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ORIGINAL ARTICLE

Prognostic Value of MiR-934 and MiR-1260a in Plasma and Early Detection of Metastasis in Patients with Cervical Cancer

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ABSTRACT

Background: **This study investigated miR-934 and miR-1260a expression in the plasma of patients with cervical cancer (CC) and their prognostic values.**
Methods: **miR-934 and miR-1260a in the plasma of 100 CC patients, 45 patients with cervical intraepithelial neoplasia (CIN), and healthy subjects were detected by RT-qPCR method, and the relationship between miR-934 and miR-1260a expression with clinicopathological characteristics of CC patients was analyzed. Kaplan-Meier and COX hazard regression models were constructed to analyze the prognostic value of miR-934 and miR-1260a in CC.**
Results: **miR-934 and miR-1260a levels in CC patients were higher than in CIN patients and healthy subjects. Patients exhibiting high levels of miR-934 and miR-1260a experienced shorter progression-free and overall survival than those with lower levels. Clinical stage, lymph node metastasis, depth of invasion, and high miR-934 and miR-1260a were influencing factors of CC patients' prognosis.**
Conclusions: **Plasma miR-934 and miR-1260a are closely related to the clinicopathological features and prognosis of CC patients and may be potential markers to predict CC prognosis.**
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KEYWORDS

miR-934, miR-1260a, cervical cancer, prognosis

INTRODUCTION

Cervical cancer (CC) is a common malignant tumor with no obvious symptoms in the early stage, but the increasing size of the tumor will compress and invade the surrounding organs and tissues, inducing corresponding symptoms [1,2]. By the time clinical symptoms of CC become severe, the disease has often advanced to the middle and late stages, potentially missing the chance for surgical intervention and leading to poor prognoses for patients. Early surgical treatment can be performed to remove the lesion tissue, prevent tumor cells from spreading or metastasizing, and improve overall survival of patients. Therefore, it is of great significance to search for an accurate diagnosis of early-stage CC. At present, the diagnosis of CC mainly relies on cervical

cytology, colposcopy and cervical biopsy, etc. However, cervical cytology is prone to false negative results resulting in missed diagnosis. As invasive examinations, colposcopy and cervical biopsy may make patients uncomfortable and have the risk of complications. Thus, discovering new efficient markers for early detection and prognostic evaluation has emerged as a key topic in CC research [3,4].

MicroRNAs (miRNAs) have been found to play a molecular biological regulatory role in a variety of malignant tumors, or can be used as potential biological markers for the diagnosis of malignant tumors [5,6]. For example, expression of circulating miR-21 is significantly elevated in gastric cancer tissues, which may be a potential biomarker for the diagnosis of gastric cancer [7]. miR-21 has been proposed as a potential serum biomarker for diagnosing malignant tumors [8]. miRNAs are useful as cancer markers due to their specific expression and high chemical stability. miR-934 is overexpressed in colorectal cancer, pancreatic cancer, and ovarian cancer, which can promote tumor malignancy and is significantly associated with poor prognosis. MiR-934 aggravates the malignancy of gastric cancer cells by targeting ZFP36 [9], and promotes the proliferation of ovarian cancer cells while blocking apoptosis by targeting BRMS1L [10]. Plasma miR-1260a could be used as a diagnostic biomarker for epithelial ovarian cancer [11] and a non-invasive biomarker and therapeutic target [12]. In recent times, researchers have conducted high-throughput second-generation sequencing on tissues from CC patients and found that miRNAs such as miR-934 and miR-1260a are up-regulated abnormally, which are considered potential new biomarkers. Based on this, the relationship between plasma miR-934 and miR-1260a with clinicopathological characteristics and prognosis of CC patients was analyzed to assess their viability as new markers for early diagnosis and prognostic evaluation.

MATERIALS AND METHODS

Clinical data

The effect size of the difference between miR-934 and miR-1260a expression in CC patients and control population was estimated, combined with the set test level (e.g., $\alpha = 0.05$) and test efficacy (e.g., $1-\beta = 0.8$). An appropriate sample size calculation formula (such as the sample size formula for comparing two sample means) was used to ensure that the study had sufficient statistical power to detect significant differences. A total of 100 CC patients, aged 32 - 76 (47.28 ± 10.75), who received preoperative neoadjuvant chemotherapy in The First People's Hospital of Jiangxia District Wuhan City from January 2021 to February 2023, were selected.

Inclusion criteria:

1) Patients met the FIGO classification criteria and WHO classification criteria and were pathologically diagnosed with CC; 2) CC was diagnosed based on histopathological results, cytological examination and high-risk human papillomavirus (HPV) DNA detection; 3) Patients provided complete clinical data and the disease was first onset; 4) No chemotherapy, radiotherapy or immunotherapy was given before surgery; 5) Patients can cooperate with this study and provide complete medical records. The NOS Scale (Newcastle-Ottawa Scale) was used, and the risk of bias in the study was assessed.

This study was approved by the ethics committee of The First People's Hospital of Jiangxia District Wuhan City (No. WH20200423) and informed consent was signed. STARD guidelines should be followed in this study.

Exclusion criteria:

1) Patients with incomplete clinical data; 2) Patients with other malignant tumors; 3) Patients with preoperative chemoradiotherapy; 4) Patients with diabetes, hypertension, and coronary heart disease.

Selection bias:

Patients were screened in strict accordance with established inclusion/exclusion criteria to ensure that all eligible patients had an equal opportunity to be included in the study. A randomized sampling method was used to select patient population who met the criteria to avoid selection bias due to subjective factors. At the same time, a control group should be formed by selecting healthy individuals matched by age and gender, to compare the expression differences of miR-934 and miR-1260a between CC patients and healthy individuals, thereby reducing potential selection bias.

Information bias:

Standardized procedures and recording forms were developed during sample collection, testing and clinical data collection. Training was uniformly provided to all personnel in the study to ensure consistent approaches to sample collection, testing, and data recording. To avoid bias from subjective factors, testing was conducted blindly, with personnel performing miR-934 and miR-126a detection without knowledge of the patients' clinical data.

Mixed bias:

Multifactorial analysis methods such as multivariate Cox regression analysis were used to include confounding factors such as patient age, pathology type, clinical stage, and treatment method that might affect the study results. These factors were corrected to eliminate the interference of confounding bias.

Reagents

The RNA extraction kit (Qiagen) was utilized to perform RT-qPCR using ABI 7500 fluorescence instrument.

Detection methods

Fasting venous blood (5 mL) was obtained from each subject, added to coagulant tubes, and centrifuged at 4,000 r/minute for 10 minutes at 4℃ and 12,000 r/minute for 15 minutes, and serum was separated and stored at -80℃. Plasma (300 μL) and 1 mL total miRNA extraction solution were mixed. Total RNA was extracted using miRNeasy rapid Blood miRNA extraction kit. In each reaction system, the CT value was the cycle count at which the fluorescence signal met the set threshold, with U6 as the internal reference. A 20 μL reaction system was used, with 40 cycles including 95℃ for 15 minutes, 94℃ for 15 seconds, 55℃ for 30 seconds, and 70℃ for 34 seconds. Finally, the fluorescence signal was analyzed by $2^{-\Delta\Delta Ct}$ method.

Follow-up

The patients in the study group were followed up for 36 months after the operation. Their Overall Survival (OS) and Progress Free Survival (PFS) after the operation were recorded and analyzed. Among them, the follow-up endpoint event of OS was all-cause death. The follow-up endpoint events for PFS are the first tumor recurrence, metastasis or all-cause death. For patients lost to follow-up without endpoint events, the last follow-up is taken as the occurrence time of the endpoint event. For patients who have not experienced endpoint events by the end of the follow-up, the end date of the follow-up is taken as the occurrence time of the endpoint event.

Statistical analysis

Data analysis was conducted with SPSS20.0, and measurement data were presented as ($x \pm s$) and compared by one-way ANOVA for multiple datasets and SNK-q test for paired datasets. *t*-test was utilized for comparisons between two groups. Enumeration data were expressed as rates and analyzed by χ^2 test. ROC curve evaluated the diagnostic value of plasma miR-934 and miR-1260a. The DeLong test for paired ROC curves was used to compare the AUCs, and the Z-statistic with its associated p-value was calculated. Kaplan-Meier method and log-rank non-parametric test were used for survival analysis, and survival curves were drawn. Multivariate survival analysis was conducted by Cox proportional risk model. $p < 0.05$ was considered to be statistically significant.

RESULTS

Plasma miR-934 and miR-1260a

Based on the GSE81137 dataset, microarray screening was performed to identify miRNA expression profiles in CC patients. A total of 45 up-regulated miRNAs and 56 down-regulated miRNAs were identified. miR-934 and miR-1260a were significantly upregulated (Figure 1). CC patients had higher levels of plasma miR-934 and miR-1260a compared to CIN patients and healthy individuals ($p < 0.01$). No significant difference was found in plasma miR-934 and miR-1260a between CIN patients and healthy subjects ($p > 0.05$, Table 1).

Comparison of differences in serum miR-934 and miR-1260a expression in CC patients with different clinicopathologic features

There were Ib2 (56 cases), IIa (18 cases), and IIb (26 cases) according to the clinical stage of CC patients. Pathological types were divided into squamous cell carcinoma (96 cases) and adenocarcinoma (4 cases). Patients were graded pathologically as G1 (32 cases), G2 (37 cases), and G3 (31 cases). There were 20 cases with lymph node metastasis (LNM) and 80 cases without LNM. In addition, 45 age-matched patients with CIN (13 patients with CIN II grade and 32 patients with CIN III grade) were selected, and 45 healthy subjects were also enrolled.

100 CC patients were divided into miR-934 and miR-1260a high expression group ($n = 50$) and miR-934 and miR-1260a low expression group ($n = 50$) with miR-934 and miR-1260a median levels as the cutoff value. High miR-934 and miR-1260a levels were correlated with HPV positivity, clinical stage, pathological grade, LNM, and depth of invasion ($p < 0.05$), but had no significant correlation with age, menopausal status, pathological type, and tumor size ($p > 0.05$, Table 2, 3).

Correlation between plasma miR-934 and miR-1260a expression and clinicopathologic features of CC patients

Both plasma miR-934 and miR-1260a in CC patients were positively correlated with HPV, clinical stages, pathological grading, LYM, and depth of invasion ($p < 0.05$) (Table 4).

The efficacy of plasma miR-934 and miR-1260a in predicting poor prognosis in patients with CC

The ROC curves of the efficacy of miR-934 and miR-1260a in predicting poor prognosis in patients with CC were plotted and the area under the curve (AUC) was calculated. The AUCs for the prediction of poor prognosis in patients with CC by miR-934, miR-1260a and the combination of both were 0.674, 0.652 and 0.883, respectively. The predictive efficacy of miR-934 and miR-1260a in combination was better than that of their respective individual prediction ($Z = 8.143$, $p < 0.001$ and $Z = 10.561$, $p < 0.001$, respectively) (Table 5, Figure 2).

Univariate analysis and multivariate analysis of prognosis of CC patients

Univariate analysis determined that clinical stage, pathological type, pathological grading, depth of invasion, and high miR-934 and miR-1260a were correlated with CC patients' prognosis ($p < 0.05$). Furthermore, multivariate analysis was conducted on the indicators with a p-value < 0.05 in univariate analysis. Clinical stages, pathological type, pathological grading, depth of invasion, and high miR-934 and miR-1260a affected CC pa-

Table 1. miR-934 and miR-1260a in plasma of all groups.

Groups	n	miR-934	miR-1260a
Control	45	1.22 ± 0.34	0.75 ± 0.21
CIN	45	1.65 ± 0.58	1.95 ± 0.65
Cervical cancer	100	4.75 ± 1.25 *, #	8.96 ± 2.68 *, #
F		14.587	13.608
p-value		< 0.001	< 0.001

* p < 0.01 vs. control group, # p < 0.01 vs. CIN group.

Table 2. Relationship between plasma miR-934 expression and clinicopathological features in patients with cervical cancer.

Clinicopathological features	n	miR-934		X^2	p-value
		High expression group (n = 50)	Low expression group (n = 50)		
Age (years)				0.171	0.664
< 45	40	19 (47.5)	21 (52.6)		
≥ 58	60	31 (51.6)	29 (48.3)		
Menopause				0.401	0.521
Yes	38	21 (55.2)	17 (44.7)		
No	62	29 (46.7)	33 (53.2)		
HPV				6.456	0.0089
Positive	67	40 (59.7)	27 (40.2)		
Negative	33	11 (33.3)	22 (66.7)		
Clinical stages				4.134	0.038
Ib2	56	23 (41.1)	33 (58.9)		
IIa + IIb	44	27 (61.3)	17 (38.6)		
Pathological type				0.311	0.524
Squamous cell carcinoma	96	47 (48.9)	49 (51.0)		
Adenocarcinoma	4	3 (75.0)	1 (25.0)		
Pathological grading				5.423	0.015
C1 + C2	68	40 (58.8)	28 (41.1)		
C3	32	11 (34.3)	21 (65.6)		
Tumor size (cm)				1.064	0.298
≤ 4	51	23 (45.1)	28 (54.9)		
> 4	49	28 (57.1)	21 (42.8)		
Lymph node metastasis				6.456	0.008
Yes	20	15 (75.0)	5 (25.0)		
No	80	35 (43.7)	45 (56.2)		
Depth of invasion				10.786	0.001
≤ 1/2 muscle layer	30	8 (26.7)	22 (73.3)		
> 1/2 muscle layer	70	44 (62.8)	26 (37.1)		

Table 3. Relationship between plasma miR-1260a expression and clinicopathological features in patients with cervical cancer.

Clinicopathological features	n	miR-1260a		X^2	p-value
		High expression group (n = 50)	Low expression group (n = 50)		
Age (years)					
< 45	39	18 (46.2)	21 (53.8)	0.172	0.634
≥ 58	61	32 (52.4)	29 (47.5)		
Menopause					
Yes	36	20 (55.5)	16 (44.5)	0.412	0.512
No	64	30 (46.9)	34 (53.1)		
HPV					
Positive	64	38 (59.4)	26 (40.6)	6.876	0.008
Negative	36	12 (33.3)	24 (66.7)		
Clinical stages					
Ib2	56	22 (39.2)	34 (60.8)	4.343	0.038
IIa + IIb	44	27 (61.3)	17 (38.6)		
Pathological type squamous cell carcinoma					
Squamous cell carcinoma	95	47 (49.5)	48 (50.5)	0.401	0.578
Adenocarcinoma	5	3 (60.0)	2 (40.0)		
Pathological grading					
C1 + C2	67	39 (58.2)	28 (41.7)	4.987	0.012
C3	33	11 (33.3)	22 (66.7)		
Tumor size (cm)					
≤ 4	51	24 (47.1)	27 (52.9)	1.054	0.298
> 4	49	28 (57.1)	21 (42.8)		
Lymph node metastasis					
Yes	19	15 (78.9)	4 (21.1)	5.983	0.007
No	81	34 (41.9)	47 (58.1)		
Depth of invasion					
≤ 1/2 muscle layer	31	7 (22.5)	24 (77.4)	12.213	0.002
> 1/2 muscle layer	69	43 (62.3)	26 (37.6)		

Table 4. Correlation between plasma miR-934, miR-1260a expression and clinicopathologic features of cervical cancer patients.

Variables	miR-934		miR-1260a	
	r_s	p-value	r_s	p-value
HPV	0.252	< 0.0001	0.274	< 0.0001
Clinical stages	0.173	0.021	0.432	< 0.0001
Pathological grading	0.143	0.061	0.275	0.0046
Lymph node metastasis	0.463	< 0.0001	0.345	< 0.0001
Depth of invasion	0.704	0.009	00.627	0.023

Correlations between variables were tested using the Spearman's rank correlation test and expressed as Spearman's correlation coefficients. p-values of < 0.05 were considered statistically significant.

Table 5. Binary logistic regression analysis for predicting factors associated with CC.

Variables	β	S.E.	Wald	OR	95% CI	p-value
miR-934	0.026	0.009	9.08	1.027	1.008 - 1.045	0.003
miR-1260a	0.039	0.007	35.34	1.039	1.026 - 1.053	0.000

β: biased regression coefficient, S.E.: standard error of the regression coefficient, OR: odd ratio, 95% CI.: 95% confidence interval. p-values of < 0.05 were considered statistically significant.

Table 6. Cox univariate and multivariate regression analysis of overall survival of patients with cervical cancer.

Factor	Univariate analysis			Multivariate analysis		
	HR	95% CI	p	HR	95% CI	p-value
Age	0.914	0.846 - 1.002	0.302	-	-	-
HPV	0.967	0.893 - 1.048	0.184	-	-	-
Clinical stages	2.283	1.833 - 2.851	0.011	1.850	1.220 - 2.736	0.032
Pathological type	1.720	1.425 - 2.704	0.035	1.310	1.085 - 1.582	0.005
Pathological grading	1.704	1.429 - 2.703	0.036	1.586	1.217 - 2.068	0.001
Lymph node metastasis	0.890	0.814 - 0.978	0.643	-	-	-
Depth of invasion	2.015	1.675 - 2.432	0.022	1.415	1.180 - 1.703	0.042
miR-934	4.162	2.850 - 5.983	0.001	3.052	2.202 - 4.235	0.005
miR-1260a	3.815	2.632 - 5.127	0.001	2.180	1.758 - 2.893	0.015

Clinical stages, pathological type, pathological grading, depth of invasion, miR-934, and miR-1260a were used for multivariate analysis.

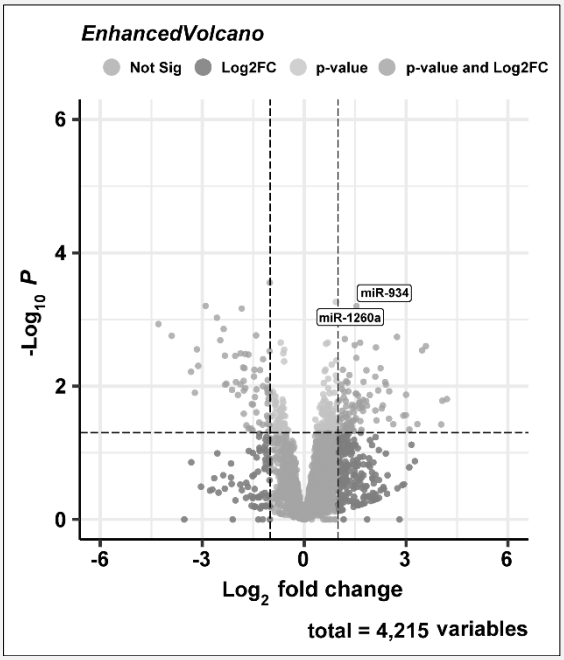

Figure 1. Microarray screening of miRNAs in CC.

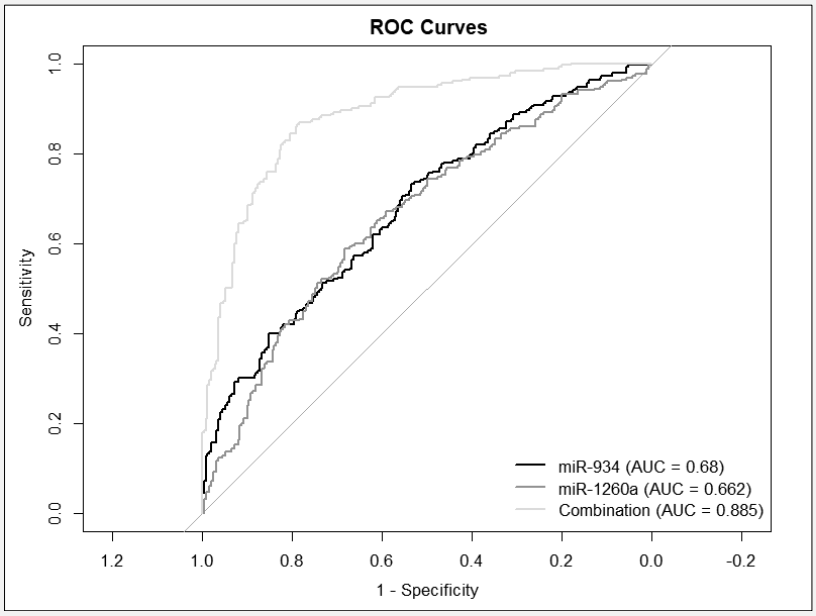

Figure 2. ROC curves of plasma miR-934 and miR-1260a for predicting poor prognosis in patients with CC.

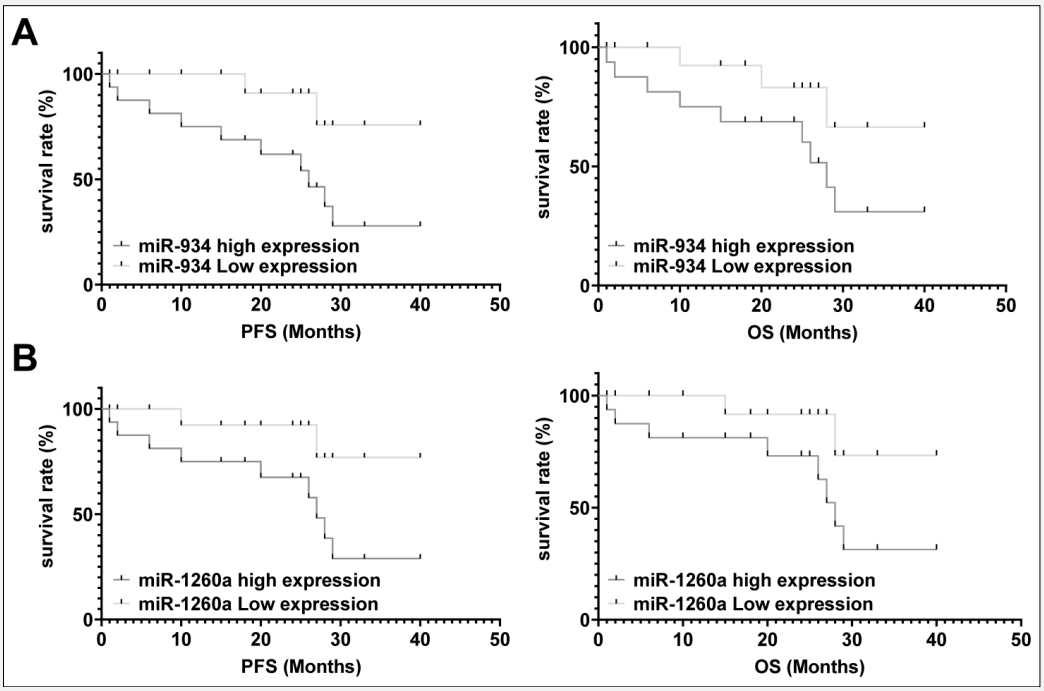

Figure 3A - B. PFS and OS of CC patients with high and low miR-934 and miR-1260a expression.

tients' prognosis (Table 6).

Relationship between plasma miR-934 and miR-1260a and prognosis in CC patients

Among CC patients, 2 cases were lost to follow-up, leading to 98% follow-up rate. Kaplan-Meier survival analysis showed that progression-free survival (PFS) and overall survival (OS) of patients with high miR-934 and miR-1260a were 55.2 ± 2.4 months and 57.92 ± 2.1 months, respectively, which was shorter than those of patients with low miR-934 and miR-1260a (63.56 ± 2.1 months, 65.54 ± 2.2 months) ($p < 0.01$, Figure 3A - B).

DISCUSSION

Globally, more than 200,000 women die from CC each year [13]. Each year, 131,500 new cases are found in China [14]. The invasion of CC cells can occur directly into surrounding tissues and organs, reaching the fornix of the vaginal wall and the uterine body, spreading sideways into pelvic tissue, and spreading forward into the bladder [15]. They can also metastasize through lymphatic vessels to the paracervical, internal iliac, external iliac, inguinal lymph nodes, and even to supraclavicular throughout the body. When the symptoms of CC appear three months later, two-thirds of patients are already in an advanced stage [16].

As a class of endogenous non-coding small RNA molecules, miRNAs are widely involved in various physiological processes such as cell proliferation, differentiation and apoptosis, and are closely related to the occurrence and development of tumors. They are expected to become a new tool for tumor diagnosis, classification and prognosis [17-19]. miR-362-3p inhibits CC migration and invasion [20]. miR-205-5p, miR-195-5p, and VEGF-A in human CC are related to venous thromboembolism [21]. Previous investigations into miR-934's role in cancer have generally pointed to miR-934 being an oncogene. miR-934, acting as an intronic miRNA of VGLL1, shows expression patterns consistent with VGLL1 in estrogen receptor-negative breast cancer [22]. Alcohol-induced dysregulation of miR-934 activates BCL-2 and promotes cell growth in head and neck squamous cell carcinoma [23]. miR-934 promotes bladder cancer cell growth and xenograft tumor growth by binding to UBE2N [24]. Advanced stages of pancreatic cancer show higher miR-934 levels, with the miR-934/PROX1 axis regulating cellular migration and invasion [25]. Moreover, miR-934 is increased in gastric cancer and enhances the tumorigenic activity of gastric cancer cells by suppressing its target gene ZFP36 [26]. Plasma miR-934 is abnormally expressed in malignant tumors, which is a potential biological indicator for early noninvasive diagnosis and postoperative monitoring [27,28]. Plasma miR-1260a is a diagnostic biomarker for epithelial ovarian cancer and metastatic melanoma [12, 29]. It has also been reported that miR-1260a enhances the proliferation, migration and invasion of chordoma cells [30]. Exosome miR-1260 may be a useful diagnostic and prognostic biomarker for epithelial ovarian cancer [31]. miR-1260a is differentially expressed in plasma post-surgical resection of metastatic melanoma and functions as a noninvasive biomarker for the disease [32]. Although miR-934 and miR-1260 have been studied in various common tumors, their role in CC has not been reported. This study showed that plasma miR-934 and miR-1260a were elevated in CC patients. It has been accepted that changes in miRNA expression profiles are associated with CC tumor staging, and detection of miRNA changes in CC patients provides diagnostic values for CC [33]. miRNAs can recognize specific target mRNAs. Tumor development is influenced by multiple factors and genes, among which the invasion and metastasis abilities of tumor cells are of great importance [34]. Single factor analysis determined that clinical stage, pathological type, pathological grading, LNM, invasion depth, and high miR-934 and miR-1260a were correlated with CC patients' prognosis. It suggests that miR-934 and miR-1260a have oncogenic roles in CC and are prognostic markers for CC. It has also been validated that miRNAs are closely associated with pathological processes such as metastasis and are considered tumor oncogenes or suppressor genes [35]. The dysregulation of miRNAs in CC is significantly associated with its onset, progression, metastasis, and prognosis, serving as potential biomarkers for early diagnosis and prognosis [36]. This study found that PFS and OS of patients with high miR-934 and miR-1260a were shorter. In addition, clinical stage, LNM, invasion depth, and high miR-934 and miR-1260a affected CC patients' prognosis. LNM is the main prognostic factor of CC, and the 5-year survival rate of patients with LNM is only 65%, while that of patients without LNM is as high as 90% [37]. Another study found that the 5-year survival rate of stage I/II CC patients with LNM was 63.4%, while the 5-year survival rate of patients without LNM was 100% [38]. This study determined that plasma miR-934 and miR-1260a in patients with LNM were significantly higher, suggesting that miR-934 and miR-1260a may be characteristic markers of CC with LNM. High miR-1260a is related to tumor progression and poor prognosis, and is a reliable indicator for monitoring patients' disease progression and survival time [39].

The current standard methods for assessing CC condition and prognosis, including CA153 and TNM staging, are limited by their clinical applicability, generalizability, and temporal and spatial limitations of gene expression. As a result, it is commonly needed to integrate multiple indexes to increase their efficacy [40]. Gene markers are currently found to be more valuable and cost-effective in CC clinical applications compared to imaging tests [41]. Additionally, research confirms that combining various indicators can efficiently overcome the shortcomings of sensitivity and specificity without complicating or increasing the cost of detection [42,43]. In this study, the efficacy of miR-934 combined with

miR-1260a in predicting the poor prognosis of CC patients was significantly higher than that of miR-934 and miR-1260a alone. The findings imply that the combined use of the two assays offers more clinical value, suggesting that miR-934 and miR-1260a should be utilized together in clinical settings to enhance the sensitivity and specificity of miR-934 and miR-1260a.

Plasma miR-934 and miR-1260a exhibit a significant association with HPV positivity, clinical stage, pathological grading, LNM, and depth of invasion in patients with CC. These miRNAs are identified as independent risk factors for poor prognosis in CC patients. Nonetheless, their precise roles require further elucidation. This study corroborates that elevated expression levels of miR-934 and miR-1260a in CC patients are linked to various clinical characteristics. However, the conclusions drawn are subject to limitations and necessitate confirmation through expanded clinical datasets and rigorous basic research trials. Additional evidence is required to elucidate the association between key signaling pathways and miR-934 and miR-1260a. The findings of this study hold substantial potential for clinical application, as miR-934 and miR-1260a may aid in the clinical diagnosis of CC, inform treatment strategies, and emerge as novel therapeutic targets. Consequently, miR-934 and miR-1260a are anticipated to serve as potential prognostic biomarkers and therapeutic targets for CC.

Availability of data and materials:

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Ethics statement:

The present study was approved by the Ethics and Scientific Committees of The First People's Hospital of Jiangxia District Wuhan City (No. WH20200423) and performed in accordance with The Declaration of Helsinki. Written informed consent was obtained from all patients prior to the study start.

Declaration of Interest:

The authors have no conflicts of interest to declare.

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