Circulation_microRNA.pdf

by

Submission date: 25-Jan-2023 03:40PM (UTC+0700)
Submission ID: 1999048638
File name: Circulation_microRNA.pdf (4.76M)
Word count: 6512
Character count: 37410

Non-coding RNA Research 7 (2022) 233–241

Contents lists available at ScienceDirect

Non-coding RNA Research

journal homepage: www.keaipublishing.com/en/journals/non-coding-rna-research

Original Research Article

Circulation microRNA expression profiles in patients with complete responses to chemoradiotherapy in nasopharyngeal carcinoma

Tirta Wardana [a,*], Siti Nur Chasanah [b], Risky Oktriani [c], Cita Herawati [d], Sumadi Lukman Anwar [e], Indwiani Astuti [f], Sofia Mubarika Haryana [g]

[a] Department of Biomedicine, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Central Java, Indonesia
[b] Graduate Student, Biomedical Science, Faculty of Medicine, Nursing, and Public Health, Gadjah Mada University, Yogyakarta, Indonesia
[c] Department of Biochemistry, Faculty of Medicine, Nursing, and Public Health, Gadjah Mada University, Yogyakarta, Indonesia
[d] Department of THT, Dharmais Hospital National Cancer Center, West Jakarta, Indonesia
[e] Department of Surgery, Faculty of Medicine, Nursing, and Public Health, Gadjah Mada University, Yogyakarta, Indonesia
[f] Department of Pharmacology and Therapy, Faculty of Medicine, Nursing, and Public Health, Gadjah Mada University, Yogyakarta, Indonesia
[g] Department of Histology and Cell Biology, Faculty of Medicine, Nursing, and Public Health, Gadjah Mada University, Yogyakarta, Indonesia

ARTICLE INFO

Keywords:
miRNA
Cancer biomarker
Nasopharyngeal carcinoma
Therapy
Plasma
Chemoradiotherapy

ABSTRACT

Background: Nasopharyngeal carcinoma (NPC) is endemic cancer in Southeast Asia with a relatively poor prognosis. Chemoradiotherapy is a primary treatment that advantages certain patients, particularly in the early stages. New predictive and prognostic biomarkers are required to guide and select the best treatment.
Aims: To evaluate the circulation expression profile of microRNAs (miRNAs) associated with responses to chemoradiotherapy in nasopharyngeal carcinoma.
Methods: Peripheral blood from 17 patients was collected before and after chemotherapy and radiotherapy. Differential expression circulating miRNAs were analyzed using microRNA Cancer Panels and were compared among patients with complete responses. Differential expression analysis using GenEx 7 Multid, statistic represented by GraphPad Prism 9. Alterations mechanism signaling pathways and biological function using IPA (Ingenuity Pathways Analysis).
Results: Using microRNAs Cancer Plate consisting of 116 miRNAs, we identified ten circulating miRNAs that were differentially expressed in NPC patients after chemoradiotherapy. Unsupervised clustering and confirmation using qRT-PCR showed that miR-483-5p, miR-584-5p, miR-122-5p, miR-7-5p, miR-150-5p were overexpressed and miRNA are miR-421, miR-133a-3p, miR-18a-5p, miR-106b-3p, miR-339-5p were significantly down-regulated after chemoradiotherapy ($p < 0.0001$). In addition, ROC analysis through AUC (Area Under Curve) with 99% confidence interval (CI) p value < 0.0001. Gene enrichment analysis of microRNAs and the targeted proteins revealed that the main involved pathways for chemoradiotherapy in NPC were cell death and survival signaling pathways.
Conclusion: qPCR profiling in circulating blood compared before and after chemoradiotherapy in nasopharyngeal carcinoma can identify pathways involved in treatment responses. miR-483-5p, miR-584-5p, miR-122-5p, miR-7-5p, miR-150-5p, miR-421, miR-133a-3p, miR-18a-5p, miR-106b-3p, miR-339-5p are differentially regulated after chemoradiotherapy in NPC.

1. Introduction

Nasopharyngeal cancer (NPC) is a head and neck cancer with relatively high incidence, mortality, and low survival rates in Southeast Asia, including Indonesia [1]. Many are found in Southeast Asia and are associated with particular ethnicities, so this cancer is unique [2]. The cause of cancer is still unclear. Although it is more commonly found in men, the relationship with gender has not been explained. Delay in diagnosis worsens the patient's condition. The anatomical location and small size are difficult to detect early on, so it is considered the cause of the low cure rate [3–5]. One of the biggest challenges in the treatment is complete response and a high rate of cancer progression after treatment.

* Corresponding author.
E-mail address: tirta.wardana@unsoed.ac.id (T. Wardana).

https://doi.org/10.1016/j.ncrna.2022.09.005
Received 27 June 2022; Received in revised form 28 August 2022; Accepted 7 September 2022
Available online 11 September 2022

The core clinical management is early diagnosis using nasopharyngeal tissue biopsy followed by radiotherapy with or without concomitant chemotherapy. In low- and middle-income countries, NPCs are usually diagnosed in late stages due to the difficulty of recognizing the disease until manifestation in the cervical lymph nodes [6–9]. In patients with advanced stages, disease recurrences are relatively high after a particular time of clinical responses with chemoradiotherapy. Clinical biomarkers to predict disease progression in NPC are still lacking.

Tumor biomarkers are essential to guide treatment, calculate disease progression risk, and design surveillance [10]. MicroRNAs (miRNAs) are small RNA (20–25 bp) involved in the modulation of gene expression through post-translational mechanisms [11,12]. Differences in miRNA expression profiles in primary tissue samples have been used in differentiating pathophysiology risk factors [13], therapeutics response [14], and prognosis of NPC [15].

Chemoradiotherapy causes apoptosis of cancer cells, thereby affecting changes in miRNA expression. Several miRNAs have been involved in chemotherapy response after receiving 5-fluorouracil + cisplatin of NPC and 5-fluorouracil sensitivity in breast cancer [16,17]. We also previously investigated the association between the expressions of chemotherapy responses f NPC and Breast cancer using cell lines and primary tissue samples [18,19]. Chemoradiotherapy might also affect circulating tumor cells, protein, free-DNA, and RNAs. This study investigated altered miRNA expression in the plasma in NPC patients with complete responses after chemoradiotherapy.

2. Material and methods

2.1. Study subjects and ethics statement

17 NPC patients who received chemotherapy based on cisplatin and radiotherapy were involved. Patients come to health facilities with complaints of nasal obstruction, ear problems, nosebleeds, headaches, and lumps in the neck. Four patients were initially diagnosed in stage II B, and 4 and 9 patients were diagnosed in stage III and IV. Plasma samples were collected before treatment 3–17 months after the chemoradiotherapy. Other eligibility criteria for this study were Early Antigen (EA) > 1, Viral Capsid Antigen (VCA) > 2, and EBNA >1.6. Response to treatment was evaluated 12 weeks after using nasopharyngeal endoscopy and CT scan. Complete response (CR) was defined as no residual disease in the smooth nasopharyngeal mucosa, no mass, and no lymph nodes with confirmation of biopsy. This study was performed after approval from Jenderal Soedirman University Ethics Committee (number 898/EC/2016). Furthermore, all participants were older than 18 years old and could provide an informed consent form when recruited, which entails using samples, acquisition, and clinical data.

2.2. Chemoradiotherapy

A combination of radiation and chemotherapy is utilized to treat progressed locoregional NPC. Chemotherapy is classified as neoadjuvant, contemporaneous, or adjuvant, whether given some time recently, amid, or after radiation. Chemotherapy is chosen separately based on the patient's characteristics. Concurrent cisplatin with illumination is the standard treatment for chemoradiation in nearby maladies. In the meantime, with cisplatin + radiotherapy taken after cisplatin/5-FU or carboplatin/5-FU, chemoradiation taken after adjuvant chemotherapy may be utilized. The docetaxel/cisplatin/5 FI, docetaxel/cisplatin regimen is utilized for neoadjuvant chemotherapy. Cisplatin/5 FU, cisplatin/epirubicin/paclitaxel, and concordant coordination with week-by-week cisplatin or carboplatin organization. The radiation measurements endorsed were 69–74 Gy to PGTVnx, 66–70 Gy to PGTVnd, 60–66 Gy to PTV1, and 50–54 Gy to PTV2, conveyed in 30 or 33 divisions. Radiation is given once day by day, five divisions per week, for 6–6.5 weeks for IMRT arranging.

Table 1
Demographical and clinical characteristics of the study population.

Characteristic	N	Percent (%)
Pre Chemotherapy	17	100
Post Chemotherapy	17	100
Median Age (Years), Range	51 (20–66)	
Sex		
Male	13	76%
Female	4	24%
Histology (WHO)		
WHO I	1	6%
WHO II	2	12%
WHO III	14	82%
Stage at diagnosis		
I	0	0
II	4	24%
III	4	24%
IV	9	53%
Pathology Anatomy		
Undifferentiated	2	12%
Non-Keratin, Undiff-Sub Type	13	76%
Non-Keratin, Differentiated	1	6%
Keratin	1	6%
EBV - EA		
Positive	15	88%
Negative	2	12%
EBV - EBNA		
Positive	15	88%
Negative	2	12%
EBV - VCA		
Positive	17	100%
Negative	0	0%

2.3. Plasma sample collection and miRNA isolation

Whole blood from patients (5 mL each) before and after therapy was collected using an EDTA vacutainer. Plasma was separated using centrifugation (1500 rpm for 10 min) and was stored at −80 °C until analysis. 200 mL of plasma was used for total RNA extraction using RNA Isolation Kit miRCURY-Biofluid (Cat No. 300 112, Exiqon). cDNA synthesis was performed using 50 mL of total RNA with cDNA Synthesis Universal kit II, 8–64 rxns (Cat No. 203 301, Exiqon) in Biorad C1000 thermal cycler (42 °C for 60 min, 95 °C for 5 min, and 4 °C). All procedures were performed following the manufacturer's recommended protocol.

2.4. Quantification microRNA panel

MicroRNA profiling was performed using real-time PCR using Cancer Focus microRNA PCR Panel. ExiLent SYBR Green master mix, 2.5 mL (Cat No. 203 402, Exiqon) consisting of 196 target primer miRNAs based on LNA (Locked Nucleic Acid). All protocols were performed following the recommended protocols provided by the manufacturer.

2.5. Data analysis

The analyses were performed using Genex 6 Pro with Exiqon qPCR wizard software MultiD. Expression analysis was performed using relative quantification of $-2^{\Delta\Delta Ct}$ [20]. Gene enrichment analysis of the differential miRNA expression was performed using Ineguinity Pathway Analysis (IPA). GraphPad Prism 9 software was used for data analysis and Figure configuration to represent the mean, standard deviation (SD), and the student t-test. ROC sensitivity and specificity analysis was constructed with a 99% confidence interval and $p < 0.05$ as a statistically significant value.

Table 2
Profile dynamic changes expression of microRNAs circulating in NPC after receiving chemoradiotherapy.

No	Variable	FC	Differences	P-value	Expression
1	hsa-miR-483-5p	−12.506	−3.645	1.00E-08	Down Expression
2	hsa-miR-584-5p	−10.558	−6.708	1.00E-08	Down Expression
3	Hsa-miR-122-5p	−6.312	−2.658	1.00E-08	Down Expression
4	hsa-miR-7-5p	−5.225	−2.386	1.00E-08	Down Expression
5	Hsa-miR-150-5p	−4.206	−2.073	1.00E-08	Down Expression
6	hsa-miR-877-5p	−4.045	−2.016	1.00E-08	Down Expression
7	Hsa-miR-215-5p	−2.935	−1.553	6.63E-07	Down Expression
8	Hsa-miR-192-5p	−2.914	−1.543	1.00E-08	Down Expression
9	hsa-miR-141-3p	−2.344	−1.229	1.01E-06	Down Expression
10	hsa-miR-16-2-3p	−2.04	−1.029	5.22E-05	Down Expression
11	hsa-miR-30e-3p	6.233	2.64	1.00E-08	Over Expression
12	hsa-miR-1	6.238	2.641	1.00E-08	Over Expression
13	hsa-miR-376a-3p	6.249	2.644	1.00E-08	Over Expression
14	hsa-miR-378a-3p	6.452	2.69	1.00E-08	Over Expression
15	hsa-miR-133b	6.674	2.739	1.00E-08	Over Expression
16	hsa-miR-339-5p	6.723	2.749	1.00E-08	Over Expression
17	hsa-miR-106b-3p	6.934	2.794	1.00E-08	Over Expression
18	hsa-miR-18a-5p	9.224	3.205	1.00E-08	Over Expression
19	hsa-miR-133a-3p	10.884	3.444	1.00E-08	Over Expression
20	hsa-miR-421	12.631	3.659	1.00E-08	Over Expression

3. Results

3.1. Patients characteristics

In this study of patients, 13 males and 4 females with a median age of 51. Staging I-II data was performed on 4 patients and 13 at III-IV. These patients received completed chemo and radiotherapy, as shown in Table 1. Based on titer EBV infection, data showed positive EBV EA (n = 15), EBV- EBNA (n = 15) and EBV-VCA (n = 17). From the histology status, the participants were dominated by WHO type III with 14 patients.

3.2. Differential expression

Clinical and pathological patient characteristics at diagnosis are summarized in Table 1. Analysis of relative expression using GenEx identified 20 of the most differentially deregulated miRNAs (details see Table 2). From these results, 10 miRNAs were down expressions ($p < 0.0001$), and 10 were up expressions ($p < 0001$). The heatmap of differentially expressed microRNAs is presented in Fig. 1.

miRNAs expression in the circulation of 17 NPC patients with complete response after chemoradiotherapy showed an inverse expression of 20 microRNAs before and after receiving therapy (Fig. 2). Five miRNAs including miR-483-5p, miR-584-5p, miR-122-5p, miR-7-5p, and miR150-5p were upregulated. Another 5 miRNAs including miR-421, miR-133a-5p, miR-18a-5p, miR-106b-3p, and miR-339-5p were down-regulated. Sensitivity and specificity analyzes were performed on 10 miRNAs that consistently experienced changes in expression after receiving chemotherapy. The analysis showed that 10 miRNAs could be suggested as candidates for assessing the response to chemo-radiotherapy in NPC patients using circulating miRNAs. ROC analysis shows the AUC (Area Under Curve) value > 0.9 with a 99% confidence interval with a significance value of $p < 0.0001$ (can be seen in Fig. 3).

3.3. Mechanism signaling pathways

The identification of potential biological mechanisms affected by the miRNAs expression dysregulation after chemoradiotherapy was carried out using IPA (Ingenuity Analysis Pathways). Differential expression of

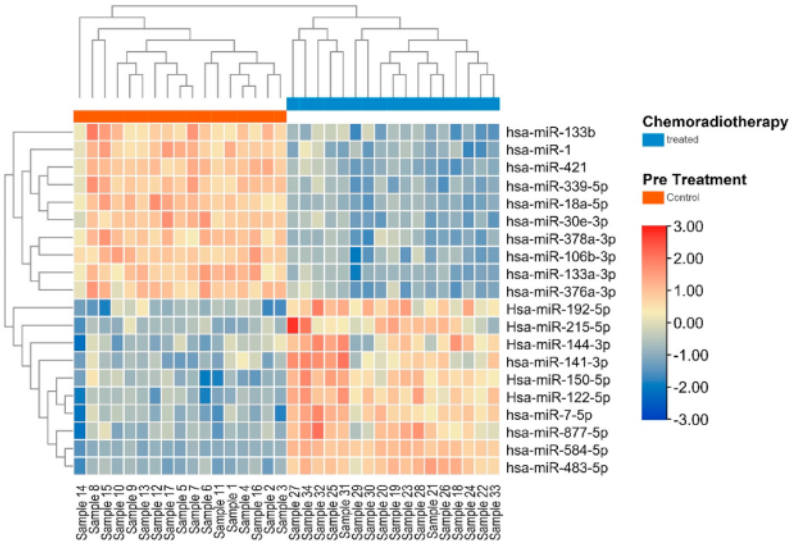

Fig. 1. Profile expression with a high significance p-value ($p < 0.0001$) using cancer focus microRNAs panel from circulating pretreatment (non-chemo-radiotherapy) and chemo-radiotherapy in NPC.

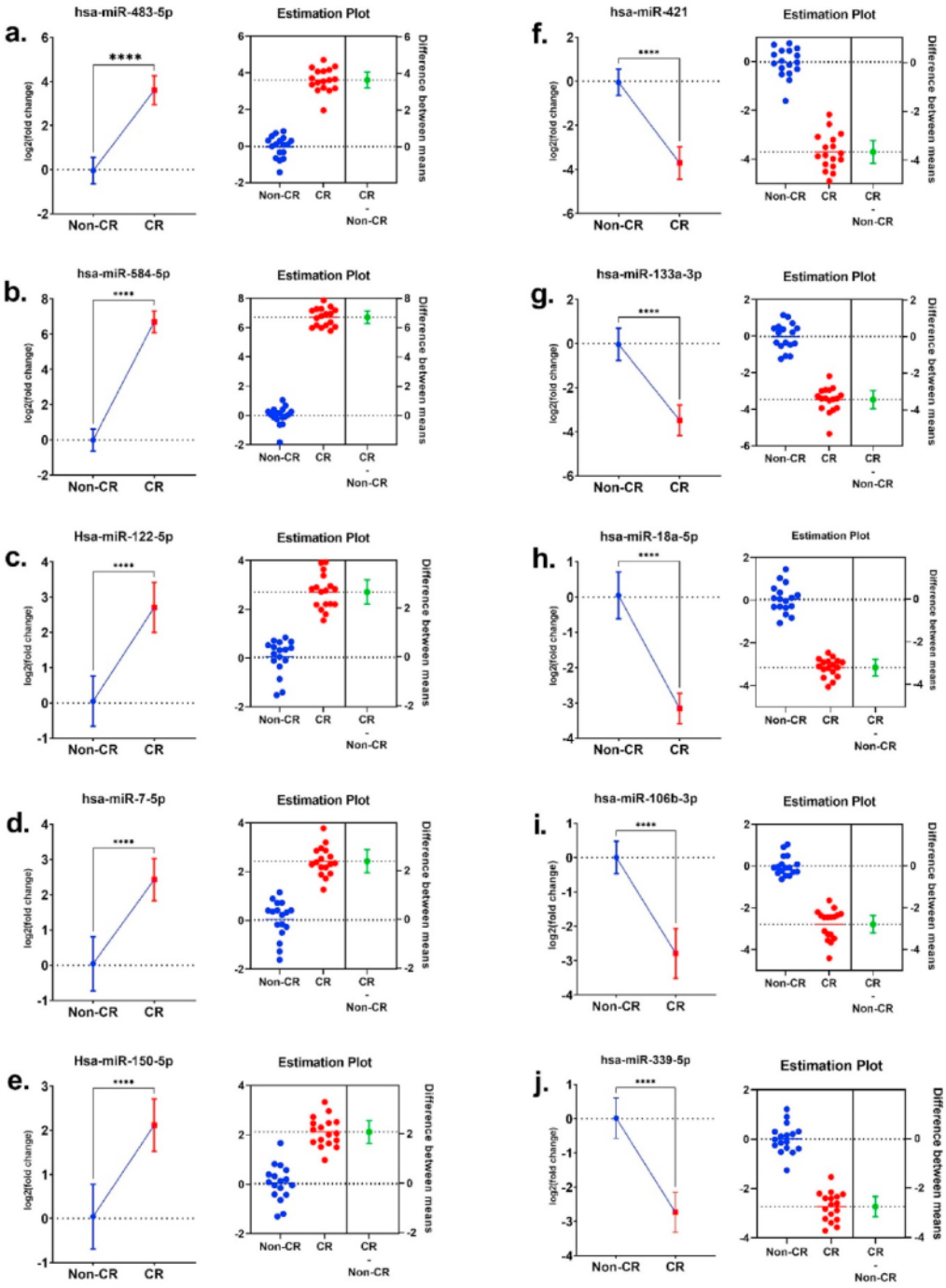

Fig. 2. Relative quantification of distribution expression of microRNAs target from pretreatment to post treatment in NPC; a. miR-483-5p; b. miR-584-5p; c. miR-122-5p; d. miR-7-5p; e. miR-150-5p; f. miR-421; g. miR-133a-3p; h. miR-18a-5p; i.miR-106b-3p; j. miR-339-5p.

deregulated miRNAs by analysis using IPA showed several impacts of cellular mechanisms with p-value <0.01 category with activation z-score of −1.131 – 2.256. Significant changes due to the impact of chemotherapy and radiotherapy affect the mechanism of cellular death and survival involved in the necrosis, apoptosis, cell viability, and cell death of carcinoma cell lines (Table 3). 13 downregulated miRNAs were involved in the cell viability processes. Six miRNAs were involved in cell death regulation, 23 miRNAs were associated with the biological pathways of apoptosis, and 25 miRNAs were involved in cell necrosis processes.

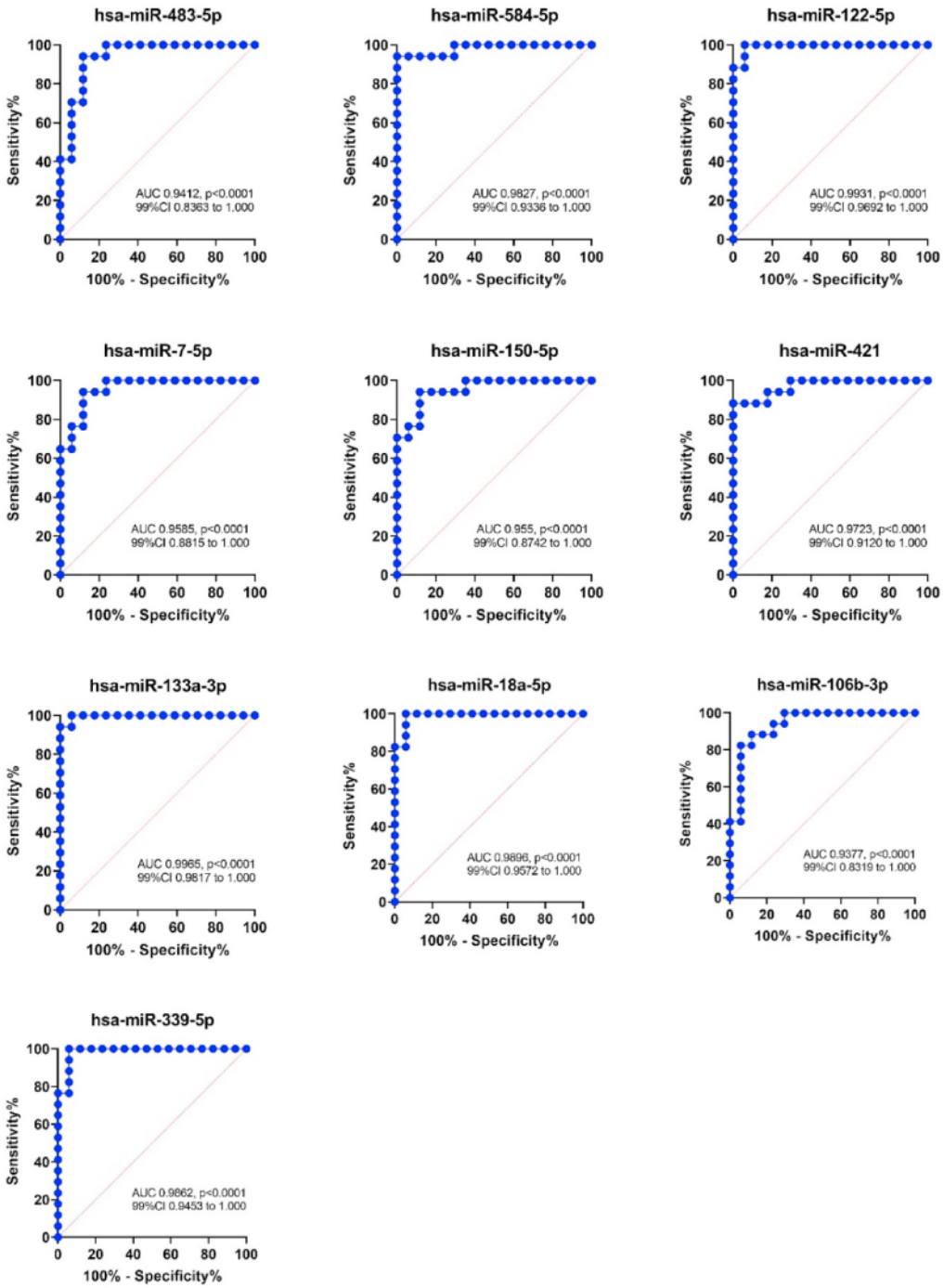

Fig. 3. Analysis sensitivity and specify after through ROC analysis through AUC (Area Under Curve) with 99% confidence interval (CI) p value < 0.0001; a. miR-483-5p; b. miR-584-5p; c. miR-122-5p; d. miR-7-5p; e. miR-150-5p; f. miR-421; g. miR-133a-3p; h. miR-18a-5p; i.miR-106b-3p; j. miR-339-5p.

4. Discussions

Chemotherapy and radiotherapy are currently the core treatment for patients with NPC. Residual disease after treatment and disease progression is frequently experienced by the patients, particularly those initially diagnosed in late stages (Table 1). Detailed molecular analysis of biological pathways involved in the complete responses after treatment might inform NPC's potential biomarker, biological pathway, and

Table 3
Mechanism cellular analysis from profiling circulating expression of microRNAs in NPC after receiving chemo-radio therapy.

Categories	Diseases or Functions Annotation	p-value	Activation z-score	Molecules	# Molecules
Cardiovascular System Development and Function, Organismal Development	Angiogenesis	0.00551	0.67	miR-122-5p,miR-125b-5p,miR-126a-3p,miR-16-5p, miR-199a-5p,miR-221-3p,miR-27a-3p,miR-320b,miR-378a-3p,miR-532-5p,miR-7a-5p	11
Cell Cycle	Interphase	0.0237		let-7a-5p,miR-132-3p,miR-16-5p,miR-186-5p,miR-21-5p, miR-27a-3p,miR-451a,miR-92a-3p	8
	Arrest in interphase	0.0162		miR-132-3p,miR-16-5p,miR-186-5p,miR-21-5p,miR-27a-3p,miR-451a	6
	G1 phase	0.0491		let-7a-5p, miR-16-5p,miR-21-5p,miR-27a-3p,miR-92a-3p	5
	Arrest in G0 phase	0.000783		miR-16-5p,miR-186-5p, miR-27a-3p,miR-451a	4
Cell Death and Survival	Necrosis	0.000624	−0.522	let-7a-5p,miR-1-3p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-132-3p,miR-141-3p,miR-142-3p,miR-145-5p, miR-146a-5p,miR-148a-3p,miR-150-5p,miR-16-5p,miR-186-5p, miR-199a-3p,miR-21-5p,miR-221-3p,miR-223-3p,miR-24-3p,miR-30c-5p,miR-320b,miR-378a-3p,miR-451a, miR-486-5p,miR-7a-5p	25
	Apoptosis	0.00351	−0.615	let-7a-5p,miR-1-3p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-132-3p,miR-141-3p,miR-145-5p,miR-146a-5, miR-148a-3p,miR-150-5p,miR-16-5p,miR-186-5p,miR-199a-3p,miR-21-5p,miR-221-3p,miR-223-3p,miR-30c-5p,miR-320b,miR-378a-3p,miR-451a,miR-486-5p,miR-7a-5p	23
	Cell viability	0.0181	2.256	miR-133a-3p,miR-141-3p,miR-145-5p,miR-150-5p,miR-16-5p,miR-186-5p,miR-21-5p,miR-221-3p,miR-24-3p, miR-30c-5p,miR-378a-3p,miR-486-5p,miR-7a-5p	13
	Cell death of carcinoma cell lines	0.00496	0.119	let-7a-5p,miR-145-5p,miR-146a-5p,miR-21-5p,miR-221-3p,miR-223-3p	6
Cell Morphology, Cellular Function, and Maintenance	Autophagy of tumor cell lines	0.0326		miR-125b-5p,miR-130a-3p,miR-23a-3p	3
Cellular Development	The epithelial-mesenchymal transition of tumor cell lines	0.0187		miR-141-3p,miR-483-5p,miR-7a-5p	3
Cellular Development, Cellular Growth and Proliferation	Cell proliferation of tumor cell lines	5.7E-12	0.694	let-7a-5p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-128-3p,miR-130a-3p,miR-132-3p,miR-133a-3p,miR-139-5p,miR-141-3p,miR-145-5p,miR-146a-5p,miR-148a-3p,miR-16-5p, miR-186-5p,miR-18a-5p,miR-192-5p,miR-197-3p,miR-199a-3p,miR-199a-5p, miR-21-5p, miR-221-3p,miR-223-3p,miR-23a-3p,miR-24-3p,miR-27a-3p,miR-30a-3p,miR-378a-3p,miR-451a,miR-708-5p,miR-7a-5p,miR-92a-3p	32
	Differentiation of thyroid precursor cells	0.00514		miR-144-3p,miR-451a,miR-486-5p	3
	Leucopoiesis	0.00477	−1.067	miR-125b-5p,miR-132-3p,miR-144-3p,miR-146a-5p, miR-150-5p,miR-16-5p,miR-18a-5p,miR-21-5p,miR-451a,miR-486-5p	10
	Myelopoiesis of leukocytes	0.00201	−1.131	miR-125b-5p,miR-144-3p,miR-16-5p,miR-21-5p,miR-451a	5
	Differentiation of myeloid leukocytes	0.00619	−1.131	miR-125b-5p,miR-144-3p,miR-16-5p,miR-21-5p,miR-451a	5
	Granulopoiesis	0.00223	−1.131	miR-125b-5p,miR-144-3p,miR-21-5p,miR-451a	4
Cellular Movement	Cell movement	0.00865	1.196	let-7a-5p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-130a-3p,miR-133a-3p,miR-139-5p,miR-141-3p,miR-145-5p,miR-146a-5p,miR-151-5p,miR-16-5p,miR-197-3p,miR-21-5p,miR-221-3p,miR-27a-3p,miR-320b,miR-532-5p,miR-7a-5p,miR-92a-3p	20
	Invasion of tumor cell lines	8E-09	0.158	miR-10a-5p,miR-122-5p,miR-125b-5p,miR-126a-3p, miR-139-5p,miR-141-3p,miR-145-5p,miR-146a-5p,miR-151-5p,miR-197-3p,miR-199a-3p,miR-21-5p,miR-221-3p,miR-223-3p,miR-451a,miR-483-5p,miR-532-5p,miR-7a-5p,miR-92a-3p	19
	Migration of cells	0.00542	1.64	let-7a-5p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-130a-3p,miR-133a-3p,miR-139-5p,miR-141-3p,miR-146a-5p,miR-151-5p,miR-16-5p,miR-197-3p,miR-21-5p,miR-221-3p,miR-27a-3p,miR-320b,miR-532-5p,miR-7a-5p,miR-92a-3p	19
	Cell movement of tumor cell lines	6.04E-06	1.092	let-7a-5p,miR-10a-5p,miR-122-5p,miR-125b-5p,miR-130a-3p,miR-139-5p,miR-141-3p,miR-145-5p,miR-151-5p,miR-16-5p,miR-197-3p,miR-21-5p,miR-221-3p,miR-27a-3p,miR-532-5p,miR-7a-5p,miR-92a-3p	17
Cellular Movement, Hematological System Development and Function, Immune Cell Trafficking, Inflammatory Response	Migration of monocytes	0.00465		miR-125b-5p,miR-133a-3p,miR-146a-5p	3
	Chromosomal aberration	0.00682		let-7a-5p,miR-125b-5p,miR-130a-3p,miR-708-5p	4

(*continued on next page*)

Table 3 (*continued*)

Categories	Diseases or Functions Annotation	p-value	Activation z-score	Molecules	# Molecules
DNA Replication, Recombination, and Repair	DNA damage	0.0139	−0.152	let-7a-5p,miR-16-5p,miR-7a-5p,miR-92a-3p	4
Inflammatory Response	Inflammation of absolute anatomical region	7.94E-08		let-7a-5p,miR-130a-3p,miR-133a-3p,miR-150-5p,miR-16-5p,miR-197-3p,miR-199a-5p,miR-210-3p,miR-221-3p,miR-223-3p,miR-23a-3p,miR-27a-3p,miR-30c-5p,miR-320b,miR-338-3p,miR-376a-3p,miR-409-3p,miR-423-3p,miR-486-5p,miR-532-5p,miR-92a-3p	21
	Inflammation of body cavity	4.88E-07		let-7a-5p,miR-130a-3p,miR-133a-3p,miR-150-5p,miR-16-5p,miR-197-3p,miR-199a-5p,miR-210-3p,miR-221-3p,miR-223-3p,miR-23a-3p,miR-27a-3p,miR-30c-5p,miR-320b,miR-423-3p,miR-486-5p,miR-532-5p,miR-92a-3p	18
Organismal Survival	Survival of organism	0.0259	−0.293	let-7a-5p,miR-122-5p,miR-141-3p,miR-142-3p,miR-16-5p,miR-221-3p,miR-223-3p,miR-92a-3p	8

potential targeted treatment [21]. The low number of patients to achieve a complete response makes it interesting to know the molecular changes.

One molecule that is known to be differentially regulated in response to the changes in cellular activities such as therapy is microRNA (miRNA). MicroRNA is responsive to the stress-like effect on hypoxia. Biological processes such as cell proliferation, apoptosis, and tumorigenesis may provide a general overview of the microenvironment affecting miRNA expression [22,23]. Therefore, discovering candidate biomarkers based on minimally invasive is expected to provide a new approach to assessing the success of treatment to increase efforts for treatment success [24].

miRNAs are molecules that play an essential role as post-transcriptional regulators by targeting hundreds of mRNAs and are involved in many disease cases, including cancer. Previous studies have reported that miRNA expression is associated with several chemotherapy responses and resistance events in esophageal cancer [14], Oral squamous cell carcinoma [25], breast cancer [19], and lung cancer [26]. In another report on nasopharyngeal carcinoma, miR-324-3p and miR-519d are deregulated by inhibiting gene translation targeting WNT2B [27] and PDRG1 [28] toward radiotherapy sensitivity. In addition, mIR-29c is known to be jointly sensitive to cisplatin-based radiotherapy and chemotherapy [18,29,30].

This study found 10 miRNAs that stably and consistently changed circulating expression after achieving a complete response to chemoradiotherapy (Figs. 1 and 2). It consisted of 5 miRNAs that significantly increased expression, namely miR-483-5p, miR-584-5p, miR-122-5p, miR-7-5p, and miR-150-5p, and 5 miRNAs that had decreased expression. Significantly, namely miR-421, miR-133a-3p, miR-18a-5p, miR-106b-3p and miR-339-5p. In the previous study, Changes in the expression response of miRNAs to therapy are closely related to the type of therapeutic agent given to influence cellular mechanisms and the response by the body [31]. It is shown by giving 5-FU to chemosensitive affect miR-494 expression and chemoresistance to miR-200c in colorectal cancer.

The sensitivity and specificity analysis showed that these 10 miRNAs are promising candidates for minimally invasive chemoradiotherapy response-based biomarkers in NPC incidence (Fig. 3). It was also shown as a miRNAs-based marker in advanced gastric cancer with high sensitivity, and the specificity of miR-338-3p and miR-142-3p was 0.86 (95% CI, 0.587–0.981; sensitivity = 70%, specificity = 100%) [32]. Some other studies performed that microRNAs were related to chemoradiotherapy in advanced rectal cancer, rectal adenocarcinoma, prostate cancer, breast cancer, and lung cancer [33–38].

The analysis results show that miRNA plays a role in multiple mechanisms and other cancer cases. miR-483-5p in adrenocortical and prostate cancer targets RBM5, which is involved in proliferation and invasion and is associated with poor prognosis [39–41]. Overexpression of mIR-584 inhibits proliferation. It induces apoptosis via the target WW domain-containing E3 ubiquitin-protein ligase 1 [42], miR-122-5p, and miR-421 inhibits cell migration and invasion by regulating DUSP4 in gastric [43,44], cancer and chemosensitizer in breast cancer [45]. miR-7-5p and miR-150-5p suppress cell proliferation and induce apoptosis of breast cancer cells [46] and colorectal cancer [47]. In another study, the down expression of miR −133a-3p has a role in promoting cell migration in bladder cancer [48] and activation of RhoA in colorectal cancer [49]. In addition, miR-18a, miR-106b-3p, and miR-339-5p modulate apoptosis and inhibit proliferation with lncRNA in carcinoma nasofaring [50], esophageal squamous cell carcinoma [51], and lung cancer [52].

To further investigate the mechanism regulation of miRNAs of this nasopharyngeal carcinoma, expressions with significance miRNAs 4 cellular regulation of cell death and survival related to chemotherapy and radiotherapy treatment. We found alterations of miRNAs related to necrosis, apoptosis, cell viability, and cell death of carcinoma cell lines. Although the mechanism analysis is related to the chemoradiotherapy response in nasopharyngeal carcinoma cases, the direct relationship with miRNAs is unclear.

There are several limitations to this study. We used a small sample size for miRNAs profiling: low survival rate and irregular treatment schedule due to unvalidated with a large cohort. Alterations expression of miRNAs on circulating can be detected by bioinformatics approaches and correlated with molecular biology change after receiving the chemoradiotherapy. Even though confirmation using the biological model and validating the differential expression of miRNAs is necessary for evaluation. Therefore, further research is needed to explain more comprehensively the mechanism and validation with a large number of samples. Meanwhile, few studies are studying the function of microRNA and circulation in the sensitivity of tumor treatment and the complete response to chemoradiotherapy for nasopharyngeal carcinoma.

5. Conclusions

In conclusion, research focused on studying changes in circulating miRNA expression in response to a combination of radiotherapy and chemotherapy is relatively limited. In this study, we created a signature profile associated with these conditions. As a result we found increased expression of miR-483-5p, miR-584-5p, miR-122-5p, miR-7-5p and miR-150-5p. In contrast, decreased expression was found in miR-421, miR-133a-3p, miR-18a-5p, miR-106b-3p and miR-339-5p.In the future, our study will validate with a larger sample size to determine the sensitivity and specificity of the obtained chemoradiotherapy biomarker candidate miRNAs.

CRediT authorship contribution statement

Tirta Wardana: Conceptualization, Methodology, Investigation, Writing – original draft, Funding acquisition. **Siti Nur Chasanah:** Investigation, Data curation, Writing – original draft. **Risky Oktriani:**

Methodology, Investigation, Data curation. **Cita Herawati:** Investigation, Resources. **Sumadi Lukman Anwar:** Validation, Writing – review & editing. **Indwiani Astuti:** Supervision. **Sofia Mubarika Haryana:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors state that there is no conflict of interest in this study.

Acknowledgments

We thank Dharmais Hospital National Cancer Center, The Genomir Team, and The Laboratory of Molecular Biology and Biochemistry Staff. The Financial support of this study by the RISBIN IPTEKDOK Scheme from The Ministry of Health, Republic of Indonesia.

Abbreviations

Hsa	Homo sapiens
NPC	Nasopharyngeal Carcinoma
miRNA	MicroRNA

References

[1] S.H. Hutajulu, D. Howdon, K.W. Taroeno-Hariadi, M.S. Hardianti, I. Purwanto, S. R. Indrasari, C. Herdini, B. Hariwiyanto, A. Ghozali, H. Kusumo, W. Dhamiyati, S. R. Dwidanarti, I. Bing Tan, J. Kurnianda, M.J. Allsop, Survival outcome and prognostic factors of patients with nasopharyngeal cancer in Yogyakarta, Indonesia: a hospital-based retrospective study, PLoS One 16 (2021), e0246638, https://doi.org/10.1371/journal.pone.0246638.

[2] Q. Wang, H. Xie, C. Jiang, Y. Zhang, Y. Li, N. Theodoropoulos, P. Boffetta, Racial and ethnic disparities in nasopharyngeal cancer with an emphasis among Asian Americans, J. Clin. Oncol. 39 (2021), https://doi.org/10.1200/jco.2020.39.28_suppl.118, 118–118.

[3] M. Adham, A.N. Kurniawan, A.I. Muhtadi, A. Roezin, B. Hermani, S. Gondhowiardjo, I. Bing Tan, J.M. Middeldorp, Nasopharyngeal carcinoma in Indonesia: epidemiology, incidence, signs, and symptoms at presentation, Chin. J. Cancer 32 (2012) 185–196, https://doi.org/10.5732/cjc.011.10328.

[4] L. Wu, C. Li, L. Pan, Nasopharyngeal carcinoma: a review of current updates, Exp. Ther. Med. 15 (2018) 3687–3692, https://doi.org/10.3892/etm.2018.5878.

[5] M.A. Wildeman, R. Fles, C. Herdini, R.S. Indrasari, A.D. Vincent, M. Tjokronagoro, S. Stoker, J. Kurnianda, B. Karakullukcu, K.W. Taroeno-Hariadi, O. Hamming-Vrieze, J.M. Middeldorp, B. Hariwiyanto, S.M. Haryana, I.B. Tan, Primary treatment results of nasopharyngeal carcinoma (NPC) in Yogyakarta, Indonesia, PLoS One 8 (2013), e63706, https://doi.org/10.1371/journal.pone.0063706.

[6] M.A.H. Abusalah, S.H. Gan, M.A.I. Al-hatamleh, A.A. Irekeola, R.H. Shueb, C. Y. Yean, Recent advances in diagnostic approaches for epstein–barr virus, Pathogens 9 (2020) 226, https://doi.org/10.3390/pathogens9030226.

[7] C.M. Stewart, P.D. Kothari, F. Mouliere, R. Mair, S. Somnay, R. Benayed, A. Zehir, B. Weigelt, S.J. Dawson, M.E. Arcila, M.F. Berger, D.W.Y. Tsui, The value of cell-free DNA for molecular pathology, J. Pathol. 244 (2018) 616–627, https://doi.org/10.1002/path.5048.

[8] R. Mlak, T. Powrózek, A. Brzozowska, I. Homa-Mlak, M. Mazurek, T. Małecka-Massalska, RRM1 gene expression evaluated in the liquid biopsy (blood cfRNA) as a non-invasive, predictive factor for radiotherapy-induced oral mucositis and potential prognostic biomarker in head and neck cancer patients, Cancer Biomarkers 22 (2018) 657–667, https://doi.org/10.3233/CBM-171062.

[9] N. Papadopoulos, Liquid biopsy for the early detection of cancer: applications in screening and minimal residual disease, Med. Genet. 30 (2018) 108, https://doi.org/10.1007/s11825-018-0176-4. http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L621355249%0Ahttp://dx.

[10] D.W. Cescon, S.V. Bratman, S.M. Chan, L.L. Siu, Circulating tumor DNA and liquid biopsy in oncology, Nat. Cancer (Que.) 1 (2020) 276–290, https://doi.org/10.1038/s43018-020-0043-5.

[11] R. Rupaimoole, F.J. Slack, MicroRNA therapeutics: towards a new era for the management of cancer and other diseases, Nat. Rev. Drug Discov. 16 (2017) 203–221, https://doi.org/10.1038/nrd.2016.246.

[12] P. Tristán-Ramos, A. Rubio-Roldan, G. Peris, L. Sánchez, S. Amador-Cubero, S. Viollet, G. Cristofari, S.R. Heras, The tumor suppressor microRNA let-7 inhibits human LINE-1 retrotransposition, Nat. Commun. 11 (2020) 5712, https://doi.org/10.1038/s41467-020-19430-4.

[13] E. Kudela, M. Samec, L. Koklesova, A. Liskova, P. Kubatka, E. Kozubik, T. Rokos, T. Pribulova, E. Gabonova, M. Smolar, K. Biringer, Mirna expression profiles in luminal a breast cancer—implications in biology, prognosis, and prediction of response to hormonal treatment, Int. J. Mol. Sci. 21 (2020) 1–20, https://doi.org/10.3390/ijms21207691.

[14] R. Hummel, C. Sie, D.I. Watson, T. Wang, A. Ansar, M.Z. Michael, M. Van Der Hoek, J. Haier, D.J. Hussey, MicroRNA signatures in chemotherapy resistant esophageal cancer cell lines, World J. Gastroenterol. 20 (2014) 14904–14912, https://doi.org/10.3748/wjg.v20.i40.14904.

[15] J.F. Barger, S.P. Nana-Sinkam, MicroRNA as tools and therapeutics in lung cancer, Respir. Med. 109 (2015) 803–812, https://doi.org/10.1016/j.rmed.2015.02.006.

[16] S. Ghafouri-Fard, A. Abak, F. Tondro Anamag, H. Shoorei, F. Fattahi, S. A. Javadinia, A. Basiri, M. Taheri, 5-Fluorouracil: a narrative review on the role of regulatory mechanisms in driving resistance to this chemotherapeutic agent, Front. Oncol. 11 (2021) 1210, https://doi.org/10.3389/fonc.2021.658636.

[17] X. Cao, J. Hou, Q. An, Y.G. Assaraf, X. Wang, Towards the overcoming of anticancer drug resistance mediated by p53 mutations, Drug Resist. Updates 49 (2020), 100671, https://doi.org/10.1016/j.drup.2019.100671.

[18] E. Savitri, I. Maharis, A. Kadir, R. Djamin, S. Mubarikaand, T. Wardana, J.S. Safri, R. Djamin, A.Q. Punagi, A. Kadir, S. Mubarika, T. Wardana, I. Maharis, A. Kadir, R. Djamin, S. Mubarikaand, T. Wardana, The expression of mir-21 and mir-29c in blood plasma of nasopharyngeal carcinoma patient post-chemoradiotherapy, Indian J. Public Heal. Res. Dev. 10 (2019) 1523–1529, https://doi.org/10.5958/0976-5506.2019.03054.7.

[19] S.L. Anwar, D.N.I. Sari, A.I. Kartika, M.S. Fitria, D.S. Tanjung, D. Rakhmina, T. Wardana, I. Astuti, S.M. 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. APJCP 20 (2019) 1223–1228, https://doi.org/10.31557/APJCP.2019.20.4.1223.

[20] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method, Methods 25 (2001) 402–408, https://doi.org/10.1006/meth.2001.1262.

[21] J. Meehan, M. Gray, C. Martínez-Pérez, C. Kay, L.Y. Pang, J.A. Fraser, A.V. Poole, I. H. Kunkler, S.P. Langdon, D. Argyle, A.K. Turnbull, Precision medicine and the role of biomarkers of radiotherapy response in breast cancer, Front. Oncol. 10 (2020) 628, https://doi.org/10.3389/fonc.2020.00628.

[22] T. Carbonell, A.V. Gomes, MicroRNAs in the regulation of cellular redox status and its implications in myocardial ischemia-reperfusion injury, Redox Biol. 36 (2020), 101607, https://doi.org/10.1016/j.redox.2020.101607.

[23] J. Dai, Y. Su, S. Zhong, L. Cong, B. Liu, J. Yang, Y. Tao, Z. He, C. Chen, Y. Jiang, Exosomes: key players in cancer and potential therapeutic strategy, Signal Transduct. Targeted Ther. 5 (2020) 1–10, https://doi.org/10.1038/s41392-020-00261-0.

[24] K.D. Davis, N. Aghaeepour, A.H. Ahn, M.S. Angst, D. Borsook, A. Brenton, M. E. Burczynski, C. Crean, R. Edwards, B. Gaudilliere, G.W. Hergenroeder, M. J. Iadarola, S. Iyengar, Y. Jiang, J.T. Kong, S. Mackey, C.Y. Saab, C.N. Sang, J. Scholz, M. Segerdahl, I. Tracey, C. Veasley, J. Wang, T.D. Wager, A.D. Wasan, M. A. Pelleymounter, Discovery and validation of biomarkers to aid the development of safe and effective pain therapeutics: challenges and opportunities, Nat. Rev. Neurol. 16 (2020) 381–400, https://doi.org/10.1038/s41582-020-0362-2.

[25] B. Liu, G. Cao, Z. Dong, T. Guo, Effect of microRNA-27b on cisplatin chemotherapy sensitivity of oral squamous cell carcinoma via FZD7 signaling pathway, Oncol. Lett. 18 (2019) 667–673, https://doi.org/10.3892/ol.2019.10347.

[26] M. Ashrafizadeh, A. Zarrabi, K. Hushmandi, F. Hashemi, E.R. Moghadam, M. Owrang, F. Hashemi, P. Makvandi, M.A.S.B. Goharrizi, M. Najafi, H. Khan, Lung cancer cells and their sensitivity/resistance to cisplatin chemotherapy: role of microRNAs and upstream mediators, Cell, Signal 78 (2021), 109871, https://doi.org/10.1016/j.cellsig.2020.109871.

[27] G. Li, Y. Liu, Z. Su, S. Ren, G. Zhu, Y. Tian, Y. Qiu, MicroRNA-324-3p regulates nasopharyngeal carcinoma radioresistance by directly targeting WNT2B, Eur. J. Cancer 49 (2013) 2596–2607, https://doi.org/10.1016/j.ejca.2013.03.001.

[28] T. Xu, D. Xiao, Oleuropein enhances radiation sensitivity of nasopharyngeal carcinoma by downregulating PDRG1 through HIF1α-repressed microRNA-519d, J. Exp. Clin. Cancer Res. 36 (2017) 1–10, https://doi.org/10.1186/s13046-016-0480-2.

[29] T. wardana, C. Herawati, R. Oktriani, S.L. Anwar, I. Astuti, T. Aryandono, S. M. Haryana, Over- and down-expression mir-29c and mir-21 after chemotherapy and radio- therapy in nasopharyngeal carcinomas and the down-regulating proteins encoding eipstein barr virus and c-Myc, J. Thee Med. Sci. (Berkala Ilmu Kedokteran). 48 (2016) 24–25, https://doi.org/10.19106/jmedsciesup004804201622.

[30] J.X. Zhang, D. Qian, F.W. Wang, D.Z. Liao, J.H. Wei, Z.T. Tong, J. Fu, X.X. Huang, Y.J. Liao, H.X. Deng, Y.X. Zeng, D. Xie, S.J. Mai, MicroRNA-29c enhances the sensitivities of human nasopharyngeal carcinoma to cisplatin-based chemotherapy and radiotherapy, Cancer Lett. 329 (2013) 91–98, https://doi.org/10.1016/j.canlet.2012.10.033.

[31] X. Yu, Z. Li, J. Yu, M.T.V. Chan, W.K.K. Wu, MicroRNAs predict and modulate responses to chemotherapy in colorectal cancer, Cell Prolif 48 (2015) 503–510, https://doi.org/10.1111/cpr.12202.

[32] X. Liu, H. Cai, W. Sheng, H. Huang, Z. Long, Y. Wang, MicroRNAs expression profile related with response to preoperative radiochemotherapy in patients with locally advanced gastric cancer, BMC Cancer 18 (2018) 1048, https://doi.org/10.1186/s12885-018-4967-4.

[33] S. Shin, Y. Jung, H. Uhm, M. Song, S. Son, J. Goo, C. Jeong, J.J. Song, V.N. Kim, S. Hohng, Quantification of purified endogenous miRNAs with high sensitivity and specificity, Nat. Commun. 11 (2020), https://doi.org/10.1038/s41467-020-19865-9.

[34] M. Kato, T. Paranjape, R. Ullrich, S. Nallur, E. Gillespie, K. Keane, A. Esquela-Kerscher, J.B. Weidhaas, F.J. Slack, The mir-34 microRNA is required for the DNA damage response in vivo in C. elegans and in vitro in human breast cancer cells, Oncogene 28 (2009) 2419–2424, https://doi.org/10.1038/onc.2009.106.

[35] S. Josson, S.Y. Sung, K. Lao, L.W.K. Chung, P.A.S. Johnstone, Radiation modulation of microRNA in prostate cancer Cell Lines, Prostate 68 (2008) 1599–1606, https://doi.org/10.1002/pros.20827.

[36] B. Muralidhar, L.D. Goldstein, G. Ng, D.M. Winder, R.D. Palmer, E.L. Gooding, N. L. Barbosa-Morais, G. Mukherjee, N.P. Thorne, I. Roberts, M.R. Pett, N. Coleman, Global microRNA profiles in cervical squamous cell carcinoma depend on Drosha expression levels, J. Pathol. 212 (2007) 368–377, https://doi.org/10.1002/path.2179.

[37] J. Yu, N. Li, X. Wang, H. Ren, W. Wang, S. Wang, Y. Song, Y. Liu, Y. Li, X. Zhou, A. Luo, Z. Liu, J. Jin, Circulating serum microRNA-345 correlates with unfavorable pathological response to preoperative chemoradiotherapy in locally advanced rectal cancer, Oncotarget 7 (2016) 64233–64243, https://doi.org/10.18632/oncotarget.11649.

[38] S. Ouró, C. Mourato, S. Velho, A. Cardador, M.P. Ferreira, D. Albergaria, R. E. Castro, R. Maio, C.M.P. Rodrigues, Potential of miR-21 to predict incomplete response to chemoradiotherapy in rectal adenocarcinoma, Front. Oncol. 10 (2020), https://doi.org/10.3389/fonc.2020.577653.

[39] Z.G. Yang, X.D. Ma, Z.H. He, Y. xin Guo, miR-483-5p promotes prostate cancer cell proliferation and invasion by targeting RBM5, Int. Braz J. Urol. 43 (2017) 1060–1067, https://doi.org/10.1590/S1677-5538.IBJU.2016.0595.

[40] P.S.H. Soon, L.J. Tacon, A.J. Gill, C.P. Bambach, M.S. Sywak, P.R. Campbell, M. W. Yeh, S.G. Wong, R.J. Clifton-Bligh, B.G. Robinson, S.B. Sidhu, miR-195 and miR-483-5p identified as predictors of poor prognosis in adrenocortical cancer, Clin. Cancer Res. 15 (2009) 7684–7692, https://doi.org/10.1158/1078-0432.CCR-09-1587.

[41] O. Chabre, R. Libé, G. Assie, O. Barreau, J. Bertherat, X. Bertagna, J.J. Feige, N. Cherradi, Serum miR-483-5p and miR-195 are predictive of recurrence risk in adrenocortical cancer patients, Endocr. Relat. Cancer 20 (2013) 579–594, https://doi.org/10.1530/ERC-13-0051.

[42] Q. Li, Z. Li, S. Wei, W. Wang, Z. Chen, L. Zhang, L. Chen, B. Li, G. Sun, J. Xu, Q. Li, L. Wang, Z. Xu, Y. Xia, D. Zhang, H. Xu, Z. Xu, Overexpression of miR-584-5p inhibits proliferation and induces apoptosis by targeting WW domain-containing ubiquitin protein ligase 1 in gastric cancer, J. Exp. Clin. Cancer Res. 36 (2017) 1–17, https://doi.org/10.1186/s13046-017-0532-2.

[43] X. Xu, F. Gao, J. Wang, L. Tao, J. Ye, L. Ding, W. Ji, X. Chen, MiR-122-5p inhibits cell migration and invasion in gastric cancer by down-regulating DUSP4, Cancer Biol. Ther. 19 (2018) 427–435, https://doi.org/10.1080/15384047.2018.1423925.

[44] H. Zhou, B. Xiao, F. Zhou, H. Deng, X. Zhang, Y. Lou, Z. Gong, C. Du, J. Guo, MiR-421 is a functional marker of circulating tumor cells in gastric cancer patients, Biomarkers 17 (2012) 104–110, https://doi.org/10.3109/1354750X.2011.614961.

[45] W. Zhang, H. Jiang, Y. Chen, F. Ren, Resveratrol chemosensitizes adriamycin-resistant breast cancer cells by modulating miR-122-5p, J. Cell. Biochem. 120 (2019) 16283–16292, https://doi.org/10.1002/jcb.28910.

[46] Y. Shi, X. Luo, P. Li, J. Tan, X. Wang, T. Xiang, G. Ren, MiR-7-5p suppresses cell proliferation and induces apoptosis of breast cancer cells mainly by targeting REGγ, Cancer Lett. 358 (2015) 27–36, https://doi.org/10.1016/j.canlet.2014.12.014.

[47] X. Chen, X. Xu, B. Pan, K. Zeng, M. Xu, X. Liu, B. He, Y. Pan, H. Sun, S. Wang, miR-150-5p suppresses tumor progression by targeting VEGFA in colorectal cancer, Aging (Albany. NY) 10 (2018) 3421–3437, https://doi.org/10.18632/aging.101656.

[48] W. Shi, T. Tang, X. Li, S. Deng, R. Li, Y. Wang, Y. Wang, T. Xia, Y. Zhang, K. Zen, L. Jin, Y. Pan, Methylation-mediated silencing of miR-133a-3p promotes breast cancer cell migration and stemness via miR-133a-3p/MAML1/DNMT3A positive feedback loop, J. Exp. Clin. Cancer Res. 38 (2019) 1–20, https://doi.org/10.1186/s13046-019-1400-z.

[49] X. Yu, D. Wang, X. Wang, S. Sun, Y. Zhang, S. Wang, R. Miao, X. Xu, X. Qu, CXCL12/CXCR4 promotes inflammation-driven colorectal cancer progression through activation of RhoA signaling by sponging miR-133a-3p, J. Exp. Clin. Cancer Res. 38 (2019) 1–18, https://doi.org/10.1186/s13046-018-1014-x.

[50] W.J. Miao, D.J. Yuan, G.Z. Zhang, Q. Liu, H.M. Ma, Q.Q. Jin, LncRNA CASC2/miR-18a-5p axis regulates the malignant potential of nasopharyngeal carcinoma by targeting RBBP8, Oncol. Rep. 41 (2019) 1797–1806, https://doi.org/10.3892/or.2018.6941.

[51] G. Qiao, C. Dai, Y. He, J. Shi, C. Xu, Effects of miR-106b-3p on cell proliferation and epithelial-mesenchymal transition, and targeting of ZNRF3 in esophageal squamous cell carcinoma, Int. J. Mol. Med. 43 (2019) 1817–1829, https://doi.org/10.3892/ijmm.2019.4107.

[52] C.Z. Gan, G. Li, Q.S. Luo, H.M. Li, miR-339-5p downregulation contributes to Taxol resistance in small-cell lung cancer by targeting α1,2-fucosyltransferase 1, IUBMB Life 69 (2017) 841–849, https://doi.org/10.1002/iub.1679.

Circulation_microRNA.pdf

ORIGINALITY REPORT

4%	%	4%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	"Systems Biology of MicroRNAs in Cancer", Springer Science and Business Media LLC, 2022 Publication	2%
2	Douglas F. Dluzen, Nicole Noren Hooten, Michele K. Evans. "Extracellular RNA in aging", Wiley Interdisciplinary Reviews: RNA, 2017 Publication	2%

Exclude quotes	On	Exclude matches	< 2%
Exclude bibliography	Off		