Profiling Plasma MicroRNA in Nasopharyngeal Carcinoma with Deep Sequencing

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BACKGROUND: The goal of this study was to establish a plasma microRNA profile by use of next-generation sequencing that could aid in assessment of patient prognosis in nasopharyngeal carcinoma (NPC).

METHODS: Two panels of NPC patients and healthy controls (HCs) were recruited for this study. We used deep sequencing to screen plasma microRNAs. Differentially expressed microRNAs were verified by quantitative real-time PCR (qPCR). Kaplan–Meier survival analysis with the log-rank test was used to compare overall survival (OS) and progression-free survival (PFS) between groups.

RESULTS: Twenty-three plasma miRNAs with differential expression levels were selected for qPCR analysis on an independent set including 100 NPC patients and 55 HCs. NPC patients with low concentrations of miR-483–5p and miR-103 had better prognosis for 5-year OS than those with high concentrations (87.5% vs 55.8%, P < 0.001; 89.9% vs 62.3%, P = 0.031). Those with low concentrations of miR-29a and let-7c had poorer prognosis (54.8% vs 82.8%, P = 0.002; 56.3% vs 84.6%, P = 0.001). A 3-signature miRNA integrated with clinical stage was further identified in an independent set. We calculated a prognostic index score and classified patients into low-, medium-, and high-risk groups. Five-year OS among the 3 groups was significantly different (90.9%, 66.7%, and 35.0%, P < 0.001). By multivariate analysis, a high-risk index score was the most significantly unfavorable prognostic factor independent of other clinical variables (P < 0.001, hazard ratio = 15.1, 95% CI = 5.2–43.9).

CONCLUSIONS: Differentially expressed plasma miRNAs as identified by next-generation sequencing can be helpful for predicting survival in NPC patients.

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Nasopharyngeal carcinoma (NPC)5 is often diagnosed at a late stage with concomitant poor prognosis (1–2). Despite large improvements in NPC treatment, management of this disease in its advanced stages is still difficult, and 5-year survival rates range from 22% to 50% (3). Although tumor biomarkers provide useful information for diagnosis and prognosis, assessment of tumor biomarkers is invasive and inconvenient, thus limiting their application (2). Minimally invasive biomarkers are urgently needed for evaluating prognosis and therapeutic response for malignancy. Recently, circulating microRNAs (miRNAs), which are very stable and RNase-resistant, have been described as minimally invasive potential tools for the early detection of disease and prediction of outcomes (4).

miRNAs are endogenous, noncoding RNAs of 19–25 nucleotides that are thought to regulate gene expression by targeting mRNAs for cleavage or translational repression (5). They are frequently deregulated in cancer and could constitute a promising group of potential diagnostic and prognostic markers due to their stability and ease of detection by techniques such as quantitative real-time PCR (qPCR) in body fluids and formalin-fixed paraffin-embedded (FFPE) tissues (6–7). Specifically, several studies have assessed miRNA expression through microarray or qPCR analysis and demonstrated that many miRNAs are deregulated in NPC tissue samples (8–9). However, little information is known regarding the systematic analysis of plasma miRNA profiles in NPC patients.

Advances in sequencing technologies have enabled the rapid identification of miRNA alterations in many diseases, and these can be used to design customized assays for monitoring disease progression. Various investigators have used miRNA microarrays to monitor disease outcomes in limited numbers of patients.
with various solid cancers, but few cases of NPC have been analyzed (10–11). The prospect of using plasma miRNAs is of great interest to both identify predisposition to cancer and guide medication for NPC patients.

Here, we describe the differential expression of miRNAs in 50 NPC patients and 50 healthy controls (HCs) using next-generation sequencing technology, as well as the development of a risk model to predict prognosis in an independent panel of 100 NPC patients.

Materials and Methods

STUDY PARTICIPANTS
All samples were obtained from confirmed cases of NPC at the time of diagnosis at the Sun Yat-sen University Cancer Center (SYSUCC) (Guangzhou, China). The first study (study 1) included 50 NPC patients and 50 HCs consecutively between January 2009 and April 2009 for screening plasma miRNAs. For the second study (study 2), we used randomly selected samples between June 2000 and May 2004 from 100 NPC patients and 55 HCs to validate miRNA expression at SYSUCC. HCs were individuals who came in for a routine physical examination and were found to be cancer-free. The characteristics of the 100 NPC patients are summarized in Table 1. The inclusion criteria for the study were a first-time diagnosis of NPC with no history of other tumors and a follow-up time of >5 years at the completion of this study. Informed consent was obtained for all participants. This study was approved by the medical research ethics committee of SYSUCC.

PLASMA COLLECTION AND RNA EXTRACTION
Whole blood samples were collected from all participants into tubes containing EDTA. Plasma was prepared by centrifugation of the samples at 1400 g for 10 min and stored at −80 °C until use. We extracted total RNA using TRizol (Invitrogen) following the manufacturer’s recommendations. More detailed information is provided in the Supplemental Data, which accompanies the online version of this article at http://www.clinchem.org/content/vol60/issue5.

SMALL RNA LIBRARY CONSTRUCTION AND SEQUENCING
We created 2 small RNA libraries according to the protocol published by Morin et al. (12). To fully investigate the plasma miRNA profile between NPC patients and HCs, sequencing was performed on the Illumina HiSeq 2000 platform [Beijing Genomics Institute (BGI)]. More details are provided in the online Supplemental Data.

Table 1. Clinicopathological features and follow-up data for 100 NPC patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>NPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>44</td>
</tr>
<tr>
<td>Median</td>
<td>44</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>44 (22–85)</td>
</tr>
<tr>
<td>Follow-up, months</td>
<td>86</td>
</tr>
<tr>
<td>Median</td>
<td>77</td>
</tr>
<tr>
<td>Mean (range)</td>
<td>77 (4–114)</td>
</tr>
<tr>
<td>Sex</td>
<td>69 (69)</td>
</tr>
<tr>
<td>Male</td>
<td>31 (31)</td>
</tr>
<tr>
<td>Female</td>
<td>31 (31)</td>
</tr>
<tr>
<td>WHO histopathological typeb</td>
<td>3 (3)</td>
</tr>
<tr>
<td>KSCCc</td>
<td>8 (8)</td>
</tr>
<tr>
<td>NKDC</td>
<td>89 (89)</td>
</tr>
<tr>
<td>NKUC</td>
<td>89 (89)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Early</td>
<td>78 (78)</td>
</tr>
<tr>
<td>Late</td>
<td>78 (78)</td>
</tr>
<tr>
<td>Treatment regimen</td>
<td>51 (51)</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>36 (36)</td>
</tr>
<tr>
<td>Radio-chemotherapy</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Chemotherapy</td>
<td>12 (12)</td>
</tr>
<tr>
<td>5-year OS rate, %</td>
<td>0.71</td>
</tr>
</tbody>
</table>

a Data are n (%) unless noted otherwise. 
b The histological grade of each NPC case was independently confirmed by 2 pathologists based on WHO classification. 
c NKUC, nonkeratinizing undifferentiated carcinoma; NKDC, nonkeratinizing differentiated carcinoma; KSCC, keratinizing squamous cell carcinoma.

BIOINFORMATICS ANALYSIS OF HIGH-THROUGHPUT DATA
Detailed information is provided in the online Supplemental Data.

ANALYSIS OF DIFFERENTIAL miRNA EXPRESSION
To identify miRNAs differentially expressed between NPC patients and HCs, we used the Bayesian method developed by Audic and Claverie (13). miRNAs displaying at least a 2-fold change difference between the 2 groups were selected for further investigation. A detailed description of this procedure is shown in the online Supplemental Data.

MATURE miRNA qPCR
In study 2, we performed qPCR to validate 23 mature miRNAs screened by sequencing with TaqMan MicroRNA assays (Applied Biosystems) (see online Supplemental Table 1). The detailed method is described in the online Supplemental Data.
Plasma miRNA Profiling in NPC

SELECTION OF CUTOFF SCORES
For each miRNA expression profile, we plotted the sensitivity and specificity of each outcome as a predictor of death for NPC with ROC curve analysis. We used ROC curves as an accuracy index for assessing the predictive power as well as evaluating the diagnostic performance of each variable. We used the curves to select cutoff scores for dichotomizing each predictor according to the maximum area under the ROC curve [score nearest to point on curve (0.0, 1.0) with maximum sensitivity and specificity]. The cutoff scores were generated with MedCalc software, version 12.2.1.

STATISTICAL ANALYSIS
To compare the sequencing data of the NPC patients and HCs, we used the Wilcoxon–Mann–Whitney test. The levels of all miRNAs with significance values <0.05 were considered statistically significant. We performed qPCR in 3 different experimental settings. The statistical analysis of miRNA concentrations by qPCR method was performed by independent-samples t-test (2-sided) with Welch correction. Hierarchical clustering analysis was performed on log2 ratio data in NPC and HC samples to choose the most significant miRNAs. We applied 2-tailed Spearman correlation to generate heat maps of the distance matrix for both miRNAs and samples by use of a hierarchical clustering algorithm on the basis of average linkage (14–15).

We defined overall survival (OS) and progression-free survival (PFS) as the period between the date of diagnosis and recurrence/metastasis, death, or last day of follow-up. Survival curves were estimated by use of the Kaplan–Meier method with the log-rank test. We used a multivariate Cox regression analysis to investigate whether the selected miRNAs were independent prognostic factors of OS and PFS in NPC patients.

Penalized maximum likelihood estimation was applied to select the optimal variables to establish a multivariate risk model for evaluating NPC patient outcome. To evaluate the joint efficacy of this multivariate risk model, we introduced a prognostic index (PI). In general, we defined the PI score in terms of the logistic regression model: $PI = \logit \{Y = 1/X\} = \beta_0 + \Sigma \beta_i \cdot |X_i| (16)$, where $Y$ is a binary outcome variable (0 or 1), $\beta_0$ is an intercept, and $\beta_i$ denotes the regression coefficients associated with the design matrix $X$ of covariates $i$. Specifically, in our study we calculated the PI score according to the formula: $PI = \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4$, where $\beta$ is a variable assignment indicated in online Supplemental Table 2. A ROC curve was generated for further analysis of the prognostic value of PI. We used the maximum Youden index to obtain optimal cutoff values for $PI_{OS}$ and $PI_{PFS}$ for prognostic assessment in NPC patients. Subsequently, ROC curve analyses for each subgroup were applied to calculate the sensitivity and specificity of PI.

Two-sided $P$ values of <0.05 were considered to indicate statistical significance. Statistical analyses were carried out by use of SPSS 17.0.

RESULTS
HIGH-THROUGHPUT SEQUENCING OF SMALL RNAs FROM NPC PATIENTS AND HCs
To derive optimal plasma miRNA profiles, we carried out high-throughput next-generation sequencing of 50 NPC patient samples and 50 HC samples. All annotated small RNAs are explained in online Supplemental Fig. 1. A total of 12 993 179 and 13 774 412 reads were sequenced from 2 small RNA libraries constructed from HCs and NPC samples, respectively. From HCs, 11 324 354 clean reads were obtained, which accounted for 87.2%, whereas 11 996 851 clean reads were obtained for NPC samples. We also sought to obtain the sequences of small RNAs shared between the 2 groups (see online Supplemental Fig. 2, A and B). To discover unknown miRNAs that have previously not been included in the miRBase v17, we took advantage of the Mireap program developed by BGI (data not shown).

DIFFERENTIALLY EXPRESSED miRNAs IN PLASMA
A total of 146 known human miRNAs were found by use of the Illumina/HiSeq2000 platform in study 1 (see online Supplemental Table 3). Following BLASTN searches and further sequence analysis, 13 miRNAs were found that were unique to NPC patients and 48 miRNAs for HC. In the plasma of NPC patients and HCs, 85 miRNAs coexisted (Fig. 1, A and B) that had previously been deposited in the miRBase database. Clustering analysis was applied to yield a heat map with a clear distinction between NPC patients and HCs. On the basis of our criteria, 92 miRNAs were identified with differential expression between NPC patients and HCs (Fig. 1C, left panel). Ultimately, only 23 plasma miRNAs with higher read counts were retained for further consideration in study 2 (Fig. 1C, right panel).

CORRELATION OF DIFFERENTIALLY EXPRESSED PLASMA miRNAs AND NPC PATIENT PROGNOSIS
To better understand the relationship between the 23 plasma miRNAs and patient survival, 100 NPC patients were enrolled with complete follow-up data. With Kaplan–Meier analysis, miR-483-5p, miR-103, miR-29a, and let-7c were measured by qPCR and eventually confirmed to be associated with NPC patient prognosis and disease progression, whereas the remaining miRNAs were not found to be significantly predictive for NPC patient outcomes. The concentrations of
miR-483-5p and miR-103 in plasma were higher in NPC than in HCs as determined by qPCR, whereas those of miR-29a and let-7c were lower in NPC, which was not consistent with sequencing results (see online Supplemental Fig. 3). Accordingly, we used qPCR to test the concentrations of the 4 significant miRNAs in 8 NPC and 8 nasopharyngitis FFPE tissue samples, respectively. The concordant results are shown in online Supplemental Fig. 4.

Specifically, NPC patients with low concentrations of miR-483-5p and miR-103 had higher 5-year OS rates than those with high concentrations of these miRNAs (82.8% vs 54.8%, \(P = 0.002\); 84.6% vs 56.2%, \(P = 0.001\)) (Fig. 2, C and D), respectively. The relationship between PFS rates and miRNA concentrations is shown in online Supplemental Fig. 5. A univariate Cox proportional hazard regression model indicated that 6 variables including DNase, clinical stage, miR-483-5p, miR-103, miR-29a, and let-7c were correlated with the OS of NPC patients (Table 2). Subsequently, miR-483-5p, miR-103, and miR-29a were independently correlated with the OS of NPC patients by means of a multivariate analysis (Table 2). The impact of clinical variables integrated with the 4 miRNA concentrations on NPC patients’ susceptibility to progression is summarized in online Supplemental Table 4. Taken together, these findings suggest that miR-483-5p,
miR-103, and miR-29a are independent prognostic factors for NPC patients.

**DYNAMIC CHANGES OF PLASMA miRNA BETWEEN NPC PATIENTS AND HCs**

With respect to the diagnostic value of the 4 miRNAs, an ROC curve was used to distinguish NPC patients from HCs in study 2. The areas under the curve (AUCs) for NPC diagnosis determined from miR-483-5p, miR-103, miR-29a, and let-7c were 0.74 (95% CI 0.65–0.83), 0.71 (0.63–0.79), 0.76 (0.69–0.84), and 0.71 (0.62–0.79) (all $P < 0.001$), respectively (Fig. 3, A–D). The 4 miRNAs had potential utility for diagnosis of NPC since the AUCs were >0.5. To better understand the dynamic changes of the 4 miRNAs in relation to diagnosis, we performed a box-plot analysis among HCs ($n = 55$) and NPC patients in early stage ($n = 22$) and advanced stage ($n = 78$). The concentrations of miR-483–5p in NPC patients in early and advanced stages were increased 8.28- and 8.3-fold, respectively, compared with HCs ($P = 0.002$, $P < 0.001$) (Fig. 3E). Similarly, NPC patients in early and advanced stages in comparison to HCs had increased concentrations of miR-103, 3.3- and 4.0-fold higher ($P = 0.011$, $P < 0.001$) (Fig. 3F). In contrast, the concentrations of miR-29a in early and advanced NPC were lower than in HCs (3.1-fold, $P = 0.006$; 4.9-fold, $P < 0.001$, respectively) (Fig. 3G). The concentrations of let-7c in advanced NPC were lower than in HCs (4.4-fold, $P < 0.001$), whereas concentrations in early NPC in comparison to HCs displayed no significant differences (Fig. 3H). In addition, none of the 4 miRNAs showed differences in concentrations between NPC patients in early and advanced stages (all $P > 0.05$) (Fig. 3E–H). These findings indicate that miR-483–5p, miR-103, miR-29a, and let-7c have good value for diagnosis, specifically for the first 3 miRNAs in early event detection ability, whereas let-7c is expressed in advanced NPC compared to HCs.

**RISK MODEL FOR PREDICTING SURVIVAL IN NPC PATIENTS**

Finally, 4 variables, including miR-483-5p, miR-103, miR-29a, and clinical stage, were analyzed in a multivariate risk model. The scores were calculated by the following equations: $PI_{OS} = 1.47(0$ or $1) + 1.14(0$ or $1) + 1.80(0$ or $1) + 1.84(0$ or $1)$; $PI_{PFS} = 1.07(0$ or $1) + 1.04(0$ or $1) + 1.6(0$ or $1) + 1.59(0$ or $1)$. The $PI_{OS}$ score had a range of 0–6.25, and the $PI_{PFS}$ score had a range of 0–5.3. Consequently, patients with low risk had OS/PFS-predictor scores of $<3.64/3.19$; those in
the medium-risk group, 3.64–4.78/3.19–3.70; those in the high-risk group, >4.78/3.70. The AUCs were compared in the analysis of sensitivity and specificity of survival prediction. The AUCs of PIOS and PIPFS were 0.87 (95% CI 0.80–0.95; P = 0.001) (Fig. 4A) and 0.84 (0.76–0.92; P < 0.001) (Fig. 4B), respectively. The PI showed a better survival prediction than the other clinical characteristics, including clinical stage. NPC patients in the low-risk group had significantly longer 5-year OS (90.9%) and 5-year PFS (83.6%) than those in the medium-risk group (66.7% and 58.2%, respectively) and the high-risk group (23.8% and 9.5%) (P < 0.001) (Fig. 4, C and D). Multivariate analysis also demonstrated that a high-risk group assignment was the most significantly unfavorable prognostic factor independent of other clinical variables (hazard ratio 15.1, 95% CI 5.2–43.9, P < 0.001). Likewise, PI was also an independent factor in evaluating PFS in NPC patients.

**Discussion**

To the best of our knowledge, this is the first study to characterize plasma small RNAs between NPC patients and HCs by use of the Illumina sequencing platform. We identified miR-483-5p, miR-103, miR-29a, and
let-7c as biomarkers associated with survival and progression in NPC patients. Furthermore, the combination of miR-483-5p, miR-103, and miR-29a with clinical stage was used to evaluate prognosis and progression with a PI score. Patients with a high-risk score had increased cancer progression and shortened survival. These findings suggest that miRNAs in plasma obtained in a minimally invasive manner may play an important role in the prediction of clinical cancer progression and prognosis of NPC.

Several publications suggest significant roles for miRNA signatures in the evaluation of cancer prognosis risk and diagnosis, such as lung cancer (17–19). Importantly, clinical stage is still a key prognostic determinant in routine clinical practice, although stage is categorized mainly with anatomical knowledge. Accordingly, an miRNA profile could complement biological information to elucidate the differences among NPC patients at the same clinical stage with much higher sensitivity and specificity (20–21). We compared our results with those of Liu et al. (21), who constructed a 5-miRNA signature and clinical stage correlation in NPC FFPE tissues, by use of a microarray containing 873 miRNA probes. They found 41 miRNAs differentially expressed between NPC and controls. In contrast, we obtained detailed plasma miRNA profiles of NPC patients and HCs including 146 human known miRNAs, 51 of 132 novel miRNAs, and 95 Epstein-Barr virus (EBV)-related novel miRNAs (data not shown) by use of the Illumina sequencing platform. Finally, 3 miRNAs integrated with clinical stage were further analyzed to perform miRNA profiling for the prediction of NPC patient outcome.

In comparison to microarray data, the use of the Illumina/HiSeq 2000 enables high-throughput sequence data and generates shorter sequence reads (typically 36 nucleotides in length). Thus, the sequencing could be focused on both discovering novel miRNAs and acquiring a highly quantitative estimate of known individual miRNA species (22). It is well known that highly stable miRNAs exist in clinical samples such as archival FFPE tissues and plasma. However, pathologic examination requires an invasive biopsy that is painful and cannot be repeated easily. Developing minimally invasive methods by integrating the recent advances in the field of miRNAs for diagnosis and prognosis of NPC is of great interest. Owing to the simplicity and reproducibility of obtaining a blood sample, the concentration of detectable miRNAs in plasma seems to be a logical way to monitor NPC patient outcome. Thus, a plasma miRNA signature in NPC patients, as shown in the present study, might be of great clinical interest as a routine testing procedure.

**Fig. 3.** ROC curves showing comparison of the sensitivity and specificity for NPC diagnosis by 4 miRNAs, with box plots representing dynamic expression changes of plasma miRNAs between HCs and NPC patients. AUCs of miR-483-5p (A), miR-103 (B), miR-29a (C), and let-7c (D) were analyzed with ROC curves indicating the potential value of the 4 miRNAs to identify NPC. t-Tests using independent samples were used to compare plasma miRNA concentrations of miR-483-5p (E), miR-103 (F), miR-29a (G), and let-7c (H) among the groups of HC (n = 55), early-stage NPC (n = 22), and late-stage NPC (n = 78), respectively.
We were unable to examine the miRNAs identified in our study with the data set of Liu et al. (21) because their data set is not available publicly. We identified a total of 4 miRNAs that were associated with OS and PFS in patients with NPC. The high concentrations of miR-483-5p and miR-103 in our study were related to poor survival outcome in NPC patients. miR-483-5p has recently been shown to accumulate in tumor cells of patients with Wilms tumors stratified as high risk (23) and was found to be upregulated in adrenocortical carcinoma compared with benign tumors (24). The high concentration of miR-103 in bladder, breast, and esophageal cancers was related to worse prognosis (25–27). The concordance of miR-483-5p and miR-103 expression suggests that they may be biomarkers related to shortened survival. On the contrary, miR-29a and let-7c concentrations were shown to be significantly lower in NPC patients. Previous studies revealed that miR-29a/b/c family members are widely downregulated and considered to be tumor suppressor genes in solid tumors, such as liver and lung cancer, mantel cell lymphoma, and brain tumors (28–29). It is noteworthy that miR-29a played the role of sustaining the epithelial polarity coordinated with suppression of tristetraprolin expression (30). This may be one of the reasons that miR-29a had lower levels of expression in NPC and was related to poor outcome in our study. Prior reports showed that the let family members were expressed in low levels in NPC and probably acted to downregulate c-Myc expression to inhibit the proliferation of NPC cells (31–32).

Logistic regression with penalized estimates may be used to develop prognostic models for binary outcomes, especially when limited data are available. Discrimination, calibration, and overall performance were taken into account in the construction of models (33).

Fig. 4. Comparisons of the sensitivity and specificity for NPC prediction of survival by PIOS (PI_OS), PIFS (PI_PFS), clinical variables, and miRNAs, with Kaplan–Meier curves of OS and PFS for NPC patients with subtypes. AUCs of PIOS, PI_PFS, clinical variables, and miRNAs for OS (A) and PFS (B) were analyzed with ROC curves. OS (C) and PFS (D) were calculated in 100 NPC patients subdivided by Pi into low-, medium-, and high-risk subtypes. Log-rank test was used to calculate P value.
ROC curve analysis was used to quantify a concordance statistic (c) in logistic regression (34). In our study, the PI, comprising 3 miRNA signatures and clinical stage, divided NPC patients into 3 groups related to survival. Such information not only may improve the predictive performance of the model, but also strengthens its clinical credibility. The risk model was applied successfully to predict survival in lung cancer (19) and patients with diffuse large B-cell lymphoma (35). Clinicians may be more inclined to use this model rather than an invasive method to predict outcomes and administer personalized treatments.

It is well known that EBV viral capsid antigen/immunoglobulin A (VCA/IgA) and early antigen/immunoglobulin A (EA/IgA) are used to screen for NPC in clinical practice. To compare the sensitivity and specificity of VCA/IgA and EA/IgA with the miRNA markers, further investigations are needed to fully understand their roles for diagnosis. This raises the prospect that the expression of miRNAs in plasma combined with either EBV VCA/IgA or EA/IgA might provide the diagnostic model with high sensitivity and specificity for NPC patients.

Our study, although interesting and relevant, has limited generalizability, since all samples were enrolled in a single center, SYUCCC. The plasma miRNA signature identified needs to be evaluated and validated in a multicenter trial of independent samples. Once validated, the plasma miRNA profile could be used to develop a clinical test. The precise mechanisms underlying the prognostic value of the risk model remain unclear, and further study on miRNA targets might provide better understanding of the roles of miRNAs in the development and progression of NPC.

In summary, we have shown that distinct plasma miRNA profiles exist in NPC patients compared with HCs. Additionally, a 3-miRNA signature integrated with clinical stage may be a robust predictor of prognosis in this disease. We acknowledge that a multicenter, prospective study is necessary to confirm our findings regarding this minimally invasive approach, which may lead to personalized therapy in the future.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


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