The potential role of up- and downregulated microRNAs in the pathogenesis of Esophageal Squamous Cell Carcinoma (ESCC)

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INTRODUCTION

- Esophageal cancer is the eighth most frequent and the sixth cancer with the highest mortality rates in the world, and the most frequent histological type is the Esophageal Squamous Cell Carcinoma (ESCC);
- The main risk factors associated with ESCC are alcohol and tobacco consumption;
- MicroRNAs (miRNAs) play an important role in the regulation of gene expression and their deregulation has been associated with different types of cancer, including ESCC;
- The knowledge about the role of microRNAs in the ESCC carcinogenesis is still limited;

OBJECTIVE

To analyze the impact of miRNA expression deregulation in ESCC development and progression.

METHODOLOGY

- The ESCC-microRNA expression profiles were obtained from two databases, TCGA (The Cancer Genome Atlas) and GEO (GEO accession: GSE43732; Gene Expression Omnibus);
- Diferentially expressed microRNA (DEM) were identified from TCGA and GEO data, using R software with DESeq and Limma packages, respectively. MicroRNAs that showed the log fold-change $\leq$ -1 or $\geq$ 1 and ajusted p value < 0.05 were considered DEM.
- The predicted targets genes for these DEMs were accessed using the miRWalk 2.0 software.
- Targets that were differentially expressed in the RNAseq and microarray studies, previously performed by the Molecular Carcinogenesis Program, were selected.
- ROC curve and Univariate analysis using TCGA and GEO data were performed in the GraphPad Prism

RESULTS

Table 1. DEMs obtained from the TCGA and GEO database simultaneously

Twenty-four Diferentially Expressed MicroRNA (DEM)

After the R sofware analyzes, 24 DEMs were identified overlapped between the two databases, 17 up-regulated and 7 down-regulated

Up-regulated microRNA

miRNA	Log Fold Change (TCGA)	FDR* p value (TCGA)	Log Fold Change (GEO)	FDR* p value (GEO)
hsa-miR-767	3,9	0,018	4,6	2,98E-02
hsa-miR-196b	2,8	0,006	2,8	6,33E-27
hsa-miR-196a	5,1	0	2,6	1,31E-12
hsa-miR-34c	3	0,018	2,5	1,51E-22
hsa-miR-424	1,9	0,001	2,5	3,86E-68
hsa-miR-450a	1,4	0,05	2,4	2,01E-31
hsa-miR-130b	1,2	0,05	2,1	9,89E-56
hsa-miR-21	1,4	0,003	2,1	4,76E-75
hsa-miR-944	2,2	0,038	1,9	6,44E-27
hsa-miR-503	2,1	0,006	1,7	2,08E-02
hsa-mir-105	3,5	0,036	1,5	4,40E-02
hsa-miR-224	3,3	0	1,3	8,98E-20
hsa-mir-3651	3,1	0,018	1,2	2,05E-46
hsa-mir-205	2,8	0	1,3	3,23E-33
hsa-mir-671	1,2	0,05	1,1	2,06E-08
hsa-miR-15b	1,1	0,05	1,3	1,87E-31

Down-regulated microRNA

miRNA	Log Fold Change (TCGA)	FDR* p value (TCGA)	Log Fold Change (GEO)	FDR* p value (GEO)
hsa-miR-338	-2,3	3,50E-05	-1,2	2,90E-26
hsa-miR-30a	-2,1	1,30E-04	-1,2	1,70E-21
hsa-miR-195	-1,4	5,00E-02	-1,2	3,50E-29
hsa-miR-133a	-3,1	3,00E-02	-1,7	9,10E-22
hsa-miR-145	-2,2	5,00E-02	-2,2	5,40E-31
hsa-miR-139	-2,1	5,40E-03	-2,5	3,30E-67
hsa-miR-375	-3,2	3,00E-02	-4	4,60E-44

Bayesian hierarchical clustering

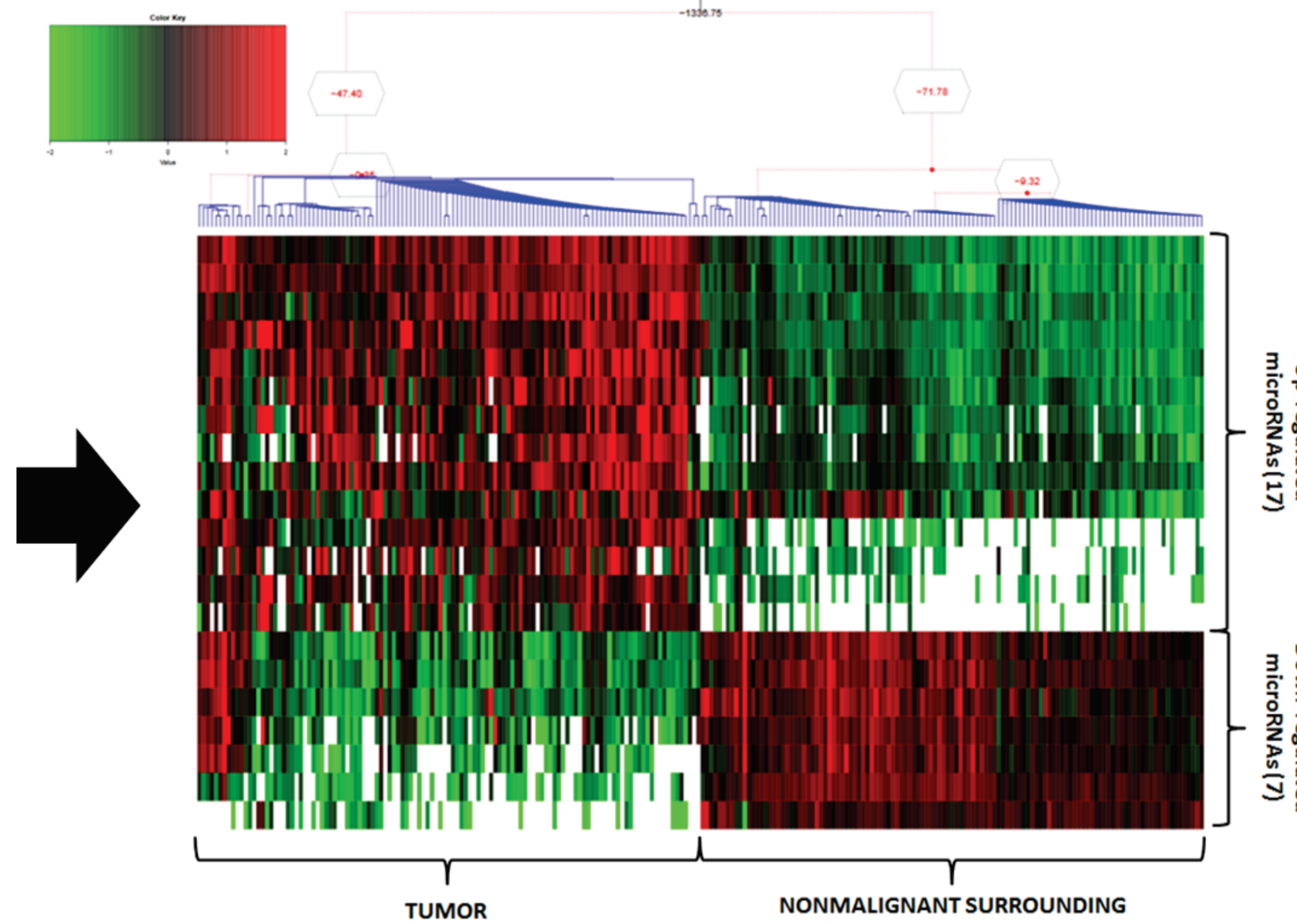The 24 selected miRNA expression profiles of paired tissues from 119 ESCC patients obtained from GEO database

Figure 1. Bayesian hierarchical clustering of ESCC and nonmalignant surrounding mucosa according to DEM expression
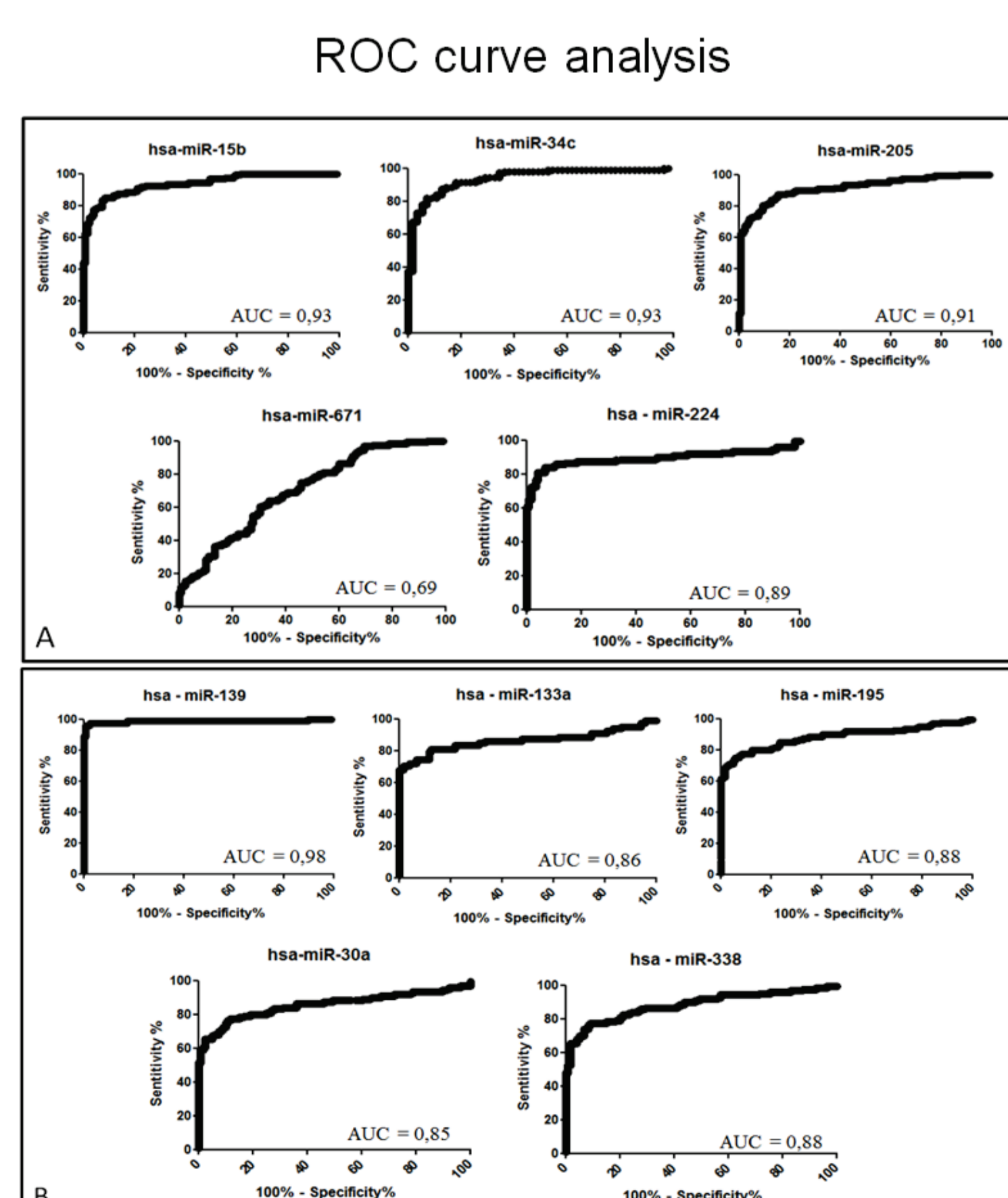
ROC curve analysis

Figure 2. ROC curve of the **A.** up-regulated miRNAs and **B.** down-regulated miRNAs.

Table 2: Target genes of DEMs probably involved in ESCC carcinogenesis.

Identification of DEMsTarget Genes Probably Involved in ESCC carcinogenesis

These targets are differentially expressed in our microarray and RNAseq data.

Up-regulated microRNA

Target Gene	microRNAs	Function	Expression Status
RORA	miR-15b miR-205 miR-34c miR-671	Tumor Supressor	Down-Regulated
MXD1	miR-15b miR-205 miR-34c	Tumor Supressor	Down-Regulated
TAB3	miR-15b miR-205	Tumor Supressor	Down-Regulated
MAPK3	miR-15b	Tumor Supressor	Down-Regulated
MAGI3	miR-205 miR-34c miR-671	Tumor Supressor	Down-Regulated
PLAGL1	miR-15b	Tumor Supressor	Down-Regulated
PDLIM2	miR-671	Tumor Supressor	Down-Regulated
EPHA1	miR-15b	Tumor Supressor	Down-Regulated
PAX9	miR-205 miR-224	Tumor Supressor	Down-Regulated
WDR26	miR-224	Tumor Supressor	Down-Regulated
CPEB3	miR-15b miR-34c	Translation regulator	Down-Regulated

Down-regulated microRNA

Target Gene	microRNAs	Function	Expression Status
STC1	miR-195, miR-30a, miR-133a	Oncogene	Up-regulated
KLF7	miR-139 miR-195 miR-133a	Oncogene	Up-regulated
NRP1	miR-139 miR-30a miR-338	Oncogene	Up-regulated
NUAK1	miR-195, miR-30a	Oncogene	Up-regulated
LRP6	miR-195, miR-30a	Oncogene	Up-regulated
CDK6	miR-139, miR-195	Oncogene	Up-regulated
CAV1	miR-30a, miR-338, miR-133a	Tumor Supressor	Up-regulated
TIMP3	miR-139 miR-30a miR-338	Tumor Supressor	Up-regulated

Table 3: Univariate survival analysis results using TCGA and GEO sample data. HR: Hazard Ratio; CI: confidence interval.

Univariate survival analysis

Data		HR (TCGA)	95% CI (TCGA)	p value (TCGA)	HR (GEO)	95% CI (GEO)	p value (GEO)
Gender	Female vs. Male	0.3271	0.1419 to 0.7542	0.009	1.225	0.6698 to 2.240	0.51
Age at diagnosis	>57.5 vs. <57.5 (TCGA); ≥ 59 vs. < 59 (GEO)	1.223	0.5956 to 2.511	0.5835	1.725	1.089 to 2.734	0.02
Tumor Stage	Early (I e II) vs. Late (III e IV)	0.3309	0.1449 to 0.7554	0.009	0.4698	0.2956 to 0.7466	0.001
hsa-miR-15b	High vs. Low Expression	0.8268	0.4060 to 1.684	0.6002	1.34	0.8458 to 2.123	0.2126
hsa-miR-205	High vs. Low Expression	1.35	0.6593 to 2.766	0.4115	0.8441	0.5295 to 1.346	0.4762
hsa-miR-224	High vs. Low Expression	0.8611	0.4215 to 1.759	0.6815	0.9323	0.5888 to 1.476	0.7649
hsa-miR-34c	High vs. Low Expression	1.408	0.6940 to 2.857	0.3431	1.274	0.7769 to 2.091	0.3369
hsa-miR-671	High vs. Low Expression	0.9624	0.4727 to 1.960	0.9159	0.7431	0.4682 to 1.179	0.207
hsa-miR-133	High vs. Low Expression	1.638	0.8037 to 3.340	0.1743	1.723	0.9853 to 3.014	0.0564
hsa-miR-139	High vs. Low Expression	2.245	1.091 to 4.622	0.028	2.25	1.239 to 4.086	0.008
hsa-miR-195	High vs. Low Expression	0.9505	0.4688 to 1.927	0.8879	1.809	1.139 to 2.874	0.012
hsa-miR-30a	High vs. Low Expression	1.658	0.8069 to 3.406	0.1688	1.217	0.7686 to 1.928	0.4017
hsa-miR-338	High vs. Low Expression	2.584	1.211 to 5.516	0.014	1.299	0.8203 to 2.057	0.2648

CONCLUSION

These results indicate that DEMs may play an important role in ESCC carcinogenesis, targeting oncogenes and tumor suppressor genes.

In addition, these microRNAs have the potential to be used as molecular markers for diagnosis and prognosis. Furthermore, *in vitro* and in vivo analysis are needed to validate these data.

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