ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
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EXPRESSION ANALYSIS OF MICRORNAS RELATED TO PLATINUM RESPONSE IN RESECTED NON-SMALL CELL LUNG CANCER

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Background: Alterations in DNA damage repair (DDR) pathways can impair cisplatin efficacy. MicroRNAs (miRNAs) actively involved in DDR regulation, have been suggested as potential biomarkers for the prediction of response to platinum- based chemotherapy (CT) in non- small cell lung cancer (NSCLC). Using bioinformatics approach we identified six miRNAs differentially expressed in patients treated with platinum-based chemotherapy. We further performed comparative expression analysis on tumor and matched normal tissues from resected NSCLC patients in order to confirm miRNAs differential expression in clinical samples. **Methods**: We assembled two miRNA microarray expression datasets from the Gene Expression Omnibus (GEO) repository including samples from NSCLC patients (n=69) treated with CT and respective data from their response to treatment (Table 1). Limma package in R was used to identify DE genes among the two dataset. RT-qPCR was used to assessed miRNA expression levels in clinical samples (N=20). **Results**: Analysis in Limma package in R revealed 112 differentially expressed miRNAs between responders (n=33) and non-responders (n=36). Meta-analysis by random-effects identified 24 miRNAs that were consistently up or down-regulated in at least two studies (Fig. 1). Survival analysis by Kaplan Meier plotter tool in NSCLC revealed twenty two miRNAs as candidates with demonstrated statistical prognostic significance. Integrated function and target pathway enrichment analysis of the miRNAs revealed significant associations for a number of pathways and functions related to DDR, such as p53, HIPPO and FOXO3. We finally identified a miRNA signature consisted by 6-miRNAs (miR-26a, miR-29c, miR-34a, miR-30-5p, miR-30e-3p and miR-497) that were down-regulated in non-responders and are involved in at least three DDR pathways. Comparative expression analysis on tumor and matched normal tissues from operable NSCLC patients treated with platinum-based chemotherapy confirmed their differential expression in clinical samples. Except miR-34a, all other miRNAs were differentially expressed among tumor and normal tissues. Among them miR-26a, miR-29c, miR-30e-5p and miR-30e-3p were down-regulated more than two folds. **Conclusions:** In summary, we developed a 6- miRNA signature that potentially predicts response to cisplatin in NSCLC and needs to be further evaluated for its prognostic significance in NSCLC patients.

Table 1. Information of Gene Expression Omnibus (GEO) datasets used for the identification of differentially expressed miRNAs, including the number of responders and non-responders and sample source.

Accession	Platform	Responders (N)	Non-responders (N)	Material	PMID	Year
GSE56036	GPL15446	17	12	Frozen tissue	25597412	2015
GSE56264	GPL16770	16	24	Frozen tissue	25142144	2014

Table 2. LogFC and *p*- values retrieved from limma and KM Plotter analysis for the 6-miRNAs.

DE miRNAs	**GSE56036**		**GSE56264**		**ADC**		**SqCC**	
	logFC	***p*-Value**	**logFC**	***p*-Value**	**HR**	***p*-Value**	**HR**	***p*-Value**
hsa-miR-26a	−1.4049	0.016655	−0.52735	0.023449	0.63	0.038	0.74	0.033
hsa-miR-29c	−1.0811	0.013295	−0.82175	0.003344	0.54	0.012	0.8	0.15
hsa-miR-30e-5p	−1.19279	0.024696			0.56	8.9 x $10^{-0.5}$	0.75	0.048
hsa-miR-30e-3p			−0.53971	0.043327	0.56	8.9 x $10^{-0.5}$	0.75	0.048
hsa-miR-34a	−1.31859	0.01225	−0.45893	0.041946	0.71	0.062	1.21	0.2
hsa-miR-497	−0.99268	0.010906	−0.82107	0.019803	0.52	0.0009	1.17	0.29

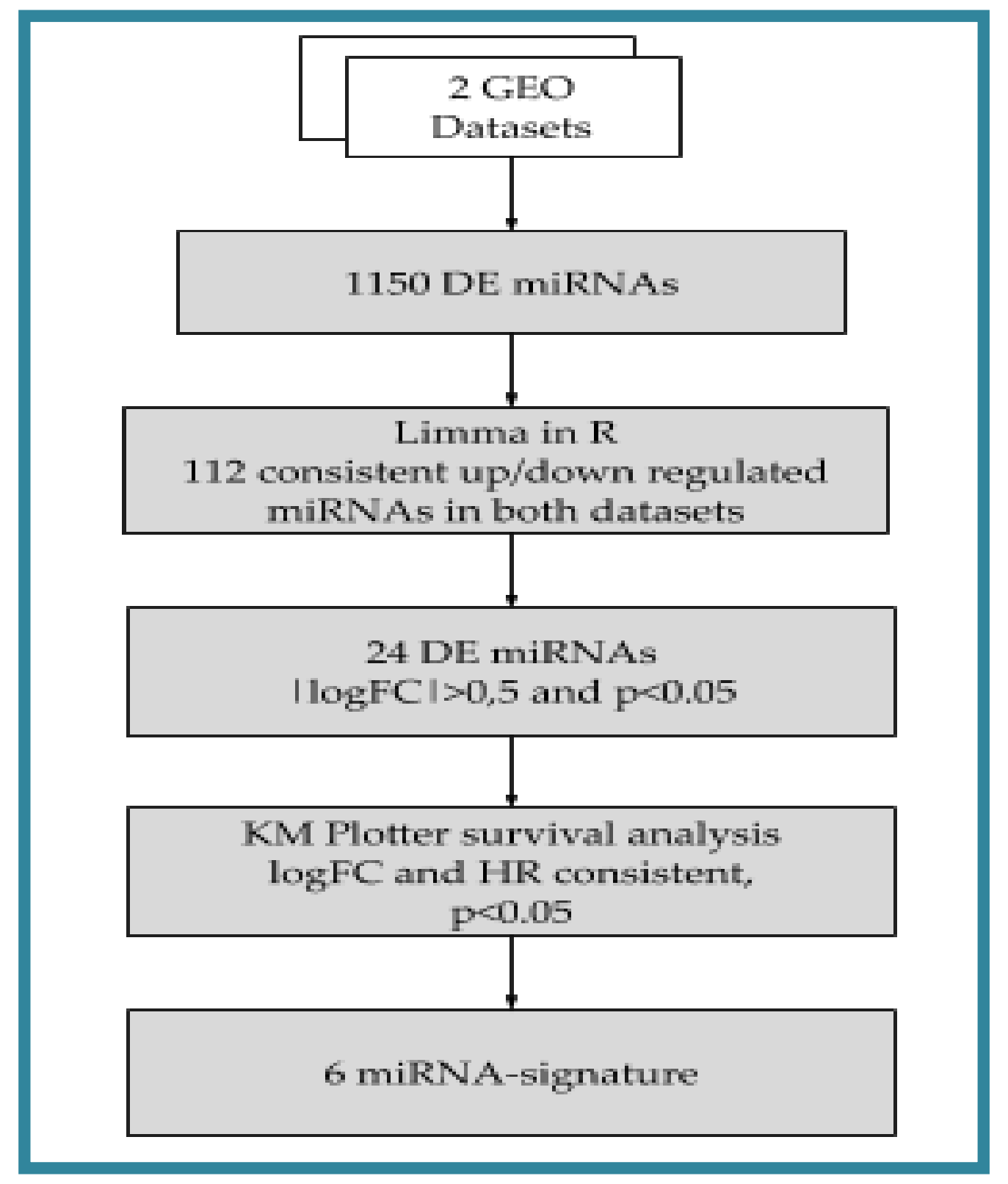
2 GEO Datasets
1150 DE miRNAs
Limma in R
112 consistent up/down regulated miRNAs in both datasets
24 DE miRNAs
|logFC|>0,5 and p<0.05
KM Plotter survival analysis
logFC and HR consistent, p<0.05
6 miRNA-signature

Figure 1. Schematic representation of the study workflow. GEO, gene expression omnibus; DE, differentially expressed; logFC, logarithm of fold change; HR, hazard ratio

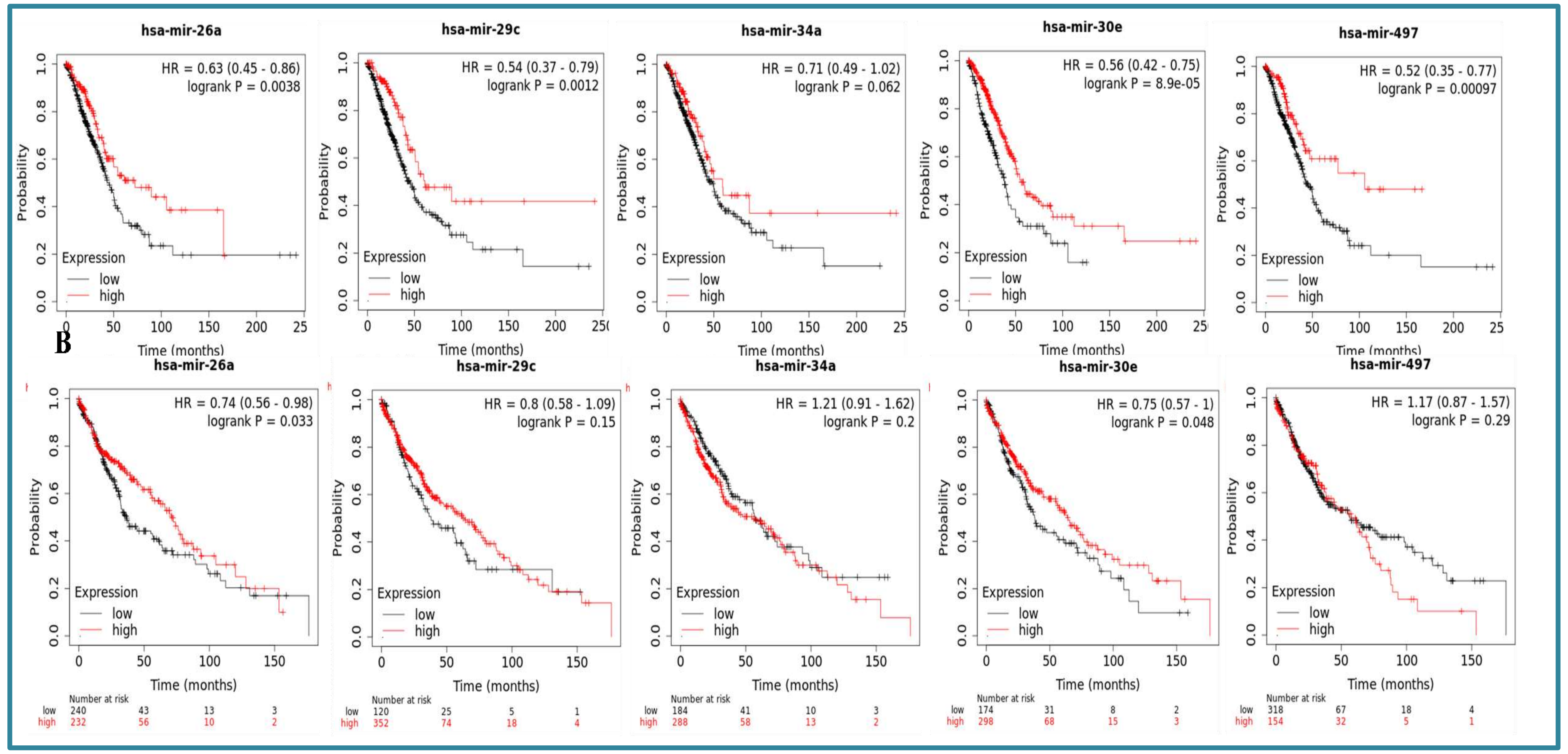

Figure 2. Survival analysis of hsa-26a, hsa-miR-29c, hsa-miR-34a, hsa-miR-30e and hsa-miR-497 in (A) Adenocarcinoma (N=504) and (B) Squamous cell carcinoma (N=472) in KM plotter dataset. Samples are categorized as high (red) and low (black) expression groups for each miRNA. Hazard ratio (HR) and p value for each miRNA associated with survival are shown within the respective plot.

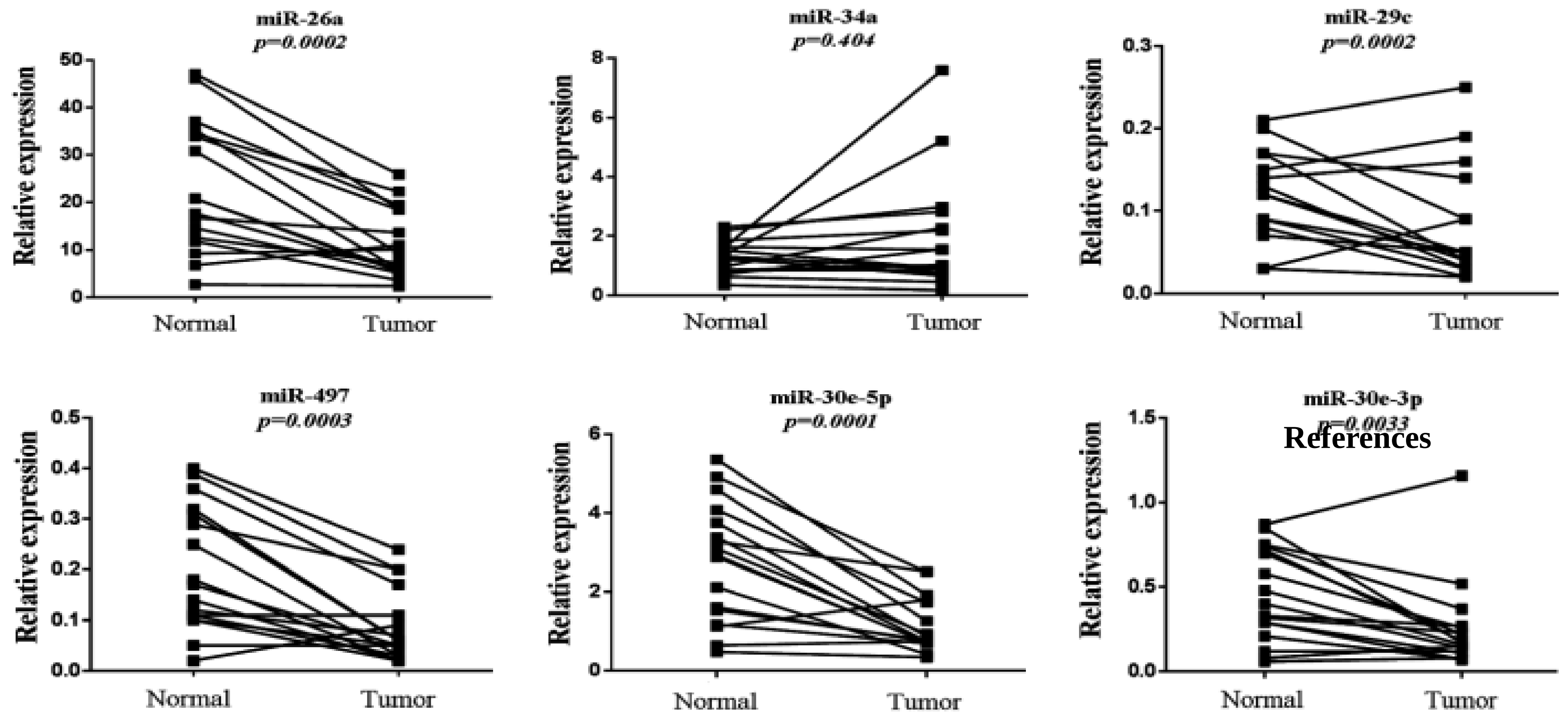

Figure 4. Relative expression of 6-miRNA signature in primary tumors and paired normal tissues. Comparison for each miRNA was performed by non-parametric Wilcoxon paired sample test. Y-axis denotes expression levels for each miRNA relative to miR-1228 assessed by 2−ΔCT.

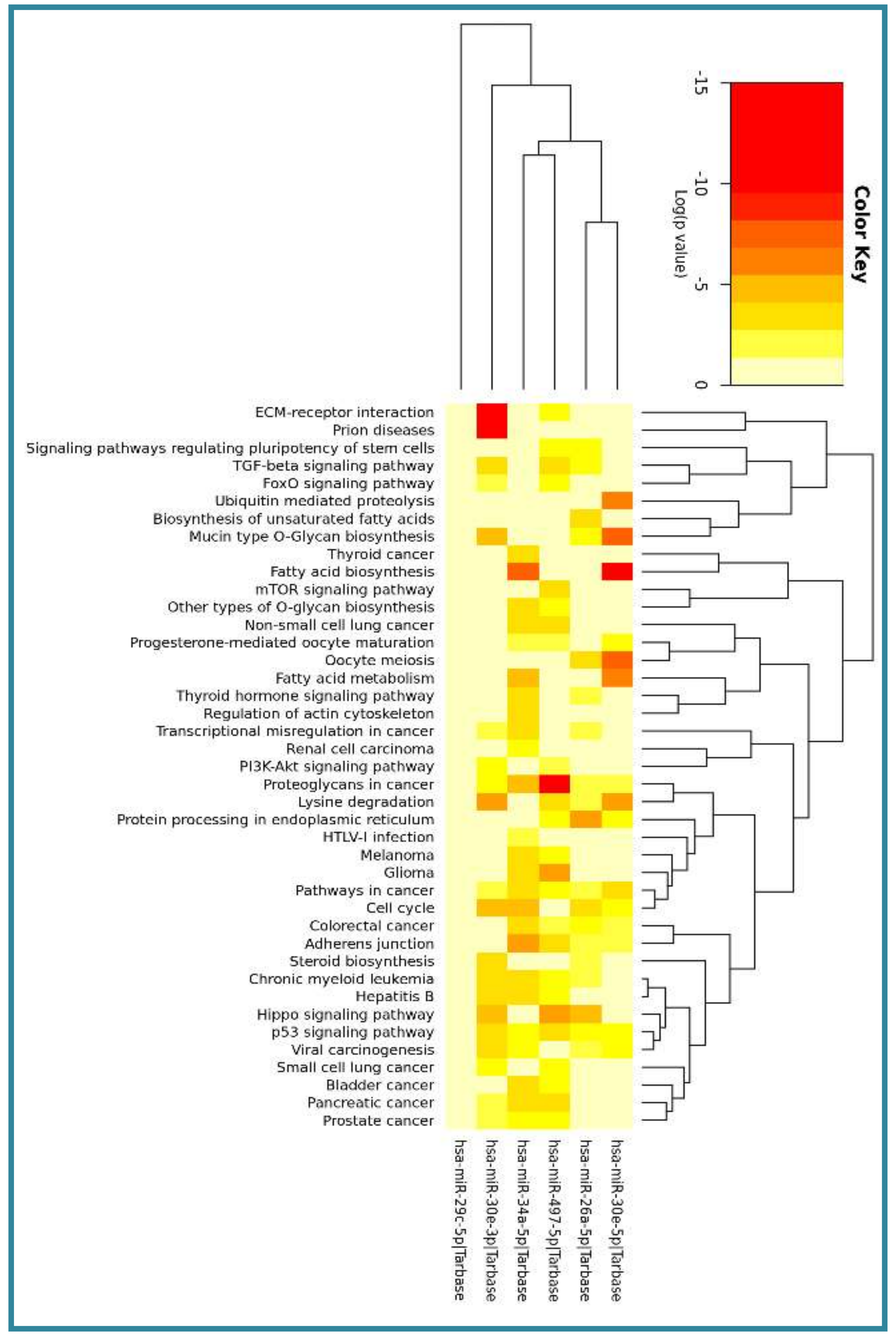

Figure 3. The heatmap exhibits significantly enriched pathways targeted from the 6-miRNAs. The color key depicts the statistical significance expressed by the log (*p*-value); as the color becomes darker, the more statistically significant the involvement of the miRNA becomes in the specific pathway.

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