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Development of a 3-microRNA signature and nomogram for predicting the survival of patients with uveal melanoma

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Abstract

Background The aim of this study was to develop a robust miRNA signature and construct a nomogram model associated with uveal melanoma (UM) to improve prognosis prediction.

Methods MiRNA and mRNA sequencing data of 80 uveal melanoma samples were downloaded from The Cancer Genome Atlas (TCGA) database. The patients were further randomly assigned to a training set (n = 40, used to identify key miRNAs) and a testing set (n = 40, used to internal verify the signature). Then miRNAs data of GSE84976 and GSE68828 were downloaded from Gene Expression Omniniub (GEO) database for outside verification. Combining univariate analysis and LASSO methods for identifying a robust miRNA biomarker in training set and the signature was validated in testing set and outside dataset. A prognostic nomogram was constructed and decision curve analyses and reduction curve analyses were performed to evaluate the clinical usefulness of the nomogram. A miRNA-mRNA network in UM was constructed and pathway enrichment of these miRNAs was conducted based on the genes in network.

Results In total, a 3-miRNA was identified and validated which can robustly predict UM patient's survival. According to univariate and multivariate analyses, age at diagnosis, tumor node metastasis (TNM) classification, stage and 3-miRNA signature significantly correlated with the survival outcomes. These characteristics were used to establish nomogram. The nomogram showed good accuracies in predicting 1 and 3 years overall survival and the decision curve revealed the clinical usefulness of our nomogram. What's more, a miRNA-mRNA network was constructed. Pathway enrichment showed that this network was largely take part in mRNA processing, mRNA surveillance pathway, spliceosome and so no.

Conclusions We developed a 3-miRNA biomarker and constructed a prognostic nomogram, which afforded a quantitative tool for predicting the survival of UM. Our finding also provided some new potential targets for the treatment of UM.

1. Introduction

Uveal melanoma (UM) is a highly aggressive form of ocular tumor in adult, which usually derives from uveal melanocytes. Despite the incidence of UM is one thousandth of 0.06-0.07, around 50% of UM patients will die from metastases[1, 2]. The liver, the lung and soft tissues are the most frequent metastatic sites. Currently, radiotherapy, chemotherapy and enucleation were widely used for treatment of UM and prevent tumor recurrence. However, there are no effective therapies for metastatic UM and the five-year survival rate of metastatic UM is very low[3, 4]. Furthermore, the best treatment remains uncertain and the mechanisms underlying the prognosis of UM are not well illustrated. Therefore, identification of novel prognostic factors for therapy targets and clarify the survival events of UM are important.

Studies of mammalian transcriptional sequences indicated that only 1.5% of the human genome encoding protein. While about 70% of human genome is the non-coding RNAs[5]. MicroRNA is a class of noncoding RNA molecule, which at length of 19–25 nucleotides. They take part in regulating the post-transcriptional expression of target mRNAs[6]. Recently, there are increasing evidences manifested that microRNA plays an important role in cell growth, development, invasion, differentiation and apoptosis. Aberrant expression of microRNA has been demonstrated that their underlying molecular mechanisms involved in tumorigenesis[7]. Moreover, previous studies showed that a complex microRNA regulatory network, rather than an individual microRNA, is involved in the regulation of metastasis of many cancers[8]. Some studies have revealed that microRNAs are associated with UM. For example, Zhou et al prior reported that microRNA-20a acts as an oncogenic microRNA to promote tumor cells growth and movement in UM[9]. Recent study of microRNA also suggested that microRNA-34a can suppress UM cell proliferation and migration [10]. Thus, it is reasonable to believe that microRNAs may be considered as prognostic biomarkers.

LASSO algorithm is a system biology-based approach. Compared with the traditional differential gene expression analysis method, LASSO performs better to integrate information at both gene expression and network topology level, which widely used in cancer biomarker research and identification of meaningful genes[11]. Additionally, nomogram represents a mathematical model, which combines plenty of important factors to predict a particular endpoint. For instance, The nomogram can combine clinical and pathological factors to estimates the probability of patients' risk of relapse and death[12, 13]. Hence, these approaches can use to predict clinical prognosis and guiding diagnosis and treatment.

Therefore, in this study, we used univariate analysis and LASSO method to identify a robust microRNA biomarker[14]. Based on the results of univariate and multivariate analyses for microRNA biomarker and clinical factors, a nomogram was established. To investigate the possible regulation of microRNA biomarker, a microRNA-mRNA network was constructed. GO and KEGG pathway enrichment of all mRNAs of network were performed. Our present study not only identifies a potential microRNA biomarker, but also construct a nomogram to better predict the survival of UM patients.

2. Methods

2.1 RNA data and clinical characteristics.

The RNA-seq data of microRNA and mRNA as well as clinical characteristics were extracted from the TCGA database of UM. MicroRNA expression profiles of GSE84976 and GSE68828 were obtained from GEO database. Next, the 80 UM samples in TCGA were randomly classified into training and testing dataset (1:1). The training dataset was used to identify key potential microRNA signature. Then testing dataset and GSE84976 dataset were used for internal and external validate respectively. Besides, the GSE68828 dataset contained 10 UM samples, including six monosomy 3 samples and four disomy 3 samples were used to explore the differential expression of signatures.

2.2 Development and validation of microRNA signature.

To explore the associations between microRNA and the overall survival time of UM patients, univariate cox regression analysis was applied to select the potential prognostic microRNAs and mRNAs (p value <0.05). Afterwards, the LASSO method was used to develop prognostic model with these prognostic microRNAs. Base on the contribution of each variable, LASSO method weights each expression level of microRNA and selects the more favorable microRNAs to construct risk system model and calculates coefficients. The risk model computes detail risk score for each patient which was further classified into high-and low-groups. Their clinical characteristics about stage and age were also divided into subgroups. The different survival curve among groups were estimated by the Kaplan-Meier methods and were compared by the log-rank test. Besides, to test the model performance, the specificity, sensitivity, Receiver operating characteristic (ROC) curves and area under receiver operating characteristic curve (AUC) values were generated[15].

2.3 Construct the nomogram.

The factors analyzed in this study are as follows: age, gender, TNM classification, stage and microRNA signature. The relationships between microRNA signature expression and clinical characteristics were also performed. The univariate and multivariate logistic regression analyses were performed to assess influence of factors on overall survival (OS). The hazard ratio (HR) was used to estimate the influence of each factor on OS. Nomogram was established in this study by using information acquired from the results of univariate and multivariate logistic regression analysis. The predictive accuracy of the nomogram was assessed by ROC curve analysis and the clinical usefulness of the nomogram was estimated by decision curve analysis and reduction curve analyses.

2.4 Prediction of microRNA-mRNA interactions and construction of network.

Firstly, the microRNA-mRNA interactions data was obtained from some trustworthy microRNA reference database including miRTarBase (http://mirtarbase.mbc.nctu. edu.tw/php/index.php), miRDB (http://www.mirdb.org/), and TargetScan (http://www.targetscan.org/vert_71/)[16]. Secondly, the microRNA-mRNA pairs were predicted using these three databases and combining with survival related mRNAs. Finally, we established matched microRNA-mRNA network. The microRNA-mRNA network was visualized by using Cytoscape 3.6.0.

2.5 Pathway enrichment analysis.

The functions of selected microRNA paired mRNA were assessed by biology process (BP) term in gene ontology (GO) enrichment analysis and Kyoto encyclopedia of genes and genomes (KEGG) enrichment analysis. Analysis of these microRNA paired mRNAs in the context of biological domain knowledge, biological functions associated the molecular network can be comprehensively understood. Pathways with p value < 0.05 were regarded as significance.

2.6 Statistical analyses.

The univariate and multivariate logistic regression, LASSO statistical algorithm and the nomogram were performed by using the R software (Version 3.5.2). Differences in clinicopathological characteristics between the training and testing cohorts were analyzed using the t test or chi-square test. P<0.05 was considered as the significant threshold in all statistical tests and 95% confidence interval (CI) also estimated.

3. Results

3.1 Processing of microRNAs and mRNAs.

Excluded tiny expression level, 15,187 mRNAs and 1581 microRNAs in 80 UM patients were acquired from TCGA after these steps. Next, these patients were randomly separated into training dataset (n=40) and testing dataset (n=40). The clinical information of training and testing dataset listed in Table 1. The statistical results indicated that no significant differences existed between two datasets.

3.2 Development and validation of microRNA signature.

Firstly, we performed univariate regression analysis and LASSO modelling to assess relationships between microRNAs and overall survival (OS) time of UM in training dataset. Finally, a 3-microRNAs biomarker was identified from 581 microRNAs. The LASSO generated coefficients for 3 microRNAs and the risk score formula as follows: -0.0596 × (expression value of has-mir-1296) + 0.1062 × (expression value of hsa-miR-199a) + -0.0461 × (expression value of hsa-miR-508). The risk score of patients was calculated by risk score formula. Moreover, patients were divided into high-risk and low-risk groups by using the optimal cut-off value of the risk scores (Figure 1A). The vital status, risk score distribution and expression value of three microRNAs in training, testing and GSE84976 datasets were presented in Figure 1. Kaplan-Meier curves showed that patients in high risk group have a shorter survival time than these in low risk group with a log-rank test of $p<0.0001$ (Figure 2A). To demonstrated the predictive ability of the 3-microRNA biomarker, the same 3 microRNAs in testing and GSE84976 datasets were used to validate the results. The patients in testing and GSE84976 datasets were classified into high-risk and low-risk groups based on training dataset. (Figure 1B, C). Kaplan-Meier curves manifested that high risk group have a shorter survival time than these in low risk group both in testing and GSE84976 datasets (log-rank $p=0.0013$ and $p=0.014$). (Figure 2B, C) The ROC curves were applied to estimate the prediction power of microRNA in training, testing and GSE84976 datasets (Figure 2D-E). Furthermore, the plot analysis of 3 microRNAs in GSE68828 dataset indicated that the expression of 3 microRNAs have significant differences between monosomy 3 samples and disomy 3 samples. (Figure 2F)

3.4 Construct the nomogram.

The univariate and multivariate cox regression were performed to estimate prognostic factors for OS. (Shown in Table 2) Age ($P=0.020$), stage ($P=0.003$) were significantly associated with OS. Kaplan-Meier curves showed that patients with old age (>=60 years) in late-stage had a significantly poor OS than the low-group. (Figure 3A,B). The AUC of age and stage was 0.553, 0.636 respectively. (Figure 3C, D). The heatmap of microRNAs signature expression and clinical characteristics indicated that 3-microRNAs biomarker were significantly associated with stage. (Figure 4). Factors considered significant in the univariate analysis or multivariate logistic analysis were enter in the nomogram according to the algorithm. Finally, age, stage, TNM classification and 3-microRNA biomarker were incorporated in the nomogram. Then, a total point summarized the points of each parameter, which can assess the 1 and 3 years, survival probabilities. (Figure 5A) The calibration curves showed that there is a good consistency between prediction and actual survival. (Figure 5B, C) The AUC of the nomogram model had higher accuracy compared with the without 3-microRNA model. (Figure 5D) Eventually, in order to estimate whether the nomogram was clinically useful, decision curve and reduction curve analyses were used to evaluate the net benefit and reduction of the models. Compared with the without 3-microRNA nomogram model, the overall nomogram model offered the better clinical utility. (Figure 5E, F)

3.5 Prediction of microRNA-mRNA interactions and construction of network.

Totally, 221 pairs of microRNA-mRNA network were constructed in UM, it was composed of 3 microRNAs and 218 mRNAs. The network presented in Figure 6A.

3.6 Pathway enrichment analysis.

In all, 217 microRNA paired mRNAs were used to performed BP term and KEGG pathway enrichment analysis. The results of BP revealed that these paired mRNAs were significantly enriched in biological functions related to regulation of transcription DNA-templated, mRNA processing, mRNA transport and so on.(The top ten shown in Figure 6B) The results of KEGG enrichment showed that paired mRNAs were significantly enriched in pathways such as spliceosome, RNA transport, endocytosis and so on.(Figure 6B)

4. Discussion

The increasing genome-wide researches proven that majority of the cellular genomes are transcribed, and there is a complex RNA network which contains lots kind of RNA molecules. But only around 2% of the transcripts own the ability to translate proteins. Recent studies have demonstrated that microRNAs take crucial regulatory roles in many biological processes of human tumor including UM[17-21]. Furthermore, increasing evidences suggested that the abnormal expression of microRNAs significantly associated with the prognosis of patients with UM and could be regarded as a potential target for treatment, like the previous demonstrated microRNAs: hsa-miR-374b-5p, hsa-miR-29c-3p and hsa-miR-211-5p[22]. However, some researchers questioned that it's not sufficient for these

molecules to accurately predict the prognosis of patients. Because they failure to take the simultaneous change of multiple microRNAs and clinical information into account. Therefore, in order to explore the prognostic value of microRNAs in UM, we conducted univariate analysis and the LASSO algorithm to identify the microRNAs biomarker, which have a significantly correlation with OS of UM in training set, testing dataset and outside dataset. Then we further used the microRNAs biomarker and associated with clinical characteristics to build a nomogram model, which manifested a good survival prediction of UM.

In this research, we distinguished a 3-microRNA biomarker to predict the prognosis of UM in TCGA dataset and found these microRNAs have significant differences between monosomy 3 and disomy 3 samples in GEO dataset. Many researches also suggested that UM with monosomy 3 is closely correlated with a dramatically poor prognosis. Thus, we could reasonably speculate that the alternation of 3-microRNA biomarker will cause the mutation of chromosomal 3 and finally lead to poor prognosis. In order to increase the prediction accuracy of UM, the 3-microRNA biomarker combined with clinical characteristics were incorporated in univariate and multivariate logistic regression. The results indicated that age (P=0.020), stage (P=0.003), 3-microRNA biomarker (P=0.032) were significantly in associated with OS. The result of Kaplan-Meier analyses indicated that patients with old age (>=60 years) and at the high risk of 3-microRNA in late-stage will have a poor prognosis. Stratified analysis revealed that this 3-microRNA biomarker was proper for predicting the stage of UM (Figure 4, P=0.036). The nomogram which can associate multiple biological variables with clinical factors to calculate the probability of clinical events[23-25]. we constructed a new nomogram depended on 80 UM patients in TCGA database, which can predict the three years overall survival rates of UM. The calibration curve for the observed 1-year, and 3-year outcomes showed that the nomogram model performed well with the ideal prediction model. What's more, compared with the without 3 microRNA model, The calibration curve including 3-microRNA biomarker were more closer to the ideal line and can better predicting 3-year OS[26]. Moreover, compared with the without 3-microRNA nomogram model, the overall nomogram model had higher accuracy and offered the better clinical utility. Therefore, our nomogram can afford a more simple and accurate tool to predict the prognosis of UM.

To better understanding the molecular functions of the three microRNAs, we constructed a microRNA-mRNA network to predict their target mRNAs. KEGG pathways and GO enrichment analysis of target mRNAs revealed that these paired genes were significantly enriched in Spliceosome pathway, RNA/mRNA transport pathway, regulation of transcription DNA-templated and so on. The results suggested that these microRNAs might take part in transcriptional and splicing regulation pathways affect the occurrence and development of UM. It has been proven that spliceosomal mutations not only exist in patients with leukemia or myelodysplastic syndrome, but can also occur in some solid tumors, including breast cancers, lung cancers and uveal melanoma[27-29]. Their presence in a variety of malignant tumors indicates that splicing and transcriptional mutations may play an important role in the definition of malignant phenotypes[30-32]. Therefore, we speculated that these microRNAs were regarded as the most important role in the prognosis of UM.

Despite we found some significant prognostic microRNAs and built a nomogram to predict the survival of UM, there are several limitations to our study. Firstly, our study was based on bioinformatics analysis, and experimental results were lack to confirm the conclusions. Additionally, the number of samples in this study is limited. Hence, further work will be needed to explore the underlying molecular mechanism.

5. Conclusion

In summary, our study highlighted a 3-microRNA biomarker and nomogram for predicting the survival of patients, which might be regarded as new promising biomarkers for UM prognosis and treatment.

Abbreviations

TCGA—The Cancer Genome Atlas database

GEO —- Gene Expression Omniniub

UM— Uveal melanoma

GO —- Gene ontology

KEGG –Kyoto encyclopedia of genes and genomes

OS —overall survival

AUC—The area under the curve

ROC–- Receiver operating characteristic curves

Declarations

Ethics approval and consent to participate

No permissions were required to use the repository data

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request

Competing interests

All authors declare that they have no competing interests.

Funding

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Author contributions

HQY was responsible for writing-original draft preparation; JT was responsible for writing-review and editing; JQL were responsible for data curation; QW was responsible for project administration and funding acquisition. All the authors commented and approved the text.

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Tables

Table 1: Clinicopathological characteristics of training and testing dataset.

	testing datasets	training datasets	P-value
n	40	40	
vital_status = DEAD (%)	12 (30.0)	11 (27.5)	1
race = white (%)	29 (100.0)	26 (100.0)	
age (mean (SD))	66.98 (13.86)	60.59 (14.28)	0.046
gender = MALE (%)	25 (62.5)	20 (50.0)	0.367
stage (%)			0.627
	0 (0.0)	1 (2.5)	
IIA	6 (15.0)	6 (15.0)	
IIB	11 (27.5)	16 (40.0)	
IIIA	13 (32.5)	12 (30.0)	
IIIB	6 (15.0)	4 (10.0)	
IIIC	1 (2.5)	0 (0.0)	
IV	3 (7.5)	1 (2.5)	
m (%)			0.492
m0	26 (66.7)	25 (64.1)	
m1	2 (5.1)	0 (0.0)	
m1b	1 (2.6)	1 (2.6)	
mx	10 (25.6)	13 (33.3)	
n = nx (%)	13 (33.3)	14 (35.0)	1
t (%)			0.498
t2a	6 (15.0)	6 (15.0)	
t2b	0 (0.0)	2 (5.0)	
t3	0 (0.0)	1 (2.5)	
t3a	11 (27.5)	14 (35.0)	
t3b	2 (5.0)	3 (7.5)	
t3c	0 (0.0)	1 (2.5)	
t4a	12 (30.0)	8 (20.0)	
t4b	6 (15.0)	3 (7.5)	
t4c	1 (2.5)	1 (2.5)	
t4d	2 (5.0)	0 (0.0)	
t4e	0 (0.0)	1 (2.5)	
age_group = younger (%)	17 (42.5)	23 (57.5)	0.264
time (mean (SD))	15.45 (13.89)	14.96 (15.23)	0.88

Table 2: Univariate and multivariate Cox regression analyses of clinicopathologic characteristics associated with overall survival in The Cancer Genome Atlas samples.

	Univariate analysis				Multivariate analysis			
	unicox_pvalue	HR	low	high	mutlicox_pvalue	exp(coef)	lower .95	upper .95
age	0.020479215*	1.05763	1.008681	1.108956	0.007036115*	1.088897894	1.023503188	1.158470866
gender	0.354130306	1.747017	0.536771	5.68598	0.531145723	1.517415813	0.411445056	5.596253296
stage	0.003822421*	8.450267	1.98981	35.88634	0.526717662	1.994431865	0.23524587	16.90894067
m	0.023885246*	7.358764	1.302368	41.57919	0.18179955	4.150961141	0.513784194	33.53641198
n	0.086394654	0.364094	0.114729	1.155456	0.009670253*	0.038987402	0.003338458	0.45530528
t	0.239473681	1.830519	0.668454	5.01276	0.485350112	1.880434126	0.319039179	11.08338013
3-miRNA	0.414197414	0.57357	0.151045	2.178039	0.03249317*	0.181783019	0.03809408	0.867459341

Figures

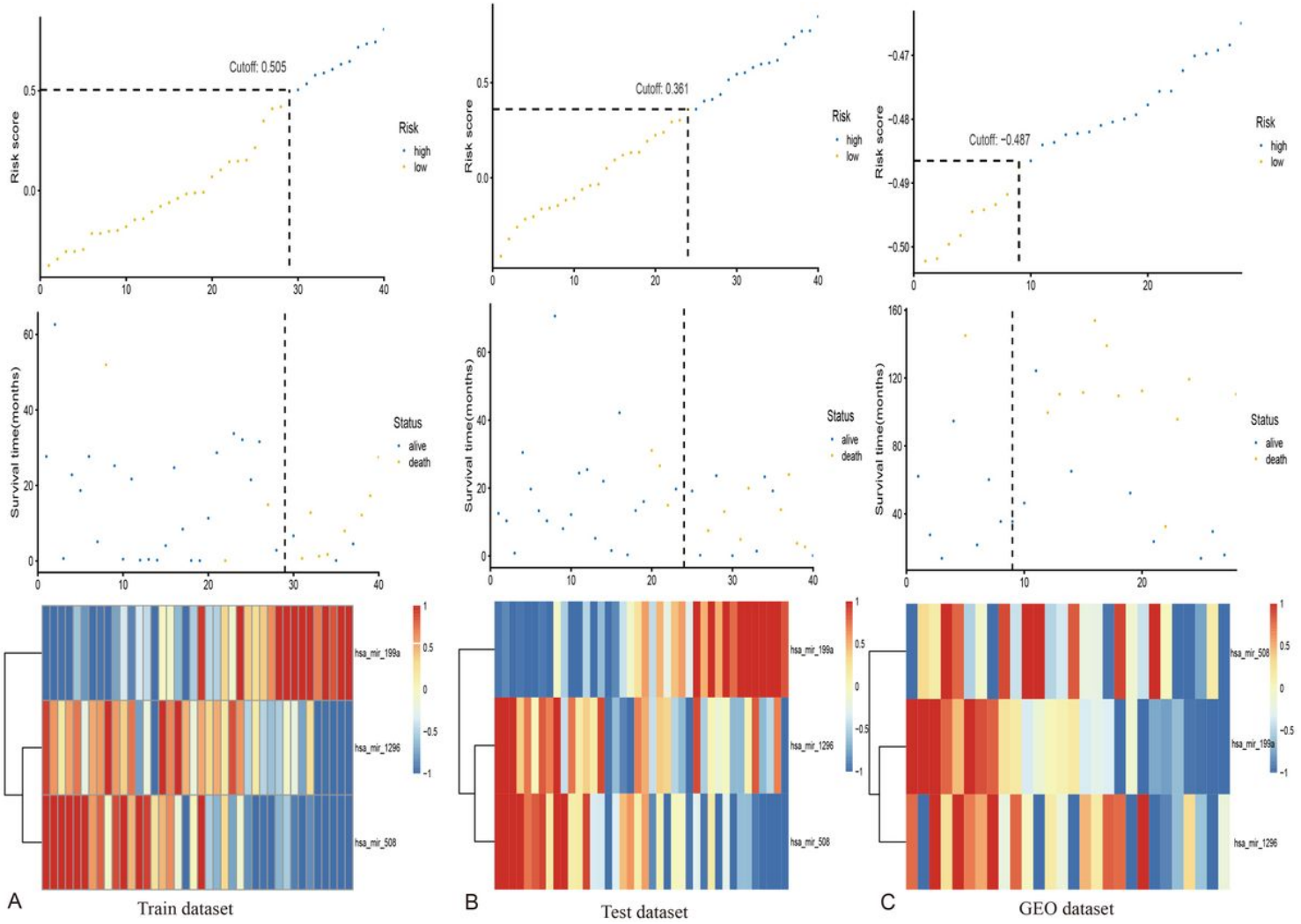

Figure 1

Risk score analysis of the training (N=40), testing (N=40) and GSE84976 datasets. (A): The distribution of 3-microRNA based risk core, patients' survival time and status, heatmap of microRNA expression biomarker in training dataset. (B): The distribution of 3-microRNA

based risk core, patients' survival time and status, heatmap of microRNA expression biomarker in testing dataset. (C): The distribution of 3-microRNA based risk core, patients' survival time and status, heatmap of microRNA expression biomarker in GSE84976 dataset.

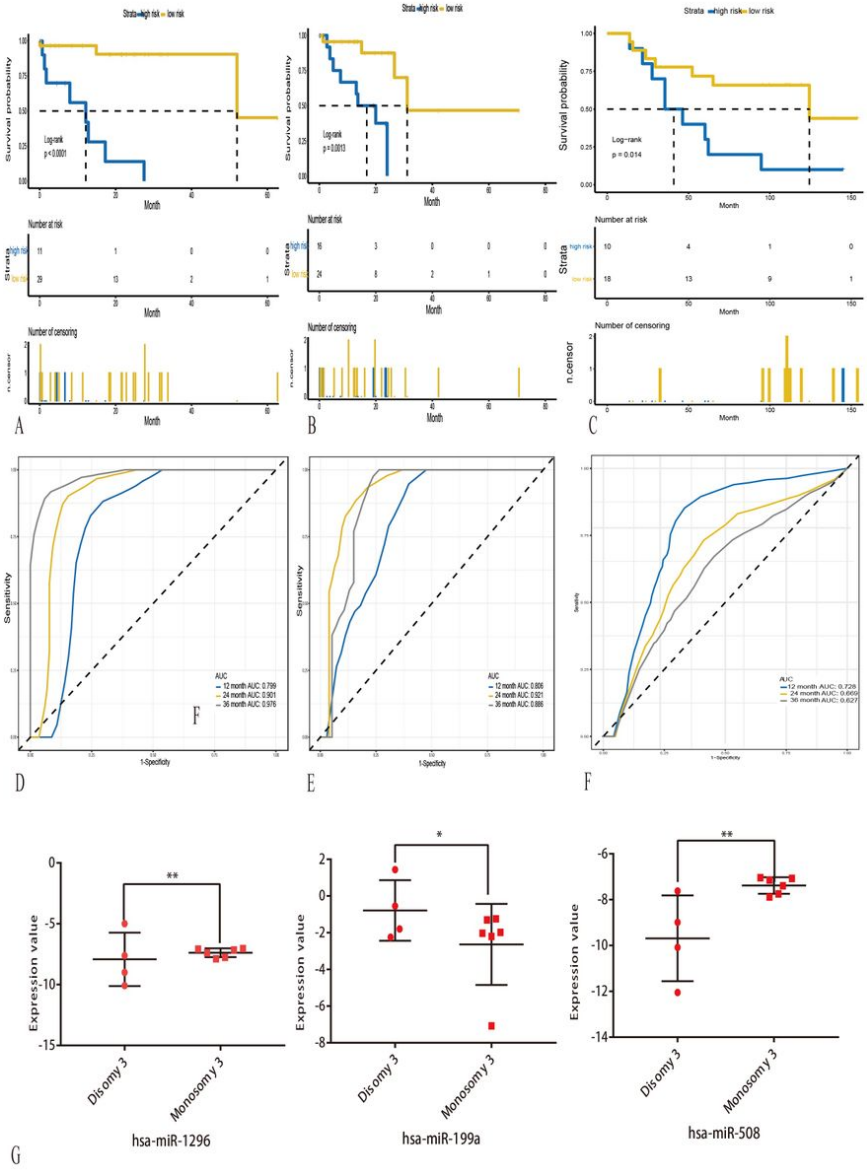

Figure 2

Kaplan-Meier survival analysis for 3-microRNA prognostic biomarker of Uveal melanoma (UM). (A-C): The Kaplan-Meier curve of the overall survival (OS) between the high-risk and low-risk groups stratified by the median risk score in training, testing and GSE84976 datasets respectively. (D-F): The receiver operating characteristic (ROC) curves of 3-microRNA in training, testing and GSE84976 datasets, respectively. (G): The plot analysis of 3 microRNAs in GES68828 dataset. * $P < 0.05$, ** $P < 0.01$.

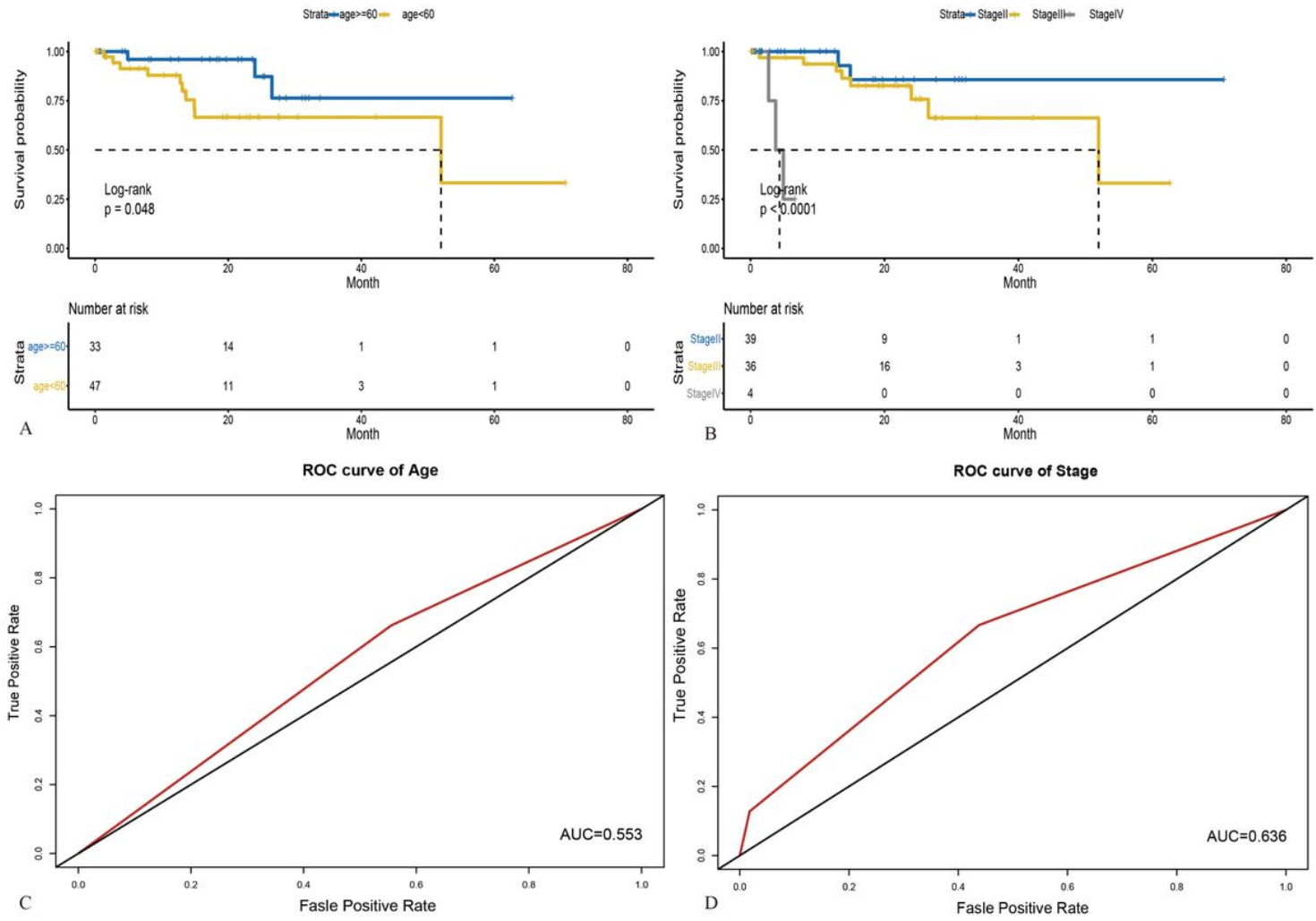

Figure 3

Kaplan-Meier survival analysis of age and stage (A,B):Kaplan-Meier survival analysis for age and stage prognostic biomarker of Uveal melanoma (UM). (C,D): The ROC analysis was used for the discrimination between subgroup.

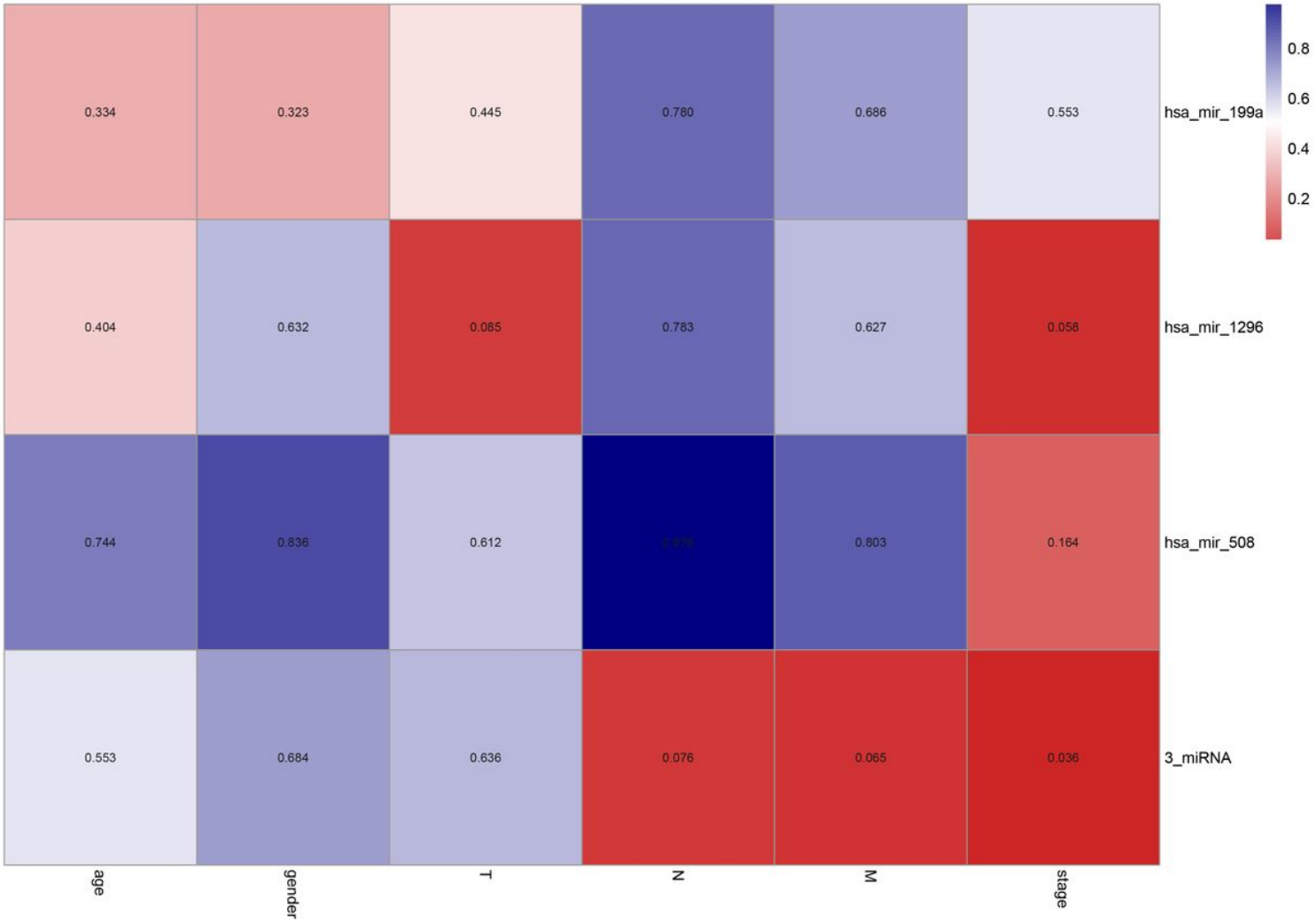

Figure 4

The heatmap of microRNAs biomarker expression and clinical characteristics (The blue and the red colors represent higher and lower P-value, respectively).

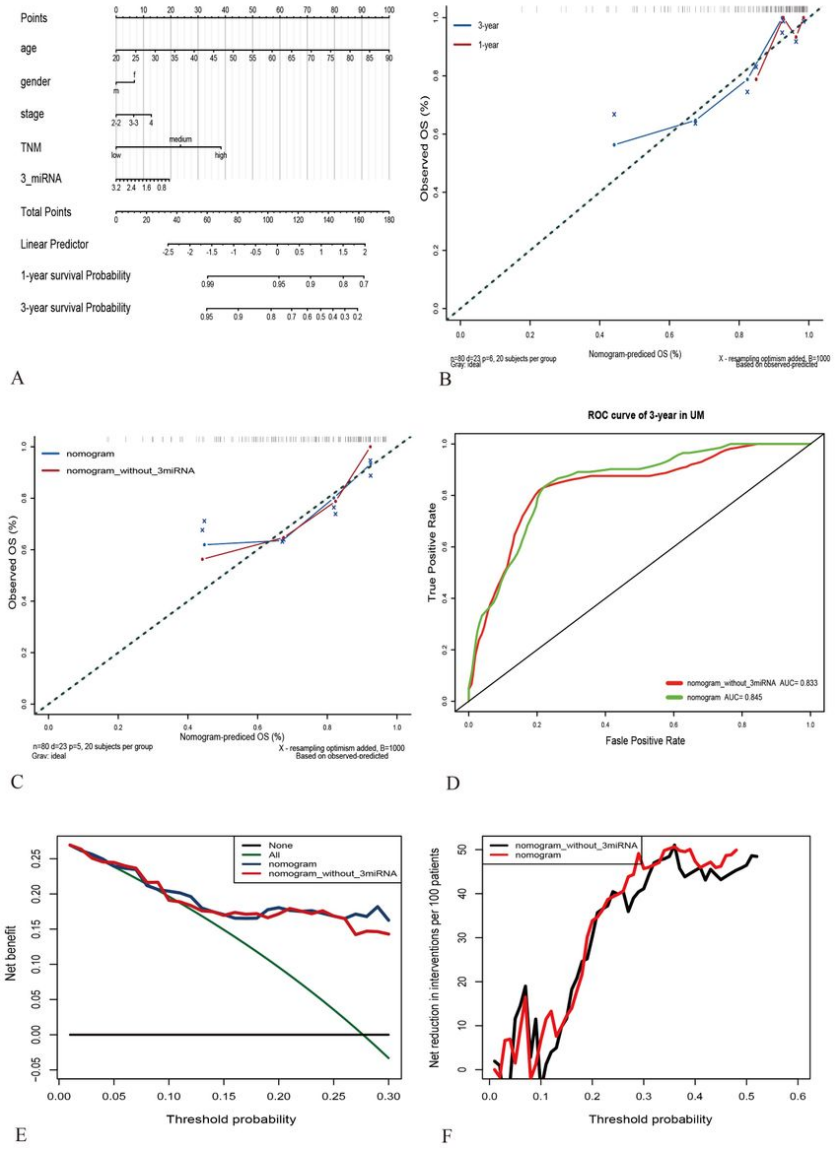

Figure 5

Construction of a nomogram for overall survival prediction in uveal melanoma. (A): The composite nomogram consists of the 3-microRNA score, TNM, stage, age and gender score. Each component generates their respective points according to the “Points” line drawn above. Add the points from 5 variables together and find the location of the total points on “Total Points” line. Then draw a vertical line from “Total Points” axis to the two lower lines which corresponds to the predicted 1-year and 3-year overall survival (OS) rates by the nomogram. (B): Calibration curves of the nomogram for the estimation of OS rates at 1-year (red solid line) and 3-year (blue solid line). (C): Calibration curves of the nomogram for the estimation of OS rates in without 3-microRNA model (red solid line) and 3-microRNA model (blue solid line). (D): ROC curve analysis for the sensitivity and specificity of the nomogram. The nomogram (green solid line) had higher accuracy compared with the without 3-microRNA model (red solid line). (E): Decision curve analysis of the nomogram for 3-year survival. The green solid line represents the assumption that all patients survive in the 3-year. The gray solid line represents the assumption that no patients survive in the 3-year. The red and blue solid line represents the nomogram without 3-microRNA model and nomogram model respectively. (F): The net reduction curves of the nomogram without 3-microRNA model (black solid line) and nomogram model (red solid line).

Figure 6 Placeholder

Figure 6

(Figure 6 is not included with this version of the Manuscript) The microRNA- mRNA network.(A): The microRNA- mRNA network is composed of 3 microRNAs and 218 mRNAs. Triangles and circles represent microRNAs and mRNAs, respectively. (B): The top ten BP term in GO and KEGG functional pathway enrichment analysis.