Translational Oncology 44 (2024) 101951

Contents lists available at ScienceDirect

Translational Oncology

journal homepage: www.elsevier.com/locate/tranon

Original Research

miRNA patterns in male LUSC patients - the 3-way mirror: Tissue, plasma and exosomes

Cecilia Bica [a,c], Ancuta Jurj [a], Antonia Harangus [b], Cristina Ciocan [a], Alin Moldovan [b], Oana Zanoaga [a], Claudia Burz [d,e], Manuela Ferracin [f], Lajos Raduly [a,*], Ioana Berindan-Neagoe [a]

[a] Research Center for Functional Genomics, Biomedicine and Translational Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, 400337, Romania
[b] Leon Daniello Pulmonology Hospital, Cluj-Napoca, Romania
[c] Research Center for Advanced Medicine-MedFUTURE, Department of Translational Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy, 400347 Cluj-Napoca, Romania
[d] Department of Immunology and Allergology, Faculty of Medicine, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania
[e] Department of Medical Oncology, The Oncology Institute "Prof. Dr. Ion Chiricuţă", 400015 Cluj-Napoca, Romania
[f] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

ARTICLE INFO

Keywords:
Lung cancer
Biomarkers
Extracellular vesicles
microRNAs

ABSTRACT

Lung cancer remains one of the leading causes of cancer-related deaths worldwide. It is classified into two main histological groups: non-small cell lung cancer (NSCLC) and small cell lung cancer. Improving the outcome of cancer patients could be possible by enhancing the early diagnosis. In the current study, we evaluated the levels of three microRNAs - miR-21-5p, miR-155-5p, and miR-181a-5p in tumor (TT) vs adjacent normal tissue (NT), as well as their expression levels in plasma and extracellular vesicles (EVs) from plasma in lung squamous cell carcinoma (LUSC) male patients vs healthy individuals as means to identify a panel of miRNAs that could serve as novel biomarkers for the diagnosis of LUSC in male patients. Matched paired tissue samples from male LUSC (n=40) patients were used for miRNA expression analysis. MiR-21-5p and miR-155-5p in tumor tissue were overexpressed, while underexpression of miR-181a-5p was observed in LUSC TT vs NT. These results were further validated in the TCGA LUSC dataset, considering 279 male samples. These alterations of miR-21-5p, miR-181a-5p, and miR-155-5p in tumor tissue are also present in plasma and plasma extracellular vesicles in LUSC male patients. In addition, ROC curves were performed to assess the sensitivity and specificity of different combinations of these miRNAs, confirming a high diagnostic accuracy for LUSC of up to 88 % in male subjects. The expression levels in tissue samples and the abundance in plasma and plasma EVs of the three miRNAs combined - miR-21-5p, miR-155-5p and miR-181a-5p – could be considered for further studies on biomarkers for the early detection of LUSC in male subjects.

Introduction

Lung cancer remains one of the leading causes of cancer-related deaths worldwide, according to GLOBOCAN 2020 [1]. Lung cancer is classified into two main histological groups: non-small cell lung cancer (NSCLC) and small cell lung cancer. NSCLC, which includes adenocarcinoma, squamous cell carcinoma and large cell carcinoma, accounts for over 80 % of all lung cancer cases. Recent therapies have improved the prognosis of lung cancer patients, but the 5-year survival remains below 20 %, which can be explained by the lack of adequate screening and early diagnosis methods [2]. Improving the outcome of cancer patients would be possible through enhancing the early diagnosis, preferentially with minimally invasive methods.

Extracellular vesicles are small vesicles (30–100 nm in size) secreted by most cells into biological fluids, such as plasma, saliva, urine and others, and their contents reflect, to a high degree, the tumor profile, giving vital information regarding cancer development and progression [3,4]. Via this cell-to-cell communication, extracellular vesicles transfer their contents, including miRNAs, RNA, DNA, proteins, and lipids and are involved in many pathological processes, including cancer [5,6]. Many explored the role of circulating extracellular vesicles and exosomal miRNAs in initiating and developing various diseases, including

* Corresponding author.
E-mail address: lajos.raduly@umfcluj.ro (L. Raduly).

https://doi.org/10.1016/j.tranon.2024.101951
Received 22 June 2023; Received in revised form 28 March 2024; Accepted 28 March 2024
Available online 1 April 2024

cancer [7].

MicroRNAs (miRNAs) are a class of non-coding RNAs with a length of 21–25 nucleotides involved in regulating gene transcription and translation. Dysregulation of miRNAs is frequently observed in cancer, including NSCLC and is involved in tumor cell proliferation, local invasion, and metastasis [8]. MiRNAs can be evaluated in normal adjacent or tumor tissue or biofluids, making these molecules a promising diagnostic tool. The most frequently upregulated miRNAs in lung cancer (oncomiRNAs) are miR-21, miR-155, miR-30d, and miR-17. In contrast, the most common downregulated miRNA is miR-181a, providing a rationale for evaluating these molecules as diagnostic or prognostic markers in lung cancer patients [9–11].

MiR-21 was identified as a potential circulating biomarker for different cancers. Recent studies described miR-21 overexpression in breast cancer patients as associated with a poor progression-free survival [12]. In NSCLC, miR-21 promotes tumorigenesis by inhibiting negative regulators of the Ras/MEK/ERK signaling pathway [13] and stimulates cell growth and invasion by inhibiting tumor suppressor PTEN [14,15]. The expression of miR-21 is increased and is associated with poor prognosis in NSCLC [16]. Moreover, a meta-analysis study demonstrated that circulating miR-21 modified levels can be valuable biomarkers of progression and diagnosis in gastric cancer patients [17]. In prostate cancer patients, it was demonstrated that miR-181a, alongside other miRNAs, can be used to separate patients based on the aggressive/nonaggressive types of prostate cancer [18].

Further studies demonstrated that low plasma levels of exosomal miR-181a-5p were associated with organ-invasive primary tumor in colorectal cancer [19]. A previous meta-analysis published by our group indicated that miR-181 downregulation is associated with poor outcomes in lung cancer patients [20]. High circulating miR-155 expression levels were observed in breast cancer patients. This overexpression was significantly diminished after a completed surgery and chemotherapy, and the study demonstrated that circulating miR-155 can represent diagnostic and therapeutic monitoring markers in the breast cancer [21]. This transcript controls metastasis and cancer progression via TGF-b/SMAD4 signaling [22]. Several reports also indicate that exosomal expression of miR-155 is involved in promoting metastasis in NSCLC [23] and colorectal cancer [24,25].

In our study, we evaluated the expression levels of a 3-miRNA panel, including miR-21-5p, miR-155-5p, and miR-181a-5p in normal and tumor tissue combined with their analysis in plasma and plasma extracellular vesicles for identifying a panel of miRNAs that could serve as novel biomarkers for the diagnosis of lung cancer. We decided to focus our study on the male population because the cohort of LUSC patients established from Hospitals in Romania mainly consisted of male patients. Therefore, we further investigated the molecular aspects of lung cancer development in male patients.

Material and methods

Patient selection

This study included 61 adult male patients (ages 48-85) with a diagnosis of LUSC who presented in the Bronchoscopy Department of the Leon Daniello Pneumology Hospital in Cluj-Napoca, Romania, with suspicion of endobronchial lung cancer at imaging testing (computer tomography or positron emission computed tomography). In addition, 28 healthy male donors were included in the study to evaluate the transcripts in plasma and plasma-derived extracellular vesicles.

The study was conducted following the Declaration of Helsinki, and all patients and healthy subjects enrolled in the study were informed about the inclusion and exclusion criteria. They signed the informed consent approved by the hospital and the institutional ethics committee of Leon Daniello Pulmonology Hospital, Cluj-Napoca, Romania, no. 264/26.06.2018 and the Ethical Committee of Iuliu Hatieganu University of Medicine and Pharmacy no. 438/24.11.2016. The study cohort comprised 61 patients, from which matched pairs of TT/NT, plasma, and plasma extrme samples were analyzed. Ten out of the 61 patients were common in all three types of samples. This study also assessed the TCGA LUSC dataset (n=279) that included data from male patients (https://www.cancer.gov/tcga). The patient's characteristics are detailed in Table 1.

Sample collection and preparation

The tumor tissue samples were collected using bronchoscopy with endobronchial biopsy, performed under local anesthesia. Normal tissue was collected from the contralateral, healthy lungs of each patient.

From the selected patients and healthy donors, four ml of peripheral whole-blood samples were collected, and the plasma samples were obtained by centrifugation of the peripheral blood at 4200 rpm for 10 min at room temperature. EVs from plasma samples were obtained using the ultra-centrifugation protocol. One ml plasma was centrifuged at 2,000 × g for 20 minutes at 4 °C followed by another centrifugation at 10,000 × g for 30 minutes at 4 °C to remove plasma debris; the remaining plasma was ultracentrifuged at 100,000 × g for 70 minutes to pellet extracellular vesicles. After ultracentrifugation, the EVs pellet was eluted in 500 µl PBS 1X.

RNA extraction

Total RNA was extracted and isolated from 40 NSCLC plasma samples and 28 plasma samples from healthy donors using the Plasma/Serum Circulating and Exosomal RNA Purification Kit - Norgen according to the manufacturer's instructions. The same extraction kit was used for 30 plasma exosome/extracellular vesicles samples from NSCLC patients and 20 plasma samples from healthy controls. Total RNA was isolated from 40 matched paired tissue samples (tumor and adjacent normal tissue) using Tri reagent (Thermo Fisher Scientific) and following the steps described in the manufacturer's protocol. RNA concentration and quality were evaluated using a NanoDrop-2000C spectrophotometer. The concentration ranged from 25 ng/µl (in plasma and extracellular vesicle samples) to 50 ng/µl (in tissue samples).

Quantitative reverse transcription PCR (qRT-PCR) analysis

Three miRNAs (miR-21-5p, miR-181a-5p, and miR-155-5p) were

Table 1
Lung squamous cell carcinoma (LUSC) patient cohorts were included in the study.

Cohorts			TCGA LUSC cohort (n = 279) No. of patients (%)	Study cohort (n = 61) No. of patients (%)
Age		<50	10 (3.6)	1 (1.6)
		50-59	34 (12.2)	14 (23)
		60-69	102 (36.6)	23 (37.7)
		70-79	107 (38.3)	17 (27.9)
		>80	22 (7.9)	6 (9.8)
		unknown	4 (1.4)	0 (0)
Sex		M	279 (100)	61 (100)
TNM	T	T1	54 (19.3)	0 (0)
		T2	169 (60.6)	7 (11.5)
		T3	47 (16.9)	16 (26.2)
		T4	9 (3.2)	38 (62.3)
	N	N0	186 (66.7)	8 (13.1)
		N1	67 (24)	4 (6.6)
		N2	20 (7.2)	41 (67.2)
		Nx/N3	6 (2.1)	8 (13.1)
	M	M0	209 (74.9)	36 (59)
		M1	4 (1.4)	25 (41)
		Mx	66 (23.7)	0 (0)
Stage		I	130 (46.6)	0 (0)
		II	105 (37.7)	3 (4.9)
		III	40 (14.3)	34 (55.7)
		IV	4 (1.4)	24 (39.4)

selected to assess their expression level profile in plasma, tissue, and extracellular vesicles by qRT-PCR. These miRNAs were investigated in a.) 40 LUSC fresh frozen matched paired tissue samples (TT/NT), b.) their plasma samples and plasma from 28 healthy controls, and c.) 30 LUSC patients and 20 healthy control plasma for extracellular vesicles.

The cDNA synthesis was performed using a 7.5 µl reverse transcription mixture containing 0.72 µl of RT primer, 50 ng of total RNA and 0.5 µl of MultiScribe Reverse Transcriptase, 0.75 µl Reverse Transcription Buffer (10×), 0.075 µl dNTPs (100 mM), 0.1 µl of RNase Inhibitor according to TaqMan MicroRNA reverse Transcription Kit (Applied Biosystems) protocol. The cDNA mixture was incubated in PCR tubes at 16°C 30 min, 42°C 30 min, and 85°C 5 min. qRT-PCR was performed in a total volume of 10 µl using 5 µl of cDNA (diluted 1:3 with nuclease-free water), 4.53 TaqMan Fast Advanced Master Mix (Applied Biosystems) and 0.47 µl primer for each miRNA in ViiA7 (Applied Biosystems) PCR machine. The reactions were set up as follows: the initial step included the UNG incubation at 50°C for two minutes and polymerase activation at 95°C for the 20s, followed by 40 cycles of 95°C for 1s (denature), 60°C (Anneal/extend) for 20s. The sequences for the miRNA primers are listed in the table (Table 2).

Statistical analysis

The expression level for each miRNA was calculated using the $2^{-\Delta\Delta Ct}$ method; U6, RNU48 and miR-16 were used for normalization; $p < 0.05$ was considered statistically significant. Additionally, a ROC (receiver operating characteristic) graphical representation was performed to assess the sensitivity and specificity of each evaluated transcript at plasma, tissue and extracellular vesicles levels using GraphPad Prism (https://www.graphpad.com/, Version 9), and the combined ROC curves were generated using the CombiROC online tool [26]. Expression level plots for miRNAs in TT vs NT samples and heatmaps were performed in R (version 4.2.1) using *ggplot* and *pheatmap* packages.

RESULTS

Extracellular vesicles isolation and characterization

EVs isolated from the plasma of 30 lung cancer patients and 20 healthy donors were characterized. NanoSight and Transmission Electron Microscopy analysis of extracellular vesicles samples showed a proper size, concentration distribution and specific disc-like structures. The extracellular vesicles size ranged from 30-150 nm (Fig. 1A), and the edges of the structures were clear and light, with concentrated staining in the central area (Fig. 1B).

miR-21-5p, miR-181a-5p, and miR-155-5p are LUSC biomarkers in tumor tissue, plasma and EVs

We tested the differential expressions of miR-21-5p, miR-181a-5p, and miR-155-5p in tissue, plasma and EVs of LUSC patients and controls.

Matched pair tissue samples from male LUSC (n=40) patients were used for miRNA expression analysis. All three miRNAs, miR-21-5p, miR-181a-5p, and miR-155-5p, showed statistically significant differential expressions between the tumor tissue (TT) compared with the adjacent normal lung tissue (NT) (log fold change > 1 and $P < 0.05$). MiR-21-5p and miR-155-5p are significantly overexpressed, while miR-181a-5p is underexpressed in LUSC TT versus NT (Fig. 2A).

To confirm the abundance of the three miRNAs, we quantified the miRNA in plasma samples from 40 male LUSC patients, comparing the expression level with the plasma profile from 28 healthy subjects. All three miRNAs, miR-21-5p, miR-181a-5p, and miR-155-5p, confirmed the levels observed in the tissue samples (log fold change > 1 and $P < 0.05$). MiR-21-5p and miR-155-5p are increased, while miR-181a-5p decreases in LUSC plasma versus plasma from healthy controls. (Fig. 2B)

Additional validation was performed by investigating the levels of the three miRNAs in plasma EVs. We assessed the levels in EV samples from 30 male LUSC patients versus EV miRNA expression from 20 healthy subjects' plasma. We observed that the same expression levels of the three miRNAs, miR-21-5p and miR-155-5p, are most abundant. In comparison, miR-181a-5p is less abundant in LUSC EVs versus normal plasma EVs (log fold change > 1 and $P < 0.05$) (Fig. 2C). When we compared the levels of the three miRNAs in plasma and plasma EVs samples from the same patients, we found that the trends of overexpression/underexpression of these miRNAs in plasma are also maintained in plasma EVs samples across LUSC patients (Fig. 3).

miR-21-5p, miR-181a-5p, and miR-155-5p are LUSC biomarkers in TCGA datasets

We validated the TCGA LUSC datasets (tumor tissue), including only samples from male patients in the analysis. As observed in Table 1, most of the samples were from patients diagnosed in stages I and II, 235 from a total of 279, while stages III and IV were only 44 (Table 1). The analysis of the expression levels of the three miRNAs in the LUSC TCGA datasets identified miR-21-5p, miR-155-5p, and miR-181a-5p dysregulated in lung cancer. The expression levels of miR-21-5p and miR-155-5p are significantly elevated, while miR-181a-5p is reduced in TT compared to NT, considering all stages (Fig. 4). These differences are already detectable in stages I+II TT vs NT (Fig. 4A) and stages III+IV TT vs NT (Fig. 4B), indicating that the expression levels of the three miRNAs are constantly altered in both early- and late-stage LUSC (Fig. 4C).

Sensitivity and specificity of miR-21-5p, miR-181a-5p, and miR-155-5p as biomarkers

A ROC curve analysis was performed to evaluate the utility of these miRNAs in lung cancer diagnostics. Fig. 5 shows the AUCs, cutoff values, specificity, and sensitivity of the three miRNAs independently. While

Table 2
MiRNA assays were used in the study.

Assay Name	Assay ID	miRNA Sequence
hsa-miR-21-5p	000397	UAGCUUAUCAGACUGAUGUUGA
hsa-miR-155-5p	002623	UUAAUGCUAAUCGUGAUAGGGGU
hsa-miR-181a-5p	000480	AACAUUCAACGCUGUCGGUGAGU
has-miR-16-5p	000391	UAGCAGCACGUAAAUAUUGGCG
U6 snRNA	001973	GTGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAGAGAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAATTCGTGAAGCGTTCCATATTT
RNU48	001006	GATGACCCCAGGTAACTCTGAGTGTGTCGCTGATGCCATCACCGCAGCGCTCTGACC

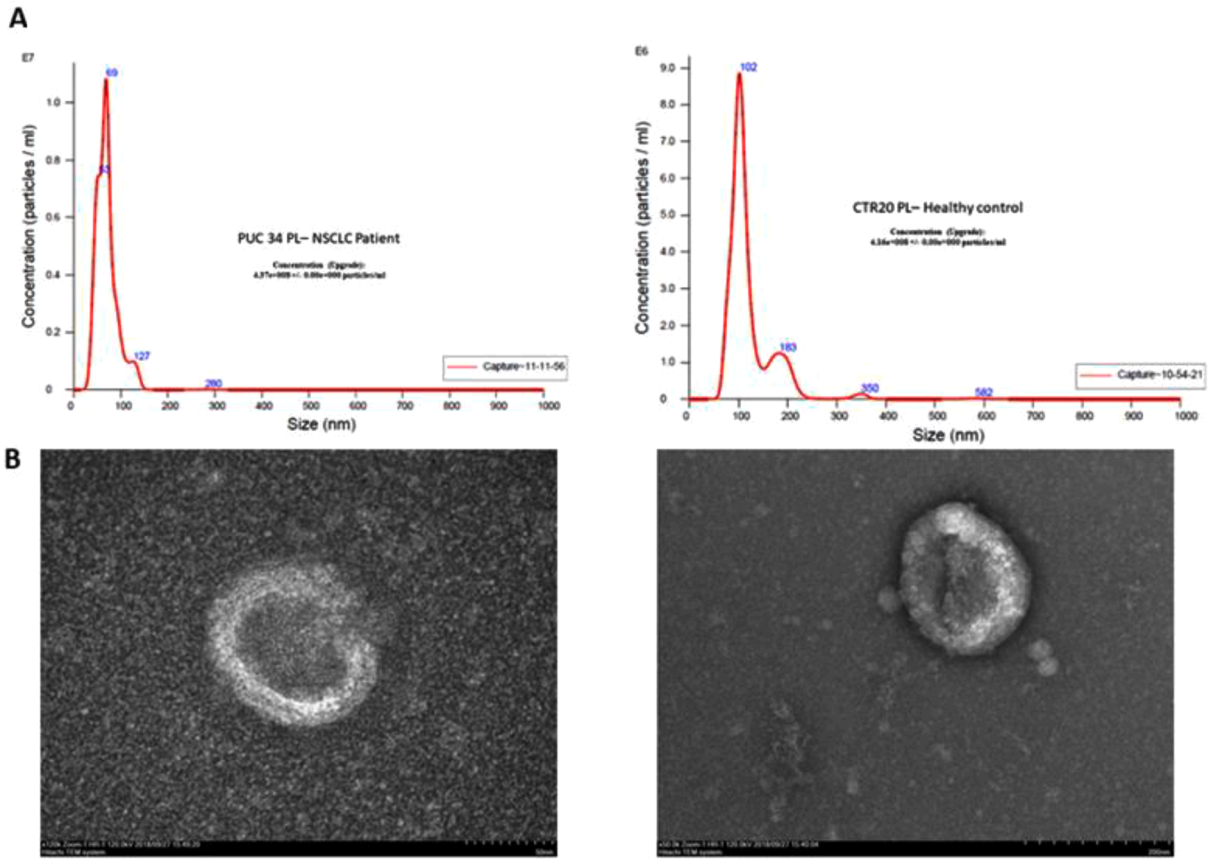
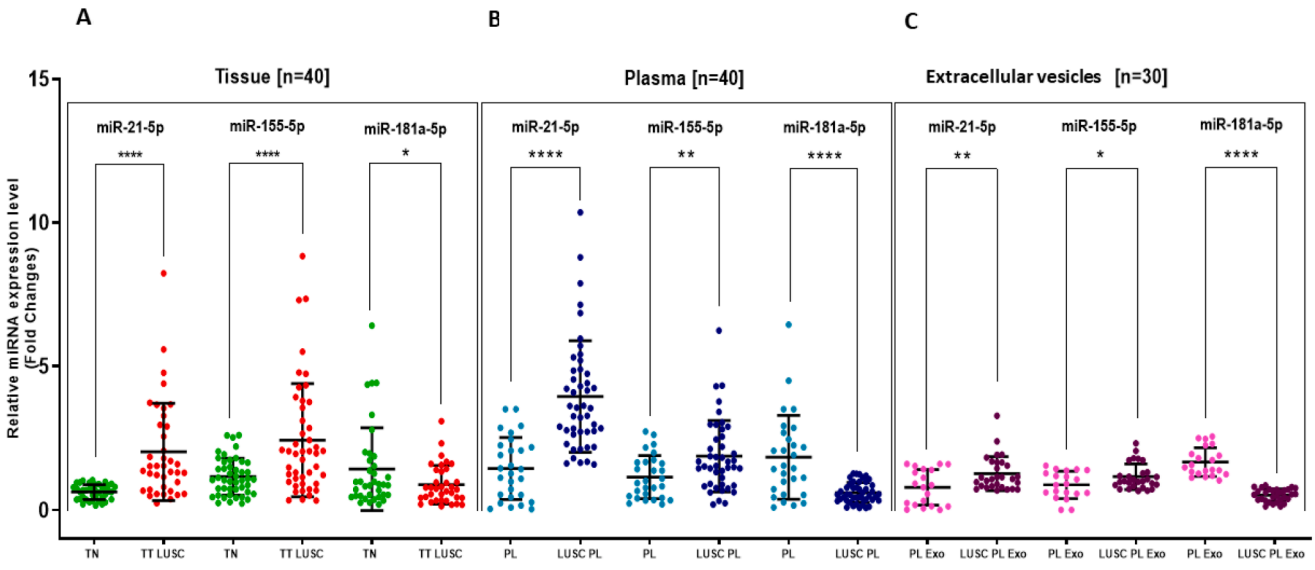


Fig. 1. Lung cancer patients' plasma extracellular vesicle size characterization by Nanosight (**A**) and Transmission electron microscopic (TEM) visualization(**B**).



miRNA	P value Tissue	P value Plasma	P value Extracellular vesicles
miR-21-5p	<0,0001	<0,0001	0,0094
miR-155-5p	<0,0001	0,007	0,0399
mir181a-5p	0,0454	<0,0001	<0,0001

Fig. 2. MiR-21-5p, miR-155-5p and MiR181a expression levels in LUSC TT vs NT (A), LUSC plasma vs plasma from healthy donors (B) and LUSC plasma EVs vs plasma EVs from healthy donors(C).

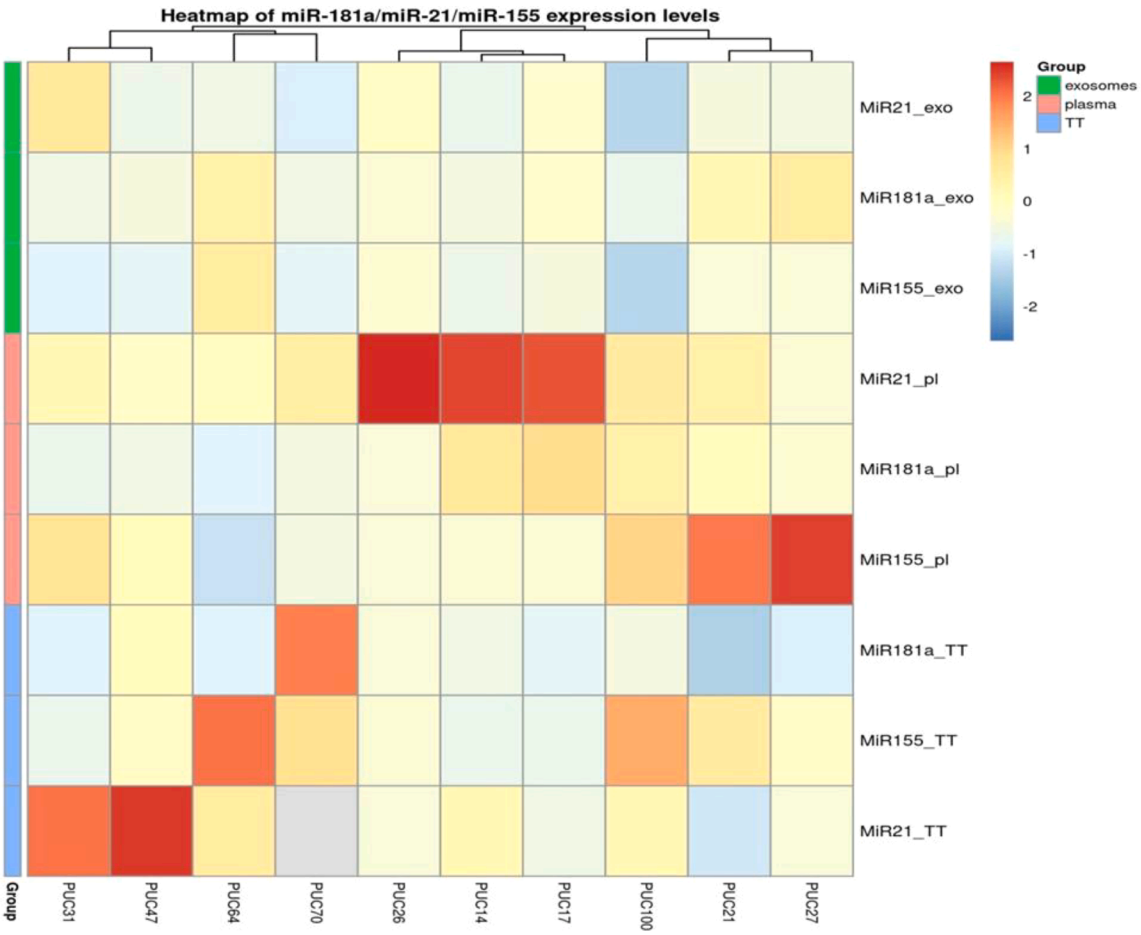


Fig. 3. Heatmap of expression levels (FC) of the three miRNAs in TT, plasma, and plasma EVs in LUSC male patients.

miR-21-5p alone recorded a satisfactory AUC (0.8-1) in each sample type, miR-181a-5p and miR-155-5p showed fair and poor AUCs between 0.5 and 0.7, respectively, except miR-181a-5p expression in plasma EVs, where the AUC=1, indicating miR-181a expression in plasma EVs has high predictive accuracy for LUSC patients. On the other hand, the combi-roc analysis revealed combinations of these miRNAs with high predictive accuracy for NSCLC. In plasma samples, miR-155-5p+miR-21-5p and miR-155-5p+miR-181a-5p had higher AUC (0.88) than the one obtained on individual transcripts (Fig. 5A, B, C).

Discussion

Aberrant miRNA expression is an essential biomarker for diagnosing, prognosis and predicting therapy resistance in NSCLC. MiRNAs could be exploited as biomarkers in cancer patients due to their stability in various specimen types, high reproducibility, and the availability of assays that can assess their levels, such as qRT-PCR or microarray. Several recent studies on lung cancer patients have suggested valuable miRNA signatures for early diagnosis in this disease [27,28]. Many studies have been conducted on lung cancer patient's tumor tissue, serum, or plasma. Because obtaining tumor tissue represents a challenge, it is essential to find minimally invasive methods with high sensibility and sensitivity to be used either for early diagnosis or to improve the survival of lung cancer patients.

Extracellular vesicles are membrane-bound, nano-sized extracellular vesicles released by cells in eukaryotic organisms. Their cargo includes DNA, RNA, proteins, and lipids. Their stability in different biological fluids, proficiency to pass over cell barriers, biocompatibility, and affinity for target cells make these vesicles useful in various cancer applications, such as drug delivery and immunotherapy [29]. Many altered miRNAs in lung cancer can be detected in extracellular vesicles, thus supporting their role as potential diagnostic and prognostic tools for this malignancy [8,30,31].

This study aimed to provide new insights into NSCLC biomarkers by analyzing the expression levels of the same three miRNAs in different biological samples: tissue, plasma, and EVs. For ten patients, matching tissue, plasma and EVs were assessed with concordant results regarding miRNA modulations in tumor vs normal/healthy samples. We observed an overall upregulation of miR-21-5p and miR-155-5p in tumors vs normal tissues and a downregulation of miR-181a-5p in our and TCGA LUSC cohorts. We also verified that these differences are detectable in early and late stages, indicating that the expression levels of the three miRNAs are an early alteration in lung cancer. This trend was also confirmed in plasma and plasma EVs compared to healthy controls.

Indeed, when we investigated the sensitivity and specificity for the three individual miRNAs, we observed that miR-21-5p recorded a satisfactory AUC (0.8-1) in all settings (tissue, plasma, EVs). At the same time, for miR-181a-5p expression in EVs vs EVs from healthy controls, the AUC=1 indicated miR-181a has high predictive accuracy for LUSC patients. The combined ROC analysis of these miRNAs revealed that their combinations could reach high predictive accuracy for NSCLC. In plasma samples, the combinations of miR-155-5p+miR-21-5p, as well as miR-155-5p + miR-181a-5p, had AUC higher (0.88) than the one obtained with individual miRNAs (Fig. 4).

The mirroring of the expression changes of miR-21, miR-181a, and miR-155 in lung tissues, plasma and EVs emphasizes the utility of these molecules in cancer diagnosis. All these results indicate that the three miRNAs combined – miR-21-5p, miR-155-5p and miR-181a-5p – can be

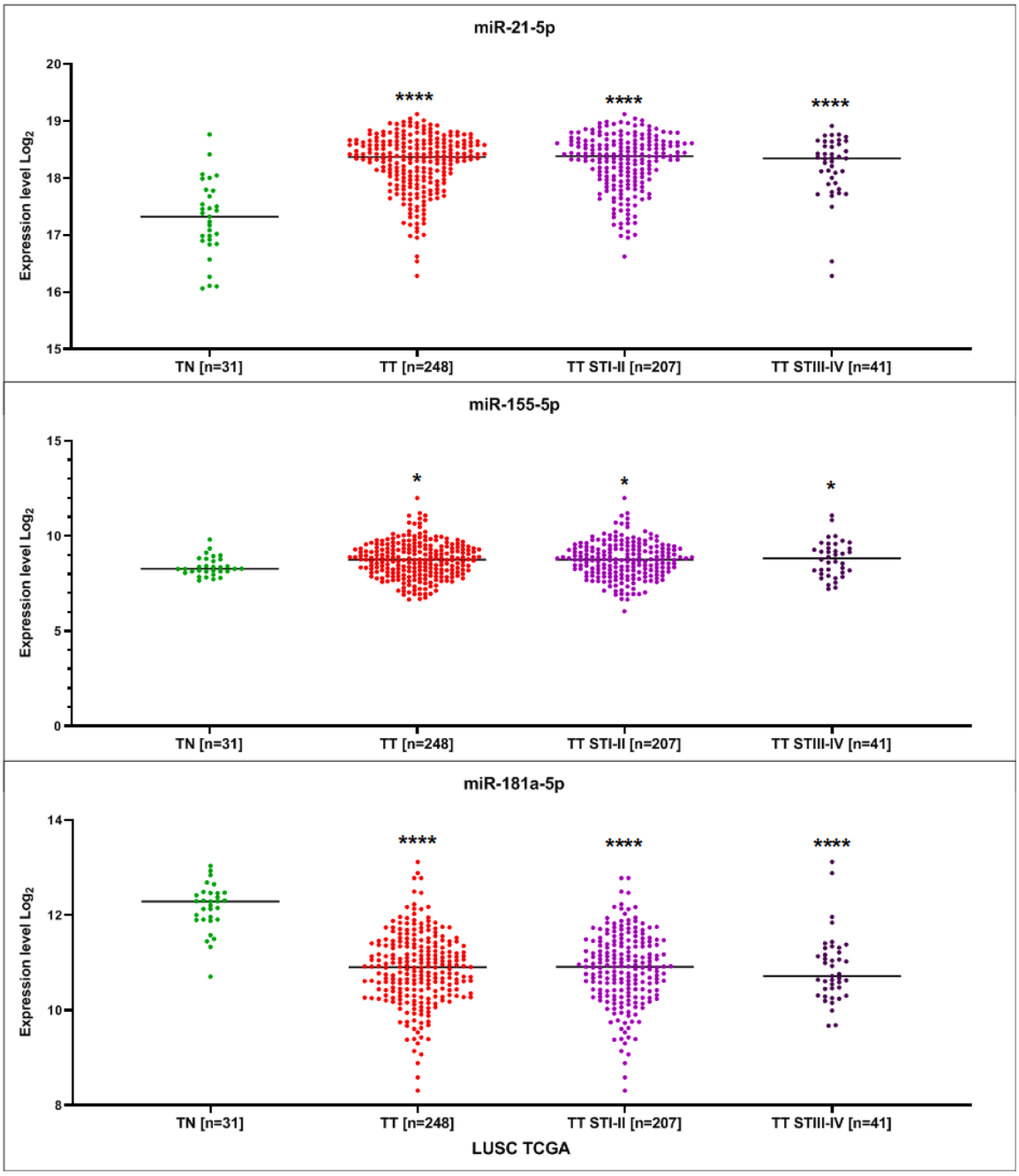


Fig. 4. Expression levels of miR-21-5p, miR-155-5p, and miR-181a-5p in TCGA LUSC datasets (male only); A) Expression levels of miR-21-5p, miR-155-5p, and miR-181a-5p in stages I+II lung tumor tissue (TT) vs normal lung tissue (NT); B) Expression levels of miR-21-5p, miR-155-5p, and miR-181a-5p in stages III+IV lung tumor tissue (TT) vs normal lung tissue (NT); C) Expression levels of miR-21-5p, miR-155-5p, and miR-181a-5p in lung tumor tissue (TT) vs normal lung tissue (NT).

considered for further studies as biomarkers for early detection of LUSC in male patients.

Conclusion

In this study, we evaluated the combination of three miRNAs – miR-21-5p, miR-155-5p and miR-181a-5p – as potential biomarkers for LUSC diagnosis in male subjects. The investigation revealed upregulation of miR-21-5p and miR-155-5p and downregulation of miR-181a, consistent in both early- and late-stages in LUSC TCGA datasets. This alteration was confirmed in a cohort of male LUSC patients in TT vs. NT.

This study confirmed that this trend in expression alteration is maintained in plasma and EVs from LUSC male patients compared to plasma and EVs from healthy controls. The finding that these circulating miRNAs have a similar abundance variation in tumor tissue makes these RNA biotypes potential clinical biomarkers detectable in liquid biopsy samples.

CRediT authorship contribution statement

Cecilia Bica: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Ancuta Jurj:** Methodology. **Antonia Harangus:** Methodology. **Cristina Ciocan:** Formal analysis. **Alin Moldovan:** Methodology. **Oana Zanoaga:** Methodology. **Claudia Burz:** Writing – review & editing. **Manuela Ferracin:** Writing – review & editing, Supervision, Project administration. **Lajos Raduly:**

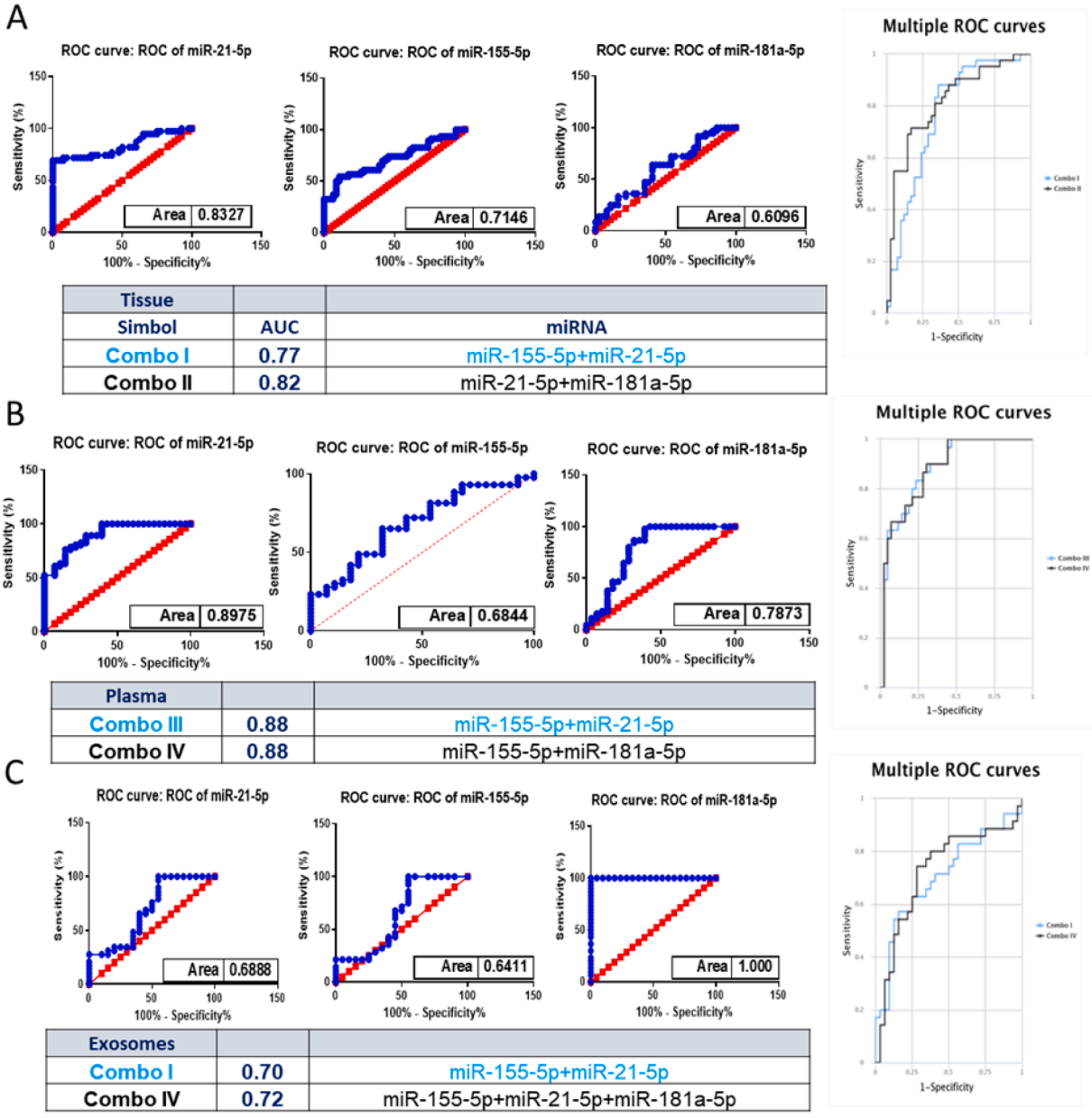


Fig. 5. ROC curves analysis using GraphPad Prism and combined ROC curves using the CombiROC online tool for miR-21-5p, miR-155-5p and miR-181a-5p for tissue (A), plasma (B) and EVs (C).

Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization. **Ioana Berindan-Neagoe:** Writing – review & editing, Supervision, Project administration.

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.

Funding

This work was granted by the Competitiveness Operational Program, 2014–2020, entitled Clinical and economical impact of personalized targeted anti-microRNA therapies in reconverting lung cancer chemo-resistance — CANTEMIR, no. 35/01.09.2016, MySMIS 103375; PDI-PFE-CDI 2021, entitled Increasing the Performance of Scientific Research, Supporting Excellence in Medical Research and Innovation, PROGRES, no. 40PFE/30.12.2021; PN-III-P1-1.1-PD-2021-0471, no. PD 44/2022, entitled MiRNA analysis in extracellular vesicles isolated from resistant and sensitive TNBC cell lines and CAFs, focuses on the miRNA's altered pattern in relationship with drug resistance.

References

[1] H Sung, J Ferlay, RL Siegel, M Laversanne, I Soerjomataram, A Jemal, F Bray, Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA: A Cancer J. Clin. 71 (3) (2021) 209–249.

[2] B Hao, T Fan, J Xiong, L Zhang, Z Lu, B Liu, H Meng, R He, N Li, Q Geng, The Prognostic significance of the histological types in patients with nonsmall cell lung cancer </=2 cm, Front. Surg. 8 (2021) 721567.

[3] D Yu, Y Li, M Wang, J Gu, W Xu, H Cai, X Fang, X Zhang, Extracellular vesicles as a new frontier of cancer liquid biopsy, Mol. Cancer 21 (1) (2022) 56.

[4] R Drula, LF Ott, I Berindan-Neagoe, K Pantel, GA Calin, microRNAs from liquid biopsy derived extracellular vesicles: recent advances in detection and characterization methods, Cancers. (Basel) 12 (8) (2020).

[5] F Andre, M Andersen, J Wolfers, A Lozier, G Raposo, V Serra, C Ruegg, C Flament, E Angevin, S Amigorena, et al., Extracellular vesicles in cancer immunotherapy: preclinical data, Adv. Exp. Med. Biol. 495 (2001) 349–354.
[6] A Jurj, O Zanoaga, C Braicu, V Lazar, C Tomuleasa, A Irimie, Berindan-Neagoe I: a comprehensive picture of extracellular vesicles and their contents. molecular transfer to cancer cells, Cancers. (Basel) 12 (2) (2020).
[7] C Li, YQ Ni, H Xu, QY Xiang, Y Zhao, JK Zhan, JY He, S Li, YS Liu, Roles and mechanisms of Exosomal non-coding RNAs in human health and diseases, Signal. Transduct. Target. Ther. 6 (1) (2021) 383.
[8] A Harangus, R Lajos, L Budisan, O Zanoaga, C Ciocan, C Bica, R Pirlog, I Simon, M Simon, C Braicu, et al., Identification of potential microRNA panels for male non-small cell lung cancer identification using microarray datasets and bioinformatics methods, J. Pers. Med. 12 (12) (2022).
[9] M Yang, H Shen, C Qiu, Y Ni, L Wang, W Dong, Y Liao, J Du, High expression of miR-21 and miR-155 predicts recurrence and unfavourable survival in non-small cell lung cancer, Eur. J. Cancer 49 (3) (2013) 604–615.
[10] M Wang, M Zhao, Q Guo, J Lou, L Wang, Non-small cell lung cancer cell-derived Exosomal miR-17-5p promotes osteoclast differentiation by targeting PTEN, Exp. Cell Res. 408 (1) (2021) 112834.
[11] Y Wu, J Zhang, S Hou, Z Cheng, M Yuan, Non-small cell lung cancer: miR-30d suppresses tumor invasion and migration by directly targeting NFIB, Biotechnol. Lett. 39 (12) (2017) 1827–1834.
[12] SL Anwar, DNI Sari, AI Kartika, MS Fitria, DS Tanjung, D Rakhmina, T Wardana, I Astuti, SM Haryana, T Aryandono, Upregulation of circulating MiR-21 expression as a potential biomarker for therapeutic monitoring and clinical outcome in breast cancer, Asian Pac. J. Cancer Prev. 20 (4) (2019) 1223–1228.
[13] ME Hatley, DM Patrick, MR Garcia, JA Richardson, R Bassel-Duby, E van Rooij, EN Olson, Modulation of K-Ras-dependent lung tumorigenesis by MicroRNA-21, Cancer Cell 18 (3) (2010) 282–293.
[14] JG Zhang, JJ Wang, F Zhao, Q Liu, K Jiang, GH Yang, MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC), Clin. Chim. Acta 411 (11-12) (2010) 846–852.
[15] X Xue, Y Liu, Y Wang, M Meng, K Wang, X Zang, S Zhao, X Sun, L Cui, L Pan, et al., MiR-21 and MiR-155 promote non-small cell lung cancer progression by downregulating SOCS1, SOCS6, and PTEN, Oncotarget. 7 (51) (2016) 84508–84519.
[16] C Bica-Pop, R Cojocneanu-Petric, L Magdo, L Raduly, D Gulei, Berindan-Neagoe I: Overview upon miR-21 in lung cancer: focus on NSCLC, Cell Mol. Life Sci. 75 (19) (2018) 3539–3551.
[17] AH Aalami, H Abdeahad, M Mesgari, Circulating miR-21 as a potential biomarker in human digestive system carcinoma: a systematic review and diagnostic meta-analysis, Biomarkers 26 (2) (2021) 103–113.
[18] B Farran, G Dyson, D Craig, A Dombkowski, JL Beebe-Dimmer, IJ Powell, I Podgorski, L Heilbrun, S Bolton, CH Bock, A study of circulating microRNAs identifies a new potential biomarker panel to distinguish aggressive prostate cancer, Carcinogenesis 39 (4) (2018) 556–561.
[19] T Bjornetro, KR Redalen, S Meltzer, NS Thusyanthan, R Samiappan, C Jegerschold, KR Handeland, AH Ree, An experimental strategy unveiling Exosomal microRNAs 486-5p, 181a-5p and 30d-5p from hypoxic Tumour cells as circulating indicators of high-risk rectal cancer, J. Extra Cell Vesicles 8 (1) (2019) 1567219.
[20] C Pop-Bica, S Pintea, R Cojocneanu-Petric, G Del Sal, S Piazza, ZH Wu, AJ Alencar, IS Lossos, I Berindan-Neagoe, GA Calin, MiR-181 family-specific behavior in different cancers: a meta-analysis view, Cancer Metastasis Rev. 37 (1) (2018) 17–32.
[21] SL Anwar, DS Tanjung, MS Fitria, AI Kartika, DNI Sari, D Rakhmina, T Wardana, I Astuti, SM Haryana, T Aryandono, Dynamic changes of circulating Mir-155 expression and the potential application as a non-invasive biomarker in breast cancer, Asian Pac. J. Cancer Prev. 21 (2) (2020) 491–497.
[22] Y Peng, CM Croce, The role of microRNAs in human cancer, Signal. Transduct. Target. Ther. 1 (2016) 15004.
[23] X Li, Z Chen, Y Ni, C Bian, J Huang, L Chen, X Xie, J Wang, Tumor-associated macrophages secret Exosomal miR-155 and miR-196a-5p to promote metastasis of non-small-cell lung cancer, Transl. Lung Cancer Res. 10 (3) (2021) 1338–1354.
[24] D Wang, X Wang, Y Song, M Si, Y Sun, X Liu, S Cui, X Qu, X Yu, Exosomal miR-146a-5p and miR-155-5p promote CXCL12/CXCR7-induced metastasis of colorectal cancer by crosstalk with cancer-associated fibroblasts, Cell Death Dis. 13 (4) (2022) 380.
[25] J Lan, L Sun, F Xu, L Liu, F Hu, D Song, Z Hou, W Wu, X Luo, J Wang, et al., M2 macrophage-derived extracellular vesicles promote cell migration and invasion in colon cancer, Cancer Res. 79 (1) (2019) 146–158.
[26] S Mazzara, RL Rossi, R Grifantini, S Donizetti, S Abrignani, M Bombaci, CombiROC: an interactive web tool for selecting accurate marker combinations of omics data, Sci. Rep. 7 (2017) 45477.
[27] F Bianchi, F Nicassio, M Marzi, E Belloni, V Dall'olio, L Bernard, G Pelosi, P Maisonneuve, G Veronesi, Di Fiore, PP: A serum circulating miRNA diagnostic test to identify asymptomatic high-risk individuals with early stage lung cancer, EMBO Mol. Med. 3 (8) (2011) 495–503.
[28] A Prelaj, C Proto, G Lo Russo, D Signorelli, R Ferrara, M Mensah, G Galli, A De Toma, G Viscardi, M Brambilla, et al., Integrating clinical and biological prognostic biomarkers in patients with advanced NSCLC treated with immunotherapy: the demo score system, Transl. Lung Cancer Res. 9 (3) (2020) 617–628.
[29] G Egger, G Liang, A Aparicio, PA Jones, Epigenetics in human disease and prospects for epigenetic therapy, Nature 429 (6990) (2004) 457–463.
[30] MA Iqbal, S Arora, G Prakasam, GA Calin, MA Syed, MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance, Mol. Aspects Med. 70 (2019) 3–20.
[31] C Pop-Bica, S Pintea, L Magdo, R Cojocneanu, D Gulei, M Ferracin, Berindan-Neagoe I: the clinical utility of miR-21 and let-7 in non-small cell lung cancer (NSCLC). A systematic review and meta-analysis, Front. Oncol. 10 (2020) 516850.