treatment. Trends Cancer 2019; 5: 475-494.

[21] Sun S, Guo W, Wang Z, Wang X, Zhang G, Zhang H, Li R, Gao Y, Qiu B, Tan F, Gao Y, Xue Q, Gao S and He J. Development and validation of an immune-related prognostic signature in lung adenocarcinoma. Cancer Med 2020; 9: 5960-5975.

[22] Afify AY. A miRNA's insight into the regenerating heart: a concise descriptive analysis. Heart Fail Rev 2020; 25: 1047-1061.

[23] Saliminejad K, Khorram Khorshid HR, Soleymani Fard S and Ghaffari SH. An overview of microRNAs: biology, functions, therapeutics, and analysis methods. J Cell Physiol 2019; 234: 5451-5465.

[24] Schneider R, McKeever P, Kim T, Graff C, van Swieten JC, Karydas A, Boxer A, Rosen H, Miller BL, Laforce R Jr, Galimberti D, Masellis M, Borroni B, Zhang Z, Zinman L, Rohrer JD, Tartaglia MC and Robertson J; Genetic FTD Initiative (GENFI). Downregulation of exosomal miR-204-5p and miR-632 as a biomarker for FTD: a GENFI study. J Neurol Neurosurg Psychiatry 2018; 89: 851-858.

[25] Zhao H and Jiang S. MiR-204-5p performs a protective effect on cerulein-induced rat pancreatic acinar cell AR42J cell damage by targeting tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein gamma and regulating pi3k/hippo pathways. Pancreas 2021; 50: 243-250.

[26] Díaz-Martínez M, Benito-Jardón L, Alonso L, Koetz-Ploch L, Hernando E and TeixidóJ. miR-204-5p and miR-211-5p contribute to BRAF inhibitor resistance in melanoma. Cancer Res 2018; 78: 1017-1030.

[27] Liu H, Wang M, Xu L, Li M and Zhao M. Neuroprotective effect of miR-204-5p downregulation against isoflurane-induced learning and memory impairment via targeting EphB2 and inhibiting neuroinflammation. Hum Exp Toxicol 2021; 40: 1746-1754.

[28] Zhou L and Ma J. MIR99AHG/miR-204-5p/TXNIP/Nrf2/ARE signaling pathway decreases glioblastoma temozolomide sensitivity. Neurotox Res 2022; 40: 1152-1162.

[29] Zhai Q, Wu H, Liu S, Zhu Y, Huang X and Wang J. ARNTL2: a key player in promoting tumor aggressiveness in papillary thyroid cancer. Transl Cancer Res 2025; 14: 522-534.

[30] Qin Y, Ci H, Wang Z, Zhang Y, Xu X and Wu Q. ARNTL2 regulated the oncogene c-myc and promoted the progression of esophageal cancer through activating ANXA2 transcription. Cancer Biol Ther 2025; 26: 2574544.

[31] Zheng W, Zhou C, Xue Z, Qiao L, Wang J and Lu F. Integrative analysis of a novel signature incorporating metabolism and stemness-related genes for risk stratification and assessing clinical outcomes and therapeutic responses in lung adenocarcinoma. BMC Cancer 2025; 25: 591-610.

[32] Guo X, Geng X, Chu Y, Gao J and Jiang L. MiR-204-5p alleviates neuropathic pain by targeting BRD4 in a rat chronic constrictive injury model. J Pain Res 2022; 18: 2427-2435.

[33] Zhang M, Cao M, Kong L, Liu J, Wang Y, Song C, Chen X, Lai M, Fang X, Chen H and Zhang C. MiR-204-5p promotes lipid synthesis in mammary epithelial cells by targeting SIRT1. Biochem Biophys Res Commun 2020; 533: 1490-1496.

[34] Luo H, Lv W, Zhang H, Lin C, Li F, Zheng F and Zhong B. miR-204-5p inhibits cell proliferation

and induces cell apoptosis in esophageal squamous cell carcinoma by regulating Nestin. Int J Med Sci 2022; 19: 472-483.
[35] Zhang P, Hou Q and Yue Q. MiR-204-5p/TFAP2A feedback loop positively regulates the proliferation, migration, invasion and EMT process in cervical cancer. Cancer Biomark 2020; 28: 381-390.
[36] Zhang J, Xing L, Xu H, Wang K, She J, Shi F, Wu H, Sun Y, Gao J and He S. miR-204-5p suppress lymph node metastasis via regulating CXCL12 and CXCR4 in gastric cancer. J Cancer 2020; 11: 3199-3206.
[37] Issa M, Tang J, Guo Y, Coss C, Mace TA, Bischof J, Phelps M, Presley CJ and Owen DH. Risk factors and predictors of immune-related adverse events: implications for patients with non-small cell lung cancer. Expert Rev Anticancer Ther 2022; 22: 861-874.
[38] Muslim Z, Stroever S, Poulikidis K, Weber JF, Connery CP, Herrera LJ and Bhora FY. Conversion to thoracotomy in non-small cell lung cancer: risk factors and perioperative outcomes. Innovations (Phila) 2022; 17: 148-155.
[39] Kim HE, Yu WS, Lee CY, Lee JG, Kim DJ and Park SY. Risk factors for pulmonary complications after neoadjuvant chemoradiotherapy followed by surgery for non-small cell lung cancer. Thorac Cancer 2022; 13: 361-368.
[40] Suazo-Zepeda E, Bokern M, Vinke PC, Hiltermann TJN, de Bock GH and Sidorenkov G. Risk factors for adverse events induced by immune checkpoint inhibitors in patients with non-small-cell lung cancer: a systematic review and meta-analysis. Cancer Immunol Immunother 2021; 70: 3069-3080.
[41] Yamaguchi T, Shimizu J, Oya Y, Watanabe N, Hasegawa T, Horio Y, Inaba Y and Fujiwara Y. Risk factors for pneumonitis in patients with non-small cell lung cancer treated with immune checkpoint inhibitors plus chemotherapy: a retrospective analysis. Thorac Cancer 2022; 13: 724-731.
[42] Simeone JC, Nordstrom BL, Patel K and Klein AB. Treatment patterns and overall survival in metastatic non-small-cell lung cancer in a real-world, US setting. Future Oncol 2019; 15: 3491-3502.
[43] Miranda TS, Heluy SL, Cruz DF, da Silva HDP, Feres M, Figueiredo LC and Duarte PM. The ratios of pro-inflammatory to anti-inflammatory cytokines in the serum of chronic periodontitis patients with and without type 2 diabetes and/or smoking habit. Clin Oral Investig 2019; 23: 641-650.
[44] Bradley JD, Hu C, Komaki RR, Masters GA, Blumenschein GR, Schild SE, Bogart JA, Forster KM, Magliocco AM, Kavadi VS, Narayan S, Iyengar P, Robinson CG, Wynn RB, Koprowski CD, Olson MR, Meng J, Paulus R, Curran WJ Jr and Choy H. Long-term results of NRG oncology RTOG 0617: standard- versus high-dose chemoradiotherapy with or without cetuximab for unresectable stage III non-small-cell lung cancer. J Clin Oncol 2020; 38: 706-714.
[45] Deng H, Liu J, Cai X, Chen J, Rocco G, Petersen RH, Brunelli A, Ng CSH, D'Amico TA, Liang W and He J. Radical minimally invasive surgery after immuno-chemotherapy in initially-unresectable stage IIIB non-small cell lung cancer. Ann Surg 2022; 275: 600-602.