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Smoking induces different expression of miR-320b and miR-10b-5p in plasma extracellular vesicles of non-small cell lung cancer patients

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ABSTRACT

Background: Previous studies found that cigarette smoke (CS) exposure could induce NSCLC malignancy and miRNA dysregulation. Yet, the association of CS-induced miRNA dysregulation and NSCLC malignancy has not been clearly understood. This study aimed to evaluate the effect of CS exposure in smokers on the expression of miR-10b-5p and miR-320b in extracellular vesicles (EVs) from NSCLC patients.
Material and methods: Bioinformatic analysis was conducted to validate miRNA candidates. Blood and tissue samples were collected from NSCLC patients (n = 21) with smoking and non-smoking history. EVs were isolated from plasma and miRNAs were extracted from the isolated EVs. The miRNAs relative expression was analyzed and compared.
Results: In silico analysis identified miR-320b and miR-10b-5p as potential biomarkers for diagnosing NSCLC in smokers. Experimental analysis revealed differential expression of EVs-associated miRNAs in NSCLC patients with smoking and non-smoking histories. EVs-associated miR-10b-5p was significantly overexpressed in smoker NSCLC patients ($p = 0.000$), while miR-320b expression was significantly lower in this group ($p = 0.018$). Additionally, smoking intensity influenced miRNA expression, with higher smoking intensity correlating with increased miR-10b-5p expression and decreased miR-320b expression. ROC analysis demonstrated that EVs were a superior source of miRNAs compared to plasma for NSCLC diagnostics. miR-10b-5p and miR-320b in EVs showed higher diagnostic performance (AUC 0.878; 0.739) compared to plasma (AUC 0.628; 0.559).
Conclusion: CS exposure induces different expression of miR-10b-5p and miR-320b in EVs of NSCLC patients with smoking history. EV-related miR-10b-5p and miR-320b showed potential to be utilized as prognostic biomarker for smokers NSCLC patients.

1. Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) being the most prevalent subtype [1–3]. Cigarette smoke (CS) exposure is a well-established risk factor for lung cancer [4], with several carcinogenic compounds in CS capable of damaging DNA through the formation of DNA adducts, thereby promoting carcinogenesis [3]. Smokers are consequently at the highest risk of developing lung cancer. Moreover, CS not only contributes to carcinogenesis but also exacerbates the progression of NSCLC. Studies have shown that nicotine can enhance NSCLC malignancy [5], and smoking history is associated with a poorer prognosis in NSCLC patients [6].

Extracellular vesicles (EVs) are membrane-bound vesicles secreted by cells and present in various bodily fluids, including plasma [7]. They play critical roles in cellular communication and intercellular signaling

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through their molecular cargo, which includes proteins and nucleic acids such as microRNAs (miRNAs) [7,8]. Several biomolecules such as proteins and nucleic acids, including micro-RNAs (miRNAs) have been found in EVs, and these have been widely studied as biomarkers for many purposes in cancer [7,9–11].

The link between smoking and dysregulated EVs profiles has been reported [12]. Notably, several studies have observed altered miRNA expression in EVs derived from smokers compared to non-smokers [12, 13]. This dysregulation may result from CS-induced DNA damage, as miRNA-coding genes are often located in genomic regions susceptible to such damage [14,15]. These findings suggest that analyzing miRNAs in EVs could help uncover mechanisms by which smoking contributes to NSCLC malignancy.

Among the miRNAs associated with NSCLC, microRNA-10b-5p (miR-10b-5p) and microRNA-320b (miR-320b), have been previously identified by several studies to be associated with NSCLC for their distinct roles in tumor molecular regulation [16–18]. These miRNAs serve different functions in tumor molecular regulation. The miR-10b-5p has been identified as an oncogenic miRNA [18], while miR-320b functions as a tumor suppressor [16,19]. However, to our knowledge, there is no study that has been conducted to analyze the association between CS exposure and the expression of these miRNAs in NSCLC. Furthermore, the possible association between smoking-induced miRNA dysregulation and NSCLC malignancy is a subject that needs to be elucidated. This study aimed to evaluate the expression of miR-10b-5p and miR-320b in EVs isolated from the plasma of NSCLC patients with and without a smoking history. Additionally, since miRNAs encapsulated in EVs are protected by their lipid bilayer membranes [20], we assessed their stability and sensitivity for analysis compared to free miRNAs in plasma.

2. Materials and methods

2.1. Bioinformatic analysis

Differential expression analysis of miRNAs was conducted using datasets GSE62182 and GSE188232 from the Gene Expression Omnibus (GEO) database (NCBI, Bethesda, MD, USA). The analysis was performed with the limma package in R. miRNAs with a p-value of <0.05 and an absolute log2 fold change ($|\log2(\text{Fold Change})|$) > 1 were considered statistically significant. Data visualization included a heatmap created with the pheatmap package and a volcano plot generated using ggplot2. Overlapping miRNAs between the two datasets were identified using Venn diagram analysis with the online tool Venny (https://bioinfogp.cnb.csic.es/tools/venny/). Targets of overlapping miRNAs were predicted using miRDB (https://mirdb.org/). Gene Ontology (GO) analysis was performed via the DAVID platform (https://david.ncifcrf.gov/), and GO terms clustering was conducted with REVIGO (http://revigo.irb.hr/).

2.2. Ethics Approval and patients recruitment

The study protocols and participant recruitment were approved by the Medical Research Ethics Committee of Dharmais Hospital National Cancer Center, Jakarta, Indonesia (041/KEPK/IV/2021). Participants, comprising fourteen smokers and ten non-smokers diagnosed with NSCLC at Dharmais Hospital National Cancer Center, were recruited between July 2021 and January 2022. All participants provided written informed consent prior to enrollment. This study was conducted in full accordance with the ethical principles of the Declaration of Helsinki and its later amendments to ensure the safety, dignity, and rights of all participants.

Demographics and clinical data were obtained from medical records at the time of sample collection and listed in Table 1. Patients included in this study had not received any anticancer therapy and did not have primary tumor in other locations prior to sample collection. Smoking degree was classified according to Brinkman Index (total cigarette consumption per day multiplied by years of smoking; Low <199, moderate: 200–599, high ≥ 600).

Table 1
Demographics and clinical characteristics of NSCLC patients.

Variable	Non-Smoker n = 10	Smoker n = 11	*P-value*
Social-demographic data (n (%))			
Age (Mean)	56.3	58.36	0.315
Age Distribution			
40	1 (10.00 %)	1 (9.09 %)	
41-50	1 (10.00 %)	1 (9.09 %)	
51-60	5 (50.00 %)	3 (27.27 %)	
61-70	2 (20.00 %)	6 (54.5 %)	
71-80	1 (10.00 %)	0	
Sex			**0.002**
Male	4 (40.00 %)	11 (100 %)	
Female	6 (60.00 %)	0	
Smoking Status			
Smoker	0	11 (100 %)	
Non-smoker	10 (100 %)	0	
Brinkman Index			
<199		1 (9.09 %)	
200-599		7 (63.64 %)	
≥600		3 (27.27 %)	
Subtype			0.314
Adenocarcinoma	9 (90.00 %)	8 (72.73 %)	
Squamous Cell Carcinoma	1 (10.00 %)	3 (27.27 %)	
Stage			.156
III	0	2 (18.18 %)	
IV	10 (100 %)	9 (81.82 %)	

2.3. Sample collection

There were 14 plasma samples and 14 tissue samples from 21 NSCLC patients were collected. Peripheral blood obtained from NSCLC patients was processed to separate plasma. Separated plasma was stored in −80 °C prior to next processing. Tissue samples were collected from patients that undergo transthoracic needle biopsy procedure. Subsequently, tissue samples were washed in Phosphate-Buffered Saline (PBS) and frozen in liquid nitrogen for further analysis.

2.4. EVs isolation

EVs were isolated with size exclusion chromatography (SEC) column (IZON qEV2/70 nm, IZON Science, Christchurch, NZ). Plasma sample was prepared as described by the manufacture protocol. Plasma was centrifuged (3000×*g*, 15 min), and supernatant was separated. Supernatant was centrifuged again (3000×*g*, 15 min) to obtain the platelet-free plasma (PFP). Separated PFP then was moved to SEC column to isolate the EVs. Separated fractions were collected as instructed in the manual. EVs fractions were pooled and concentrated with qEV Concentration Kit (IZON Science, Christchurch, NZ).

2.5. Transmission electron microscopy (TEM)

10 µl of the pooled EVs fraction was placed on a 200-mesh copper grid and fixed with 1 % glutaraldehyde in cold PBS, then incubated for 5 min. The grid containing the sample was placed in 10 µl 2 % uranyl oxalate (w/v) and then observed under the Tecnai G2 20 S-Twin (FEI, Oregon, US).

2.6. Flowcytometry

100 µl of EVs fraction were incubated with 3 µl of APC-anti $CD63^+$ antibody (BioLegend®, San Diego, USA) and 3 µl of FITC-anti $CD81^+$ antibody (BioLegend®, San Diego, USA). Samples were incubated for 1 h at room temperature, and measured with FACSAria™ III Cell Sorter (BD Biosciences, NJ, USA).

2.7. Particle size analyzer (PSA)

Samples of the EVs fraction were measured with Zetasizer Nano ZS (Malvern Panalytical, Malvern, UK). Measurements were made at 25 °C.

2.8. RNA isolation and quantitative polymerase chain reaction (qPCR)

Total RNA of tissue and whole plasma samples were extracted using TriPure™ Isolation Reagent (Roche Diagnostics, Basel, Switzerland). EVs RNA was extracted using qEV RNA Extraction Kit (IZON Science, Christchurch, NZ). Extracted RNA then converted to cDNA using miRCURY® LNA® RT Kit (Qiagen, Hilden, Germany) and incubated with Veriti™ 96-well Thermal Cycler (Thermo Fischer Scientific, MA, USA) as instructed in the manufacturer's protocol. For qPCR, 2 µl cDNA was diluted 60x as instructed in the manual, then mixed with miRCURY® LNA® SYBR® Green PCR Kit (Qiagen, Hilden, Germany) and primers in

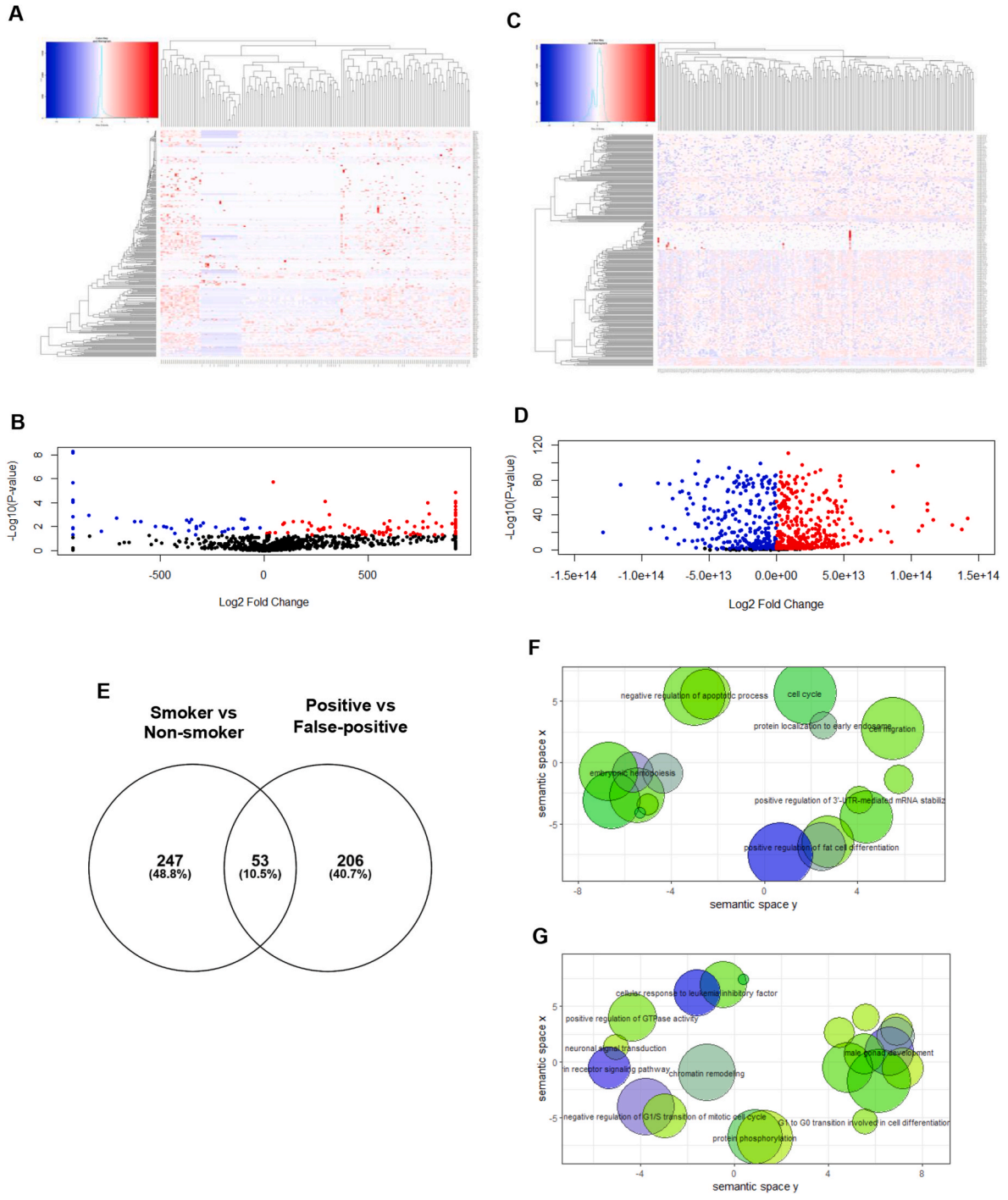

Fig. 1. Bioinformatics Analysis of miRNA Expression in NSCLC. A-B) Heatmap and volcano plot showing miRNA expression differences in NSCLC patient tissues with smoking and non-smoking history, derived from the GSE62182 dataset. C-D) Heatmap and volcano plot comparing miRNA expression in plasma samples from positive NSCLC patients and false-positive cases, based on the GSE188232 dataset. E) Venn diagram illustrating the 53 overlapping miRNAs identified from the top 300 miRNAs in both datasets. F) Gene Ontology (GO) clustering analysis highlighting the biological functions associated with miR-320b. G) GO clustering analysis detailing the functional pathways linked to miR-10b-5p.

a 10 µl reaction volume. qPCR analysis was done using 7500 Fast Real-time PCR System (Applied Biosystem, MA, USA). 18s served as an internal reference, and the relative expression of the miRNAs was assessed using the ΔΔCt method. Each sample was assessed in triplicates.

2.9. Statistical analysis

Data were analyzed using the GraphPad Prism 9 software program (GraphPad Software, San Diego, USA) and Microsoft Excel (Microsoft, Washington, USA). Data were presented as the mean with ± SD. An independent T-test was utilized for comparing two groups. The receiver operating characteristic (ROC) curve was used to evaluate the sensitivity of miRNA in the analysis. P-values of <0.05 were considered to be statistically significant for all comparisons.

3. Results

3.1. Bioinformatics analysis of the potential association between miR-320b and miR-10b-5p in smoking-related NSCLC

To validate our hypothesis that miR-320b and miR-10b-5p are associated with smoking-related NSCLC, publicly available datasets

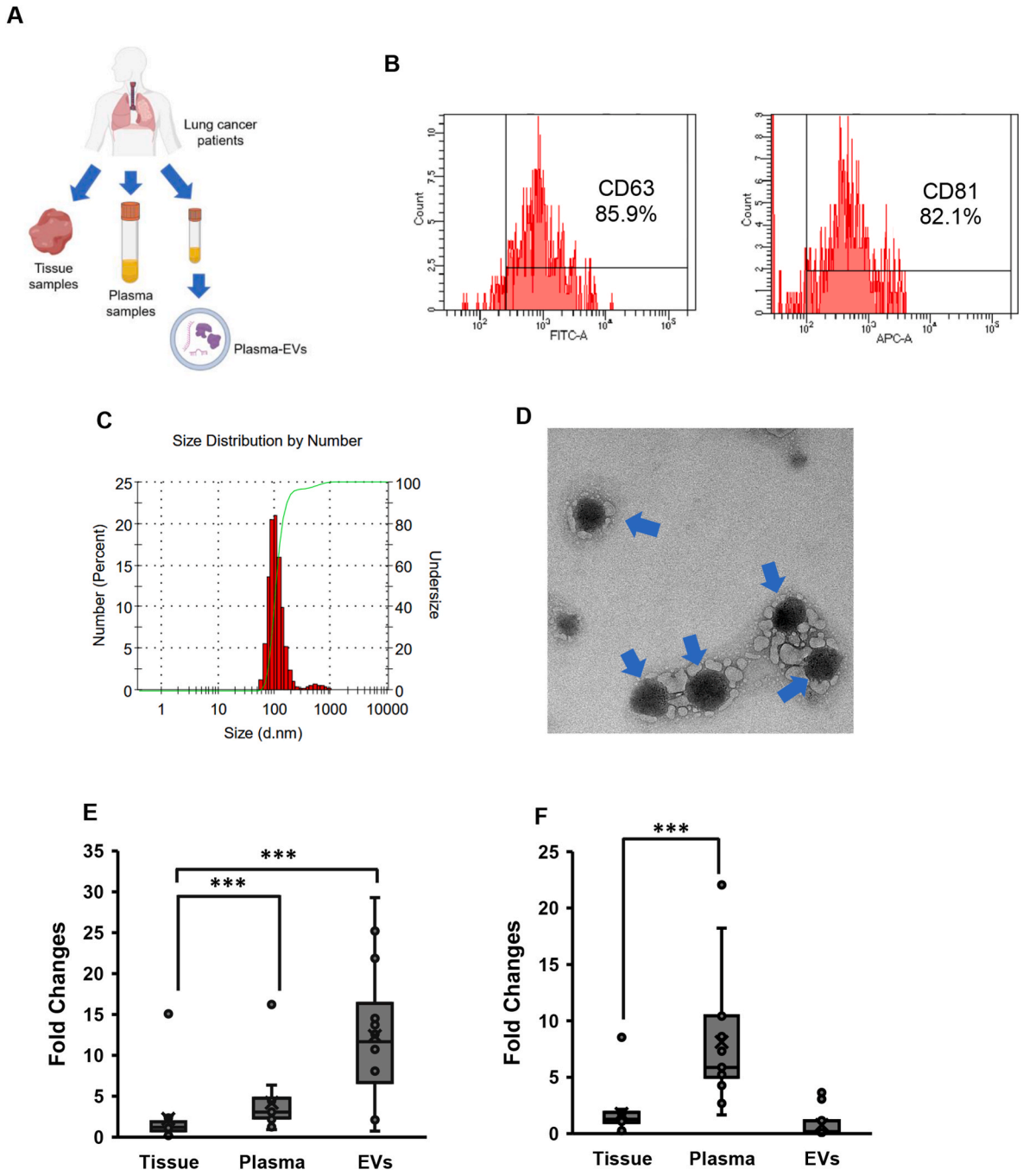

Fig. 2. Characterization of Isolated EVs. A) Flow cytometry analysis of isolated EVs using a singlet gate on a forward scatter versus side scatter dot plot. Filled histograms represent EVs stained with monoclonal antibodies specific to CD63 and CD81. B) Particle size distribution analysis, performed using a particle size analyzer, reveals a predominant population with diameters ranging from 90 to 110 nm. C) Transmission electron microscopy (TEM) showing round, dark vesicles approximately 100 nm in diameter. E) Relative expression of miR-320b in tissue, plasma, and EVs, n = 14, *p < 0.05, **p < 0.01, ***p < 0.005. F) Relative expression of miR-10b-5p in tissue, plasma, and EVs, n = 14, *p < 0.05, **p < 0.01, ***p < 0.005.

GSE188232 and GSE62182 were obtained from the GEO database. GSE188232 includes miRNA expression profiles comparing lung cancer patients and false-positive cases [21], while GSE62182 provides data comparing lung cancer patients with and without a history of smoking [22]. The GSE188232 dataset revealed 726 differentially expressed miRNAs (Fig. 1A and B) in lung cancer patients compared to false-positive cases. Similarly, the GSE62182 dataset identified 1,625 differentially expressed miRNAs (Fig. 1C and D) in lung cancer patients based on smoking history.

We selected the top 300 most significantly different miRNAs ($p < 0.05$) from each dataset and identified 53 overlapping miRNAs (Fig. 1E). Among these, miR-320b was present in the shared list, while miR-10b-5p was significantly differentially expressed between the positive and false-positive groups in the GSE188232 dataset.

To gain insight into their functional roles, we performed GO analysis of the predicted target genes for both miRNAs. The target genes of miR-320b were found to be enriched in pathways related to apoptosis regulation, the cell cycle, and cell migration (Fig. 1D). On the other hand, the target genes of miR-10b-5p were associated with pathways involved in G1-G0 transition, chromatin remodeling, and G1/S phase transition (Fig. 1E).

3.2. Characterization of plasma EVs

To confirm our initial hypothesis and the bioinformatic analysis results, we conducted experimental studies using tissue, plasma, and EVs samples from patients. (Fig. 2A). EVs were isolated from patients' plasma and was characterized using flow cytometry, PSA, and TEM (Fig. 2A–D). Flow cytometry was performed to confirm the presence of EVs by detecting the surface markers CD63 and CD81. The analysis with anti-CD63 and anti-CD81 antibodies demonstrated that the pooled EVs fractions contained a population positive for these EVs-specific protein markers (Fig. 2B). Particle size analysis revealed that the EVs fractions predominantly consisted of particles measuring 90–110 nm in diameter (Fig. 2C). Further visualization using TEM confirmed the morphology of the vesicles as round-shaped, consistent with previously reported EVs morphologies isolated from plasma [23,24], with electron-dense structures approximately 100 nm in size (Fig. 2D).

3.3. Relative expression levels of miR-10b-5p and miR-320b in tumor tissue, plasma, and EVs

The relative expression levels of miR-10b-5p and miR-320b were analyzed using RT-qPCR in 14 tumor tissue samples, 14 plasma samples, and 14 EVs samples. The results, as shown in Fig. 2E and F, highlight the significant differences in miRNA expression among sample types.

miR-320b expression was significantly elevated in EVs ($p = 0.000$) and plasma ($p = 0.002$) compared to tumor tissue (Fig. 2E). Conversely, miR-10b-5p exhibited higher expression in plasma compared to tumor tissue and EVs ($p = 0.000$) (Fig. 2F).

3.4. Decreased expression of miR-320b in EVs from NSCLC patients with smoking history

To evaluate the impact of CS exposure on miR-320b expression, we analyzed its relative levels in plasma and EVs from NSCLC patients with and without a smoking history. No significant differences in miR-320b expression were observed in plasma samples (Fig. 3a). However, miR-320b expression in EVs was significantly lower in smokers compared to non-smokers ($p = 0.018$, Fig. 3b).

Smoking intensity further influenced miR-320b expression levels in EVs but not in plasma (Fig. 3c–d). Higher smoking intensity was associated with reduced miR-320b expression, with moderate smokers ($p = 0.009$) and heavy smokers ($p = 0.000$) showing significantly lower levels compared to light smokers. Receiver operating characteristic (ROC) curve analysis highlighted the superior diagnostic performance of miR-320b in EVs compared to plasma. In EVs, miR-320b exhibited an area under the curve (AUC) of 0.739, with a sensitivity of 73.33 % and a specificity of 54.55 %, compared to plasma (AUC: 0.559; sensitivity: 61.11 %; specificity: 53.33 %; Fig. 3e–f).

3.5. Increased expression of miR-320b in EVs from NSCLC patients with a smoking history

We performed a similar analysis on the same group for miR-10b-5p expression. Our results suggested that miR-10b-5p expression was influenced by smoking history, with significant differences observed only in EVs and not in plasma (Fig. 4A–B). In EVs, miR-10b-5p expression was significantly higher in smokers compared to non-smokers ($p = 0.000$, Fig. 4B). Smoking intensity further modulated miR-10b-5p levels in EVs, with expression increasing as smoking intensity rose (Fig. 4C–D). Moderate smokers ($p = 0.009$) and heavy smokers ($p = 0.001$) exhibited significantly higher miR-10b-5p expression compared to light smokers. ROC analysis demonstrated the diagnostic capability of miR-10b-5p in EVs-associated miRNAs, with an AUC of 0.878, a sensitivity of 78.95 %, and a specificity of 73.68 %, compared to plasma (AUC: 0.628; sensitivity: 63.16 %; specificity: 60 %; Fig. 4e–f). These results underscore the utility of plasma-derived EVs as a reliable source for miRNA analysis in NSCLC diagnostics, highlighting miR-10b-5p and miR-320b as promising biomarker candidates (Fig. 5).

4. Discussion

Smoking is a well-established major risk factor for NSCLC due to the carcinogenic compounds present in CS. Smokers face an increased risk of developing NSCLC, reported to be up to 30 times higher than non-smokers [25]. However, additional risks associated with smoking and NSCLC malignancy have received less attention. Notably, nicotine has been shown to promote malignancy in lung cancer cells, suggesting the potential for worse outcomes in NSCLC patients with a smoking history [5]. This underscores the need to investigate the mechanisms by which CS exacerbates NSCLC malignancy to improve understanding and management of the disease. In this study, we identify two miRNAs, miR-10b-5p and miR-320b, which may contribute to the increased malignancy of NSCLC in smokers, highlighting their potential roles in this context.

Our bioinformatic analysis, utilizing two publicly available databases, confirmed that these miRNAs are associated with NSCLC, as they were identified among the differentially expressed miRNAs in NSCLC-positive patients (Fig. 1). Notably, miR-320b was also found to be differentially expressed in patients with a smoking history, further supporting its potential as a biomarker candidate. Additionally, GO analysis aligned with previous studies [16–19,26] which suggest that these miRNAs are involved in critical biological processes such as cell cycle regulation, cell migration, and apoptosis. Dysregulation of these miRNAs may disrupt key cellular processes, potentially promoting tumor growth and progression in NSCLC. These findings strengthen the evidence for their role as potential biomarkers and their involvement in NSCLC pathogenesis.

Consistent with our *in silico* results, our experimental study confirmed that miR-10b-5p and miR-320b are differentially expressed in tissue and EVs from NSCLC patients with a smoking history compared to non-smoking patients. As we analyzed the expression of these two miRNAs across three biological sources: tissue, plasma, and plasma EVs, we observed that miR-10b-5p was more highly expressed in plasma, whereas miR-320b showed higher expression in plasma EVs.

Previous studies have demonstrated that these miRNAs play different roles in cellular regulation. miR-320b is known for its tumor-suppressive function in NSCLC [16], as well as in other cancers [27,28]. In contrast, miR-10b-5p has been identified as an oncogenic miRNA, particularly in gastric cancer [29]. The high expression of miR-10b-5p in plasma suggests an increased abundance of this miRNA in a vesicle-free state. This

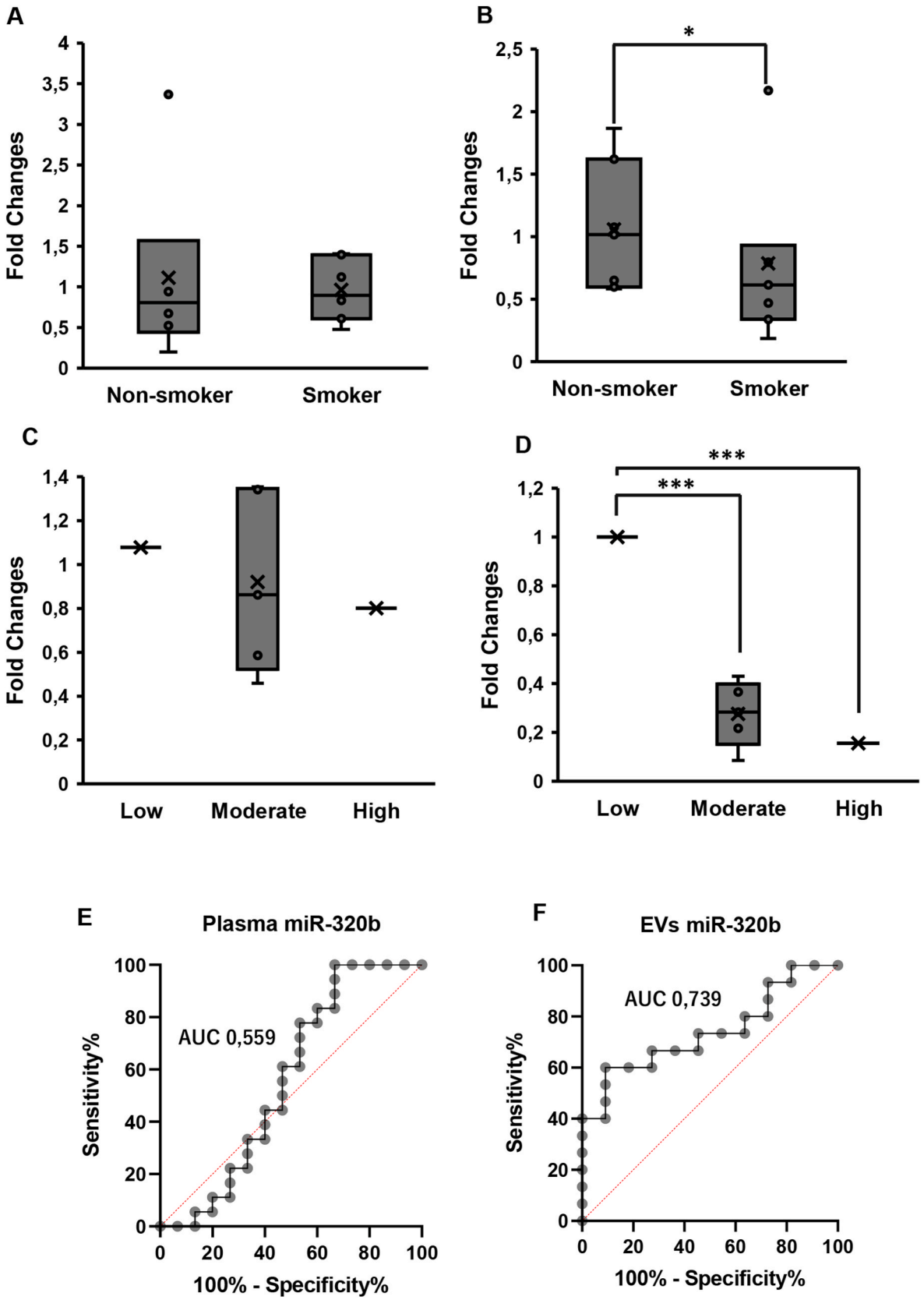

Fig. 3. Relative expression of miR-320b in non-smoker and smoker NSCLC patients. A) Relative expression of miR-320b in plasma samples from non-smoker and smoker NSCLC patients, n = 14, *p < 0.05, **p < 0.01, ***p < 0.005. B) Relative expression of miR-320b in EVs samples from non-smoker and smoker NSCLC patients, n = 14, *p < 0.05, **p < 0.01, ***p < 0.005. C-D) Expression levels of miR-320b in plasma (c) and EVs (d) from smoker NSCLC patients categorized by their Brinkman Index (Low: <199; Moderate: 200–599; High: ≥600), *p < 0.05, **p < 0.01, ***p < 0.005. E) Receiver Operating Characteristics (ROC) curve analysis of miR-320b in plasma samples (Area Under Curve (AUC) 0.559, sensitivity 61.11 %, specificity 53.33 %). F) ROC curve analysis of miR-320b in EVs samples (AUC 0.739, sensitivity 73.33 %, specificity 54.55 %).

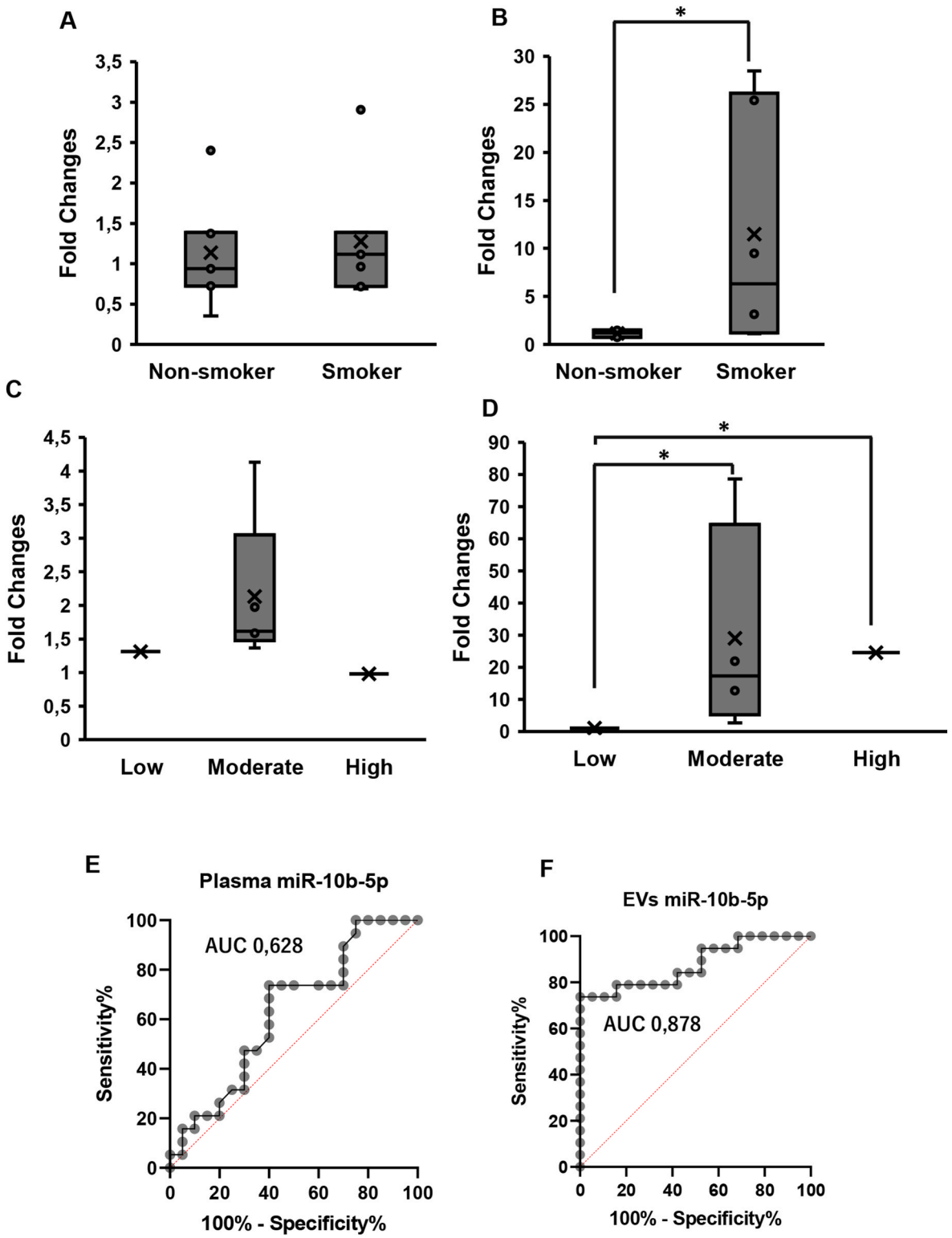

Fig. 4. Relative expression of miR-10b-5p in non-smoker and smoker NSCLC patients. A) Relative expression of miR-10b-5p in plasma samples from non-smoker and smoker NSCLC patients, n = 14, *p < 0.05. B) Relative expression of miR-10b-5p in EVs samples from non-smoker and smoker NSCLC patients, n = 14, *p < 0.05. C-D) Expression levels of miR-10b-5p in plasma (c) and EVs (d) from smoker NSCLC patients categorized by their Brinkman Index (Low: <199; Moderate: 200–599; High: ≥600), *p < 0.05. E) ROC curve analysis of miR-320b in plasma samples (AUC 0.628, sensitivity 63.16 %, specificity 60 %). F) ROC curve analysis of miR-10b-5p in EVs samples (AUC 0.878, sensitivity 78.95 %, specificity 73.68 %).

abundance may be linked to its oncogenic activity. It has been reported that miR-10b-5p can inhibit E-cadherin (*CDH1*) expression [18]. Given that most patients in this study had stage IV NSCLC with metastases, elevated miR-10b-5p levels may contribute to tumor progression by suppressing *CDH1* expression. Furthermore, the high expression of miR-10b-5p observed in tumor cells may account for its elevated levels in plasma, as tumor cells are known to secrete miRNAs into the bloodstream [30]. Additionally, the presence of metastatic tumor cells in

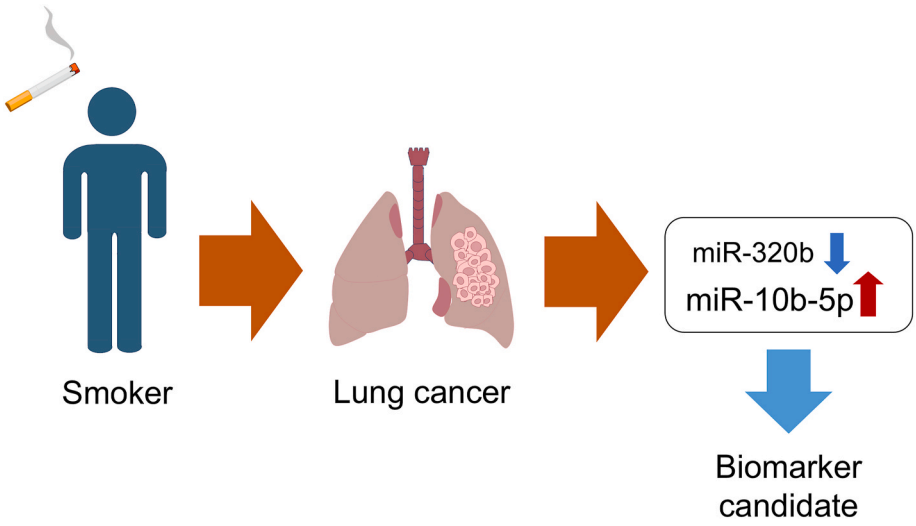

Fig. 5. Schematic Illustration of miR-320b and miR-10b-5p as Biomarker Candidates for NSCLC Diagnosis in Smokers.

plasma may further amplify miR-10b-5p levels, explaining its greater abundance in plasma compared to tissue.

Regarding the tumor-suppressive role of miR-320b [16], the observed low expression in cancer tissue may suggest a diminished ability of tissue cells to counter tumor growth effectively. This reduction may indicate a tumor-driven mechanism to evade suppression by actively secreting miR-320b into the plasma. This could explain the observed high expression of miR-320b in plasma. Interestingly, miR-320b showed elevated levels in EVs, suggesting that it may originate from surrounding cells in the tumor microenvironment. This implies a potential mechanism by which the tumor microenvironment communicates with tumor cells via EVs, possibly as an effort to suppress tumor growth by supplying miR-320b. These findings suggest a complex interplay between tumor cells and the tumor microenvironment in regulating miRNA expression and function, warranting further investigation.

Our further findings reveal that EVs-associated miR-10b-5p and miR-320b are differentially expressed in NSCLC patients with a smoking history compared to those without. Specifically, miR-10b-5p was overexpressed, while miR-320b was underexpressed in smokers. These results provide further evidence of EVs-associated miRNA dysregulation induced by CS exposure, consistent with previous studies [12,13,15]. The overexpression of miR-10b-5p in EVs from NSCLC patients with a smoking history may be linked to reduced *CDH1* expression, as reported in previous studies [31]. Given that *CDH1* is a known target of miR-10b-5p, our results imply that EVs-associated miR-10b-5p may contribute to the suppression of *CDH1* expression in smokers, potentially promoting tumor progression.

Conversely, the lower expression of miR-320b in EVs from smokers aligns with patterns observed in previous studies [32]. Considering the role of EVs in cellular communication, this reduction suggests that CS exposure may impair the tumor-suppressive capacity of EVs cargo. Together with the observed miR-10b-5p overexpression, these findings indicate that CS-induced dysregulation of EVs-associated miRNAs contributes to NSCLC malignancy.

Additionally, CS exposure is known to induce changes in the tumor microenvironment that facilitate tumor progression [33]. Our findings suggest that these changes may be mediated, in part, through EVs released by cells in the tumor microenvironment. Furthermore, our results showed that miRNA dysregulation in EVs worsens with higher levels of smoking (Figs. 2D and 3D), providing evidence that prolonged CS exposure exacerbates this dysregulation. Hence, our study provides further evidence that smoking NSCLC patients could potentially have worse outcomes and prognoses compared to non-smoking patients.

Building on these findings, our study highlights the potential of miR-10b-5p and miR-320b as prognostic biomarkers for NSCLC patients through liquid biopsy. Both miRNAs demonstrated high diagnostic accuracy, with miR-10b-5p showing an AUC of 0.878 and a sensitivity of 78.95 %, and miR-320b showing an AUC of 0.739 and a sensitivity of 73.33 %. These results suggest that miR-10b-5p and miR-320b, particularly in EVs, could serve as valuable biomarkers for prognosis in NSCLC patients, especially those with a smoking history. Moreover, our findings indicate that EVs-associated miRNAs have higher sensitivity compared to plasma-derived miRNAs, reinforcing the utility of EVs as a superior source of biomarkers for liquid biopsy. Although isolating EVs from plasma requires additional steps, the higher sensitivity they provide makes them a preferable choice for clinical applications.

These results contribute to the ongoing development of NSCLC management strategies and have implications for future clinical applications. However, this study has several limitations that must be addressed in future research. The clinical utility of miR-10b-5p and miR-320b as prognostic biomarkers has not been fully established in this study. Therefore, longitudinal cohort studies are required to confirm their potential in clinical settings. Furthermore, the specific molecular mechanisms by which CS exposure affects the expression of EVs-associated miR-10b-5p and miR-320b remain unclear, and further investigation is needed to elucidate these pathways.

5. Conclusion

In conclusion, our study demonstrates that smoking induces differential expression of miR-10b-5p and miR-320b in EVs from NSCLC patients. These findings provide new insights into the relationship between smoking and NSCLC and underscore the potential of EVs-associated miR-10b-5p and miR-320b as prognostic biomarkers.

Ethical statement

The studies involving human participants were reviewed and approved by the Medical Research Ethics Committee of Dharmais Hospital National Cancer Center, Jakarta, Indonesia (041/KEPK/IV/2021). Written informed consent was obtained from all patients.

Authors contributions

Conceived and designed the analysis: Widjaja Lomanto MY, Wanandi SI, Jayusman AM, Lukmanto D, Sutandyo N.

Samples collection: Widjaja Lomanto MY, Prayitno YH.

Collected the data: Widjaja Lomanto MY.

Contributed data or analysis tools: Widjaja Lomanto MY, Wanandi SI, Jayusman AM, Sutandyo N.

Writing and editing: Widjaja Lomanto MY, Wanandi SI, Jayusman AM, Lukmato D, Prayitno YH, Sutandyo N.

Funding Acquisition: Sutandyo N.

Performed the analysis: Widjaja Lomanto MY.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT to assist with grammar checking and language refinement. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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.

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