Roles and functions of exosomal miRNA in abdominal aortic aneurysm

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Research

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Abstract

Background and objective: abdominal aortic aneurysms（AAAs）are the permanent dilatation of the abdominal aorta, ruptured AAA is a serious threat to the patient's life. It’s hardly known about exosomal miRNAs in AAA. The main purpose of this article is to screen miRNAs which differentially expressed in exosomes from normal people and patients with AAA, and to understand the mechanism of work.

Material and methods: The plasma of healthy control group and patients with AAA were collected, and the RNAs from exosomes was isolated and sequenced. The mature miRNA sequence of miRBase 21 database was used to identify the known type and expression of miRNAs. DEGseq software was used to analyze the types of miRNAs with significant difference between the experimental group and the control group (P ＜ 0.05, | log 2 (FoldChange) | ≥ 1). The targets of miRNAs were detected by miRTarBase, miRDB, TargetScan and miRWalk software. Targets were analyzed based on Kyoto encyclopedia of genes and genomes（ KEGG ）biological pathway and gene ontology（ GO ）functional enrichment analysis. Conclusion: miRNAs in exosomes regulate in the progress of AAA by activating PI3K-Akt/mTOR and MAPK pathway, and its mechanism needs more research. Background and objective: abdominal aortic aneurysms（AAAs）are the permanent dilatation of the abdominal aorta, ruptured AAA is a serious threat to the patient's life. It’s hardly known about exosomal miRNAs in AAA. The main purpose of this article is to screen miRNAs which differentially expressed in exosomes from normal people and patients with AAA, and to understand the mechanism of work. Material and methods: The plasma of healthy control group and patients with AAA were collected, and the RNAs from exosomes was isolated and sequenced. The mature miRNA sequence of miRBase 21 database was used to identify the known type and expression of miRNAs. DEGseq software was used to analyze the types of miRNAs with significant difference between the experimental group and the control group (P ＜ 0.05, | log 2 (FoldChange) | ≥ 1). The targets of miRNAs were detected by miRTarBase, miRDB, TargetScan and miRWalk software. Targets were analyzed based on Kyoto encyclopedia of genes and genomes（ KEGG ）biological pathway and gene ontology（ GO ）functional enrichment analysis.

Conclusion: miRNAs in exosomes regulate in the progress of AAA by activating PI3K-Akt/mTOR and MAPK pathway, and its mechanism needs more research.

Introduction

AAA refers to the abdominal aortic dilatation with a diameter of 3.0 cm or more. Most AAA are asymptomatic before rupture. Although the risk of rupture varies greatly, it depends on the size of the aneurysm. The risk of death associated with rupture is as high as 81%. The prevalence of AAA is 4–7% in men and 1–2% in women over 65 years old. At present, the treatment options for the prevention of aortic rupture are limited to surgical repair, and there is inefficient drug treatment to prevent progressive growth or rupture. The pathological features of AAA include vascular endothelial cell damage and inflammatory cell infiltration, extracellular matrix loss and vascular smooth muscle cell (VSMC) apoptosis.

Extracellular vesicle (EV) was first to discover in sheep reticulocyte test in 1983,which be divided into two types: ectosomes and exosomes. The exosomes originate from the vesicles of the late endosome and the molecular diameter is about 40–160 nm. Almost all types of cells can secrete exosomes which are widely distributed in body fluid. Exosomes contain proteins, nucleic acid and lipids, which can participate in the regulation of important cellular physiological activities. Small RNA (sRNA) is a class of non-coding RNA molecules with a length of about 20–30 nucleotides. The post-transcriptional gene regulation is a new type of gene regulation mechanism in organisms. microRNA (miRNA) is the main component of sRNA. miRNA guides the RNA-induced silencing complex(RISC)to degrade mRNA or hinder its translation by pairing with the target gene mRNAs. miRNAs are closely related to a variety of diseases, including cancer, vascular diseases, immune system diseases so on. Studying the difference of miRNA expression in healthy and diseased state can be used to detect and monitor the disease process, and may become a biomarker of early diagnosis and prognosis of the disease, and look for potential regulatory mechanisms through its target genes.Therefore, exosomal miRNAs may be a target for early screening and treatment of AAA.

Material And Methods

1.1 **Sample collection:** Plasma samples from 3 patients with AAA and 3 healthy people were provided by the First Affiliated Hospital of Zhengzhou University. All patients with AAA were confirmed by CTA that the diameter of the abdominal aorta was larger than 3 cm (Table 1). healthy group without AAA was selected as the control group. The blood is collected into the EDTA anticoagulant tube and gently mixed to ensure exposure to the wall of the tube coated with EDTA.

Table 1. Clinical characteristics of patients with AAA and organ donors

	Patient 1	Patient 2	Patient 3	Control 1	Control 2	Control 3
Sex	Male	Male	Male	Female	Male	Female
Hypertension	-	-	+	-	-	-
Diabetes	+	-	-	-	-	-
Dyslipidemia	-	-	-	-	-	-
CAD	+	-	-	-	-	-
COPD	+	-	-	-	-	-
Renal dysfunction	-	-	-	-	-	-
MAAA(mm)	58.2	39.7	53	-	-	-

Abbreviations: CAD coronary artery disease, COPD chronic obstructive pulmonary disease, MAAA maximum abdominal aortic diameter.

1.2 **Exosome separation:**

Firstly, the plasma sample was centrifuged at 2000 g at room temperature for 20 min, the residual cells and fragments were removed. Then, the supernatant was absorbed into the new tube, centrifuged at 10000 g at room temperature for 20 min again. According to the agreement, the supernatant was fully mixed with 1/3 volume Ribo Exosome Isolation Reagent（RIBOBIO，China）standing at 4 ℃ for 30 min, followed by 15000 g centrifugation for 2 min to remove the supernatant. Phosphate buffered saline was used to re-suspend exosome particles.

1.3 **Exosome identification:** Using Zetasizer Nano-ZS (Malvern Panalytical, UK), the molecular diameter distribution determined by Nanoparticle Tracking Analysis (NTA) software is mainly composed of 20-200

nm particles. Fluorescent direct labeling CD63 and CD81 antibody (BD, USA) were used for staining, and unstained exosome was labeled as NC as negative control. Accuri C6 flow cytomenter (BD, USA) was used for flow cytometry analysis (83%), which proved that the isolated EV contained abundant exosomes. The results are shown in Fig 1. Characterization of EV particle diameter is shown in Table 2.

Table 2. Characterization of EVs particle diameter

	Sample
Average diameter(nm)	48.89
Polydispersity index(DPI)	0.306
Major peak of particle diameter (nm)	74
Percentage of 20-20nm Diameter (%)	89.5

Polydispersity index (PDI) is a dimensionless value that represents the distribution of particle size. PDI values of 0.08–0.7 indicate moderate dispersion system and optimum application scope of algorithm.

1.4 **RNA separation:** Total RNA was extracted from exocrine by Trizol reagent (Invitrogen, Life Technologies).

1.5 **RNA detection:** According to the manufacturer's agreement, the concentration of RNA was quantified by NanoDrop2000 spectrophotometer (Thermo Science, USA). The OD260/280 ratio of samples is between 1.8 and 2.1, which is acceptable. RNA integrity and genomic DNA contamination were detected by denaturing agarose gel electrophoresis. The sampled 2 ul was analyzed by Aligent2200 bioanalyzer (Agilent, USA). The RNA map of Agilent bioanalyzer showed RNA peaks around 25 nt and 200 nt, but not 18 s and 28 s rRNA peaks. This indicates that the plasma exosomal RNA is mainly small RNA. The result is shown in Fig 2.

2.6. **cDNA library construction and high-throughput sequencing:** The 3 ' and 5' connectors were linked to RNA, including primers. Then reverse transcription and PCR amplification were performed. Amplification conditions: 95 °C for 15 second, then 94 °C for 15 second, 55 °C for 30 second, and 70 °C for 34 second.

3. **Analysis of high-throughput sequencing data**

The clean reads was obtained by removing the joint sequence, length 17 nt and low quality reads. Clean reads were compared with human genome by Burrow-Wheeler Aligner software. Comparing and annotating clean data with a variety of RNA databases[11]. Using Rfam 11.0 for yRNA, rRNA, snRNA, snoRNA and tRNA. MiRBase 21 for miRNA, piRNABank for piRNA[12].

Compared with the entire reference sequence of miRBase 21, the number of miRNA expressions in each sample was obtained and standardized as number of mapped reads per million clean reads (RPM)[13]. The analysis of differential miRNA expression is an independent hypothesis statistical test for thousands of miRNAs. This multiple test has the problem of high false positive. In order to highlight the difference in expression, the P value needs to be corrected. We use DEGseq to correct the P value to get the Q value. The lower the Q value is, the more significant the difference in miRNA expression is[14]. The significant

difference of miRNA expression was determined by DEGseq software. Setting P □ 0.05, Q□0.05, |log 2 (FoldChange) | ≥ 1. Drawing the volcano map□Fig 3□ and heat map□Fig 4□ of the overall miRNA expression differences among the samples.

4. **GO and KEEG enrichment analysis of predicted miRNA targets.**

Targets of miRNA with significant differences were predicted by TargetScan, miRDB, miRTarBase and miRWalk software, and the intersections were selected[15,16]. The targets were annotated in the KEGG biological pathway database, and the biological pathway enrichment analysis of targets was carried out by using Fisher Exact Test, with the threshold of P□0.05[17]. According to GO gene annotation, all genes of this species were selected as background genes, and hypergeometric method was used to calculate high frequency annotation(P □ 0.05)[18].

Results

DEGseq helps us to screen out 85 species of miRNA with significant differences in expression, including 42 up and 43 down□Table 3□. In order to study the potential biological function and pathway of miRNA, the predicted targets were used to analyze the enrichment of GO biological process and KEGG pathway.

Table 3. miRNAs with significant differences in expression between AAA plasma EVs and the control.

UP	P	Q	log 2(FoldChange)
hsa-miR-136-3p	0.006486231	0.039600145	1.002103458
hsa-miR-15b-5p	2.65E-07	2.90E-06	1.03912509
hsa-miR-503-5p	0.001517921	0.010671442	1.039687152
hsa-let-7d-3p	1.32E-42	4.25E-41	1.058578006
hsa-miR-381-3p	2.61E-11	3.29E-10	1.060009965
hsa-miR-194-5p	2.63E-24	5.65E-23	1.073034563
hsa-miR-335-3p	0.000428733	0.003315532	1.080773254
hsa-miR-26b-5p	1.00E-114	4.46E-113	1.214053867
hsa-miR-548av-3p	4.46E-06	4.31E-05	1.242465603
hsa-miR-182-5p	2.45E-21	4.59E-20	1.277990137
hsa-miR-548o-3p	1.33E-05	0.000124243	1.286726222
hsa-miR-101-3p	0	0	1.329639736
hsa-miR-3613-5p	0.001381321	0.009890939	1.352703446
hsa-miR-10a-5p	1.35E-189	7.45E-188	1.37296967
hsa-miR-16-5p	4.15E-87	1.66E-85	1.381452179
hsa-miR-128-3p	0	0	1.403450868
hsa-miR-451a	0	0	1.413491698
hsa-miR-374a-5p	3.15E-05	0.000289861	1.42406343
hsa-miR-654-3p	2.67E-14	3.82E-13	1.427963013
hsa-miR-495-3p	0.000252977	0.002009958	1.459930639
hsa-miR-889-3p	0.000156114	0.001312262	1.469186968
hsa-miR-323b-3p	1.69E-14	2.45E-13	1.519170838
hsa-miR-340-5p	2.38E-91	9.86E-90	1.579479204
hsa-miR-27a-3p	7.55E-244	5.15E-242	1.608163185
hsa-miR-15a-5p	2.45E-06	2.41E-05	1.621860467
hsa-miR-335-5p	9.88E-28	2.55E-26	1.622374025
hsa-miR-20a-5p	4.39E-55	1.54E-53	1.749019993
hsa-miR-130b-5p	1.70E-26	4.11E-25	1.751577975
hsa-miR-183-5p	1.68E-21	3.26E-20	1.770817151
hsa-miR-10b-5p	0	0	1.791807496
hsa-miR-369-3p	0.000112555	0.000981679	1.92335335
hsa-miR-29c-3p	0.000590089	0.004416147	1.924957869
hsa-miR-485-3p	0.000749617	0.005538572	1.945544953
hsa-miR-340-3p	1.33E-08	1.58E-07	1.952264928
hsa-miR-95-3p	0.004289268	0.0271888	1.962369457
hsa-miR-411-5p	5.43E-07	5.68E-06	1.990337635
hsa-miR-548a-3p	0.000708163	0.005265829	2.108591859
hsa-miR-708-3p	0.003322231	0.021650496	2.323869811
hsa-miR-32-5p	1.79E-17	2.81E-16	2.731414365
hsa-miR-1277-5p	2.03E-05	0.000188667	3.387248809
hsa-miR-4424	0.000128688	0.001089625	3.890874175
hsa-miR-411-3p	0.001767204	0.012129923	4.130487773
DOWN			
hsa-miR-34a-5p	0.000177914	0.001463687	-3.046120546
hsa-miR-193b-5p	3.72E-13	5.02E-12	-2.664364369
hsa-miR-4669	4.36E-06	4.25E-05	-2.559769622
hsa-miR-1228-5p	0.002210866	0.014910493	-2.496472058
hsa-miR-483-5p	1.25E-17	1.99E-16	-2.493661586
hsa-miR-4533	0.00135209	0.009741763	-2.491742668
hsa-miR-4665-5p	1.37E-07	1.53E-06	-2.432811033
hsa-miR-122-5p	0	0	-2.332912796
hsa-miR-133a-3p	4.23E-24	8.91E-23	-2.20908903
hsa-miR-6741-5p	0.000253904	0.002003595	-1.86796741
hsa-miR-769-5p	1.48E-39	4.51E-38	-1.784025967
hsa-miR-326	6.29E-19	1.07E-17	-1.738426609
hsa-miR-145-5p	0.001351524	0.00979855	-1.712748423
hsa-miR-4286	0.008382572	0.049359306	-1.712403945
hsa-miR-362-5p	0.000550722	0.004175408	-1.683809373
hsa-miR-3180	7.90E-08	9.03E-07	-1.583801845
hsa-miR-3180-3p	7.90E-08	9.03E-07	-1.583801845
hsa-miR-185-3p	3.96E-19	6.97E-18	-1.581849303
hsa-miR-1273h-5p	8.89E-08	1.00E-06	-1.548717034
hsa-miR-760	2.99E-18	4.95E-17	-1.529550839
hsa-miR-139-3p	5.36E-21	9.71E-20	-1.506557086
hsa-miR-23a-5p	0.001578021	0.011027138	-1.491642034
hsa-miR-1908-5p	1.24E-22	2.57E-21	-1.478859686
hsa-miR-125b-2-3p	0.004046277	0.025789459	-1.420798608
hsa-miR-193a-5p	7.39E-69	2.77E-67	-1.382986889

hsa-miR-17-3p	0.002645456	0.017435961	-1.368972962
hsa-miR-181c-3p	0.001693148	0.011760791	-1.281909037
hsa-miR-127-5p	0.007491731	0.044795919	-1.247234469
hsa-miR-148a-5p	1.03E-12	1.35E-11	-1.246975046
hsa-miR-6852-5p	6.44E-09	7.79E-08	-1.213316205
hsa-let-7i-5p	0	0	-1.199155756
hsa-miR-4433b-3p	3.16E-22	6.33E-21	-1.185507131
hsa-miR-4446-3p	2.46E-08	2.85E-07	-1.160385936
hsa-miR-422a	0.006595908	0.03985028	-1.12097872
hsa-miR-378i	2.13E-53	7.28E-52	-1.113027101
hsa-miR-221-3p	7.12E-282	5.50E-280	-1.111446277
hsa-miR-877-5p	0.004664764	0.029408293	-1.101055298
hsa-miR-505-3p	0.001739329	0.012009652	-1.083972994
hsa-miR-378g	0.00055385	0.004171855	-1.068686663
hsa-miR-378d	2.02E-21	3.84E-20	-1.067144872
hsa-miR-125a-3p	0.000389685	0.003033789	-1.051629056
hsa-miR-1301-3p	8.85E-18	1.43E-16	-1.028117391
hsa-miR-320c	7.60E-127	3.67E-125	-1.026330669

Abbreviations: hsa Homo sapiens.

GO analysis consists of three independent ontologies: biological process, molecular function and cellular component. Through the classification of genes with different functions, gene annotation and functional enrichment analysis were carried out（Fig 5）. The results of biological process showed that targets were involved in the process of biological regulation and metabolism. Cellular component enrichment results showed that targets were significantly enriched in intracellular organelle and membrane- bounded organelle. In addition, target genes are rich in molecular function entries, including nucleic acid binding, metal ion binding and catalytic activity. KEGG results showed that the targets were rich in PI3K–Akt signaling pathway, MAPK signaling pathway, Rap1 signaling pathway, mTOR signaling pathway, AMPK signaling pathway, Ras signal pathway, cancer pathway, and so on（Fig 6.）

Discussion

AAAs are an important cause of death in adults with aortic rupture. In recent years, with the development of surgical techniques, the treatment of AAA is becoming more and more minimally invasive. EVAR has become the first choice for AAA (male ≥ 5.5 cm, female ≥ 5 cm). Small AAA(male ＜ 5.5 cm, female ＜ 5 cm) are recommended for regular monitoring[7]. However, there is no reliable drugs that can limit the growth and rupture of AAA. Therefore, it is necessary to determine the pathways that can easily lead to the formation of aneurysms, which is very important for the discovery of new therapeutic targets.

Exosomes are the carrier of intercellular information. Proteins, lipids and RNA are transported from exosomes into receptor cells, effectively altering their biological responses. Our previous research proved that exosomal AFAP1-AS1 induces drug resistance of trastuzumab by binding to AUF1. Exosomal miRNA is stable and convenient for body fluid detection, so it can be used as a diagnostic marker. Such as exosomal miR-22-3p and miR-320a were significantly increased in the patients with endometriosis, the two miRNAs may be potential diagnostic biomarkers for endometriosis. In addition, the exosomes' ability

to transport functional vesicles to diseased cells also makes it a therapeutic carrier. Combining with new ligands, exosomes are expected to become the next generation of nanocapsules for precision medicine.

Exosome plays an important role in the progression of AAA. Recent study has shown that macrophage-derived exosomes prompt the expression of metalloproteinases (MMP-2) in VSMC to regulate the progression of AAA through Jun N-terminal kinase (JNK) and P38 pathways. However, it's little known about the role of exosomal miRNA in AAA. By comparing the expression of exosomal miRNA in plasma samples of healthy people and AAA population, we found that there were 85 significantly different miRNAs, suggesting that miRNAs in exosomes work in the development of AAA. We sorted out these miRNAs and found that the mechanisms of some miRNAs in AAA have been reported. Down-regulated miR-145 affect the progression of AAA by regulating the target gene Rac2, which prevents atherosclerotic plaque calcification by inhibiting the expression of Rac1-dependent macrophage interleukin-1β. Most AAA was considered to be one of the terminal manifestations of atherosclerosis[7]. Gan et al. reported that the overexpression of miR-15b reduces ACSS2 acts on AAA through lipid biosynthesis and inflammatory response. Up-regulation of miR-15a-5p is involved in the AAA by affecting the expression of cyclin-dependent kinase inhibitor 2B (CDKN2B), which was considered closely related to VSMC apoptosis. Down-regulated expression of miRNA-125b and miR-193b in AAA leads to the up-regulation of ALOX5 expression, which leads to the increase of leukotriene production and promote inflammation and injury of aortic wall. The overexpression of miR-29c-3p inhibits aortic fibrosis through targeting transforming growth factor β2(TGFβ2)and MMP2, which has a protective effect on the occurrence of AAA. In a word, the above results not only present that the isolated miRNA is reliable, but also prove that exosomal miRNA is crucial in the AAA. However, because a single mRNA is regulated by a variety of miRNAs, its mechanism is complex. As diagnostic marker of AAA, exosomoal miRNA needs more research to identify.

Interestingly, our study found that hsa-let-7i-5p was down-regulated in the AAA group, while hsa-let-7d-3p in the same family was up-regulated. Ana et al. reported that hsa-let-7i-5p regulate β-amyloid peptides by targeting metallopeptidase domain 10, amyloid protein-binding protein 2 and β-amyloid precursor. Zhang et al. found that hsa-let-7i-5p and its target gene Was1 mediate macrophage phagocytosis. A recent study confirmed that hsa-let-7i-5p is associated with endothelial progenitor cell dysfunction. It is hardly known about the role of hsa-let-7d-3p, and a study suggested that it may be associated with primary Sjogren's syndrome. Structurally, seed regions are based on the 2–8 nucleotide sequence at the 5 'end, which identify the 3' untranslated regions (UTR) of mRNA. The seed regions of the same family are highly conservative and the base difference is small. Studies showed that the complementary probability of miRNA 3' UTR and target genes is also the structural basis of different miRNA expression in the same family. It is an interesting phenomenon, showing that there is a competitive inhibition relationship. Besides, the mechanism of mir-193a and mir-125a is not understood, but the expression is also down-regulated, so it may have similar effects to mir-193b and mir-125b.

In addition, some enriched signaling pathways have been confirmed to be related to vascular endothelial cell senescence and VSMC dysfunction. MTOR is a serine/threonine kinase that regulates cell growth and metabolism. Its function is realized by two complexes, mTORC1 and mTORC2. An article showed that

mTORC1-S6K1-eNOS-uncoupling promotes the senescence of vascular endothelial cells. PI3K/Akt as the classic upstream pathway of mTOR, a study confirmed that PI3K/Akt-mTORC-S6K1 promotes VSMC apoptosis by regulating oxidative emergency response and telomere function . It is one of the pathological manifestations of AAA. Study also showed that AMPK may indirectly activate SIRT1 to inhibit mTORC1-S6K1 pathway, which may become a target to treat AAA. MAPK signaling pathway is involved in many important cellular reactions, and its activation leads to the phosphorylation of intracellular substrates or targets. There are 14 known MAPKs, including extracellular signal-regulated kinase(ERK1, ERK2, ERK3, ERK4, ERK5, ERK7), P38α, β, γ, δ, JNK1, 2, 3, NLK. As mentioned earlier, the promotion of MMP-2 expression by JNK and P38 indicates the role of MAPK signaling pathway in AAA. A study proved that the activation of MAPK pathway regulates the contraction of angiotensin converting enzyme II to VSMCs, which eventually leads to VSMCs proliferation, differentiation and inflammation. Therefore, exosomal miRNAs may regulate the progression of AAA involved in VSMC through PI3K-Akt/mTOR and MAPK pathway. However, there are some shortcomings in our article, such as the insufficient number of samples, the action mechanism of most exosomal miRNAs is not understood. Further research is needed.

Conclusion

Our study shows that there is a difference in the expression of miRNA in exosomes between healthy people and AAA patients, and some known miRNAs have been confirmed to act on target genes in the form of exosome in peripheral blood, such as miR-145, miR-15b, miR-15a-5p, miR-29c-3p ,miR-193band miR-125b. These miRNAs may regulate PI3K-Akt/mTOR and MAPK pathway to cause vascular smooth muscle dysfunction and induce AAA.

Declarations

Authors' contributions

CH and LZ designed the study and revised the manuscript. ZYB, LC and GYM performed the experiment. WSW and CGP analyzed the data. JZY, HZH, XP and LSR collected the samples. ZYB and WSW were two major contributors in writing the manuscript. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Zhengzhou University. All methods were carried out in accordance with the relevant regulations. All studies obtained the

informed consent of the donor.

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Not Applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Consent for publication

Written informed consent for publication was obtained from the patients and donors next-of-kin.

Competing interests

The authors declare that they have no competing interests.

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Figures

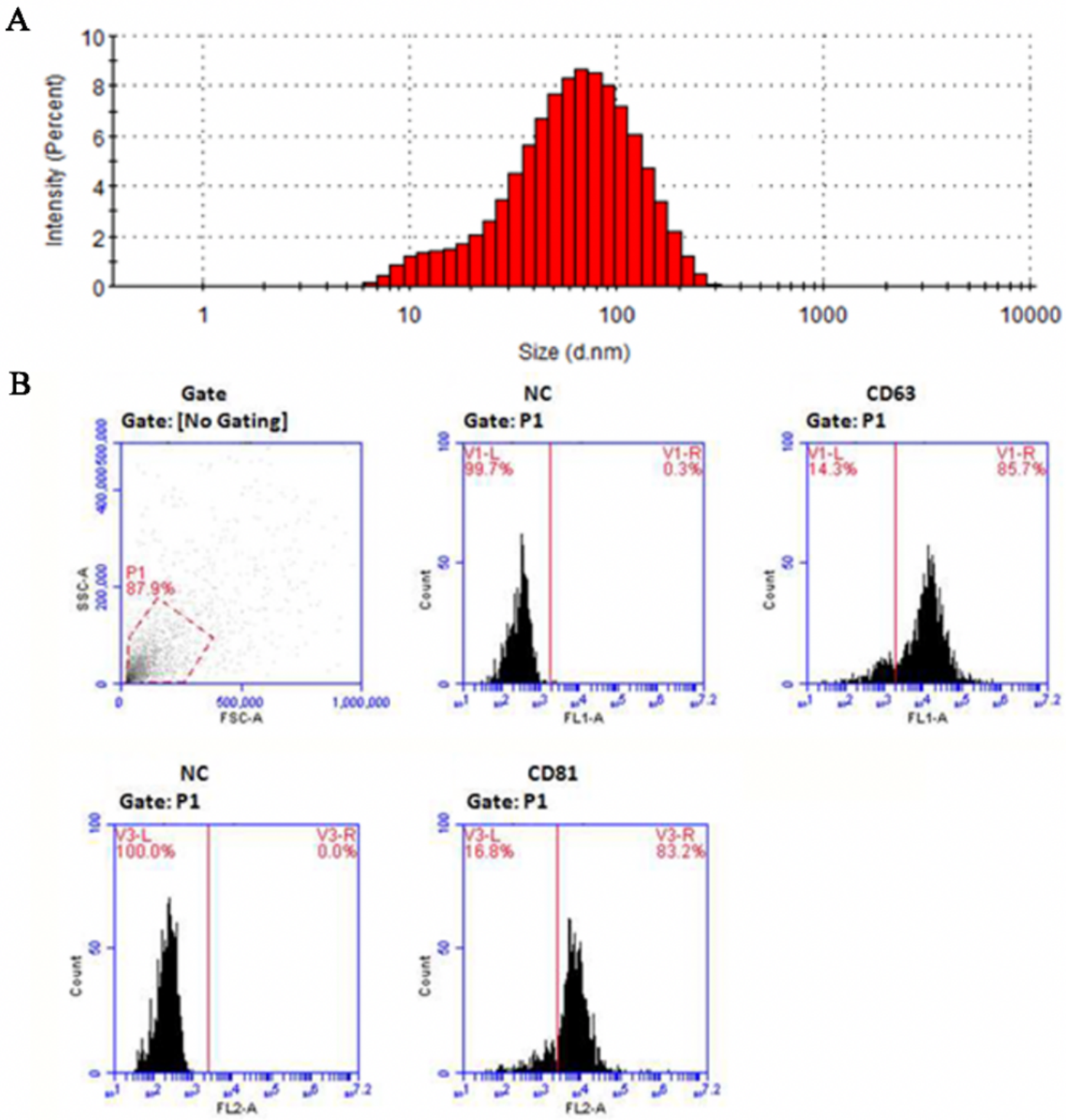

Figure 1

Characterization of EVs from plasma samples of AAA patients and controls. A Size distribution of plasma EVs by NTA. B Plasma EVs were analyzed by flow cytometry for the exosomal markers antibodies CD63 and CD81.

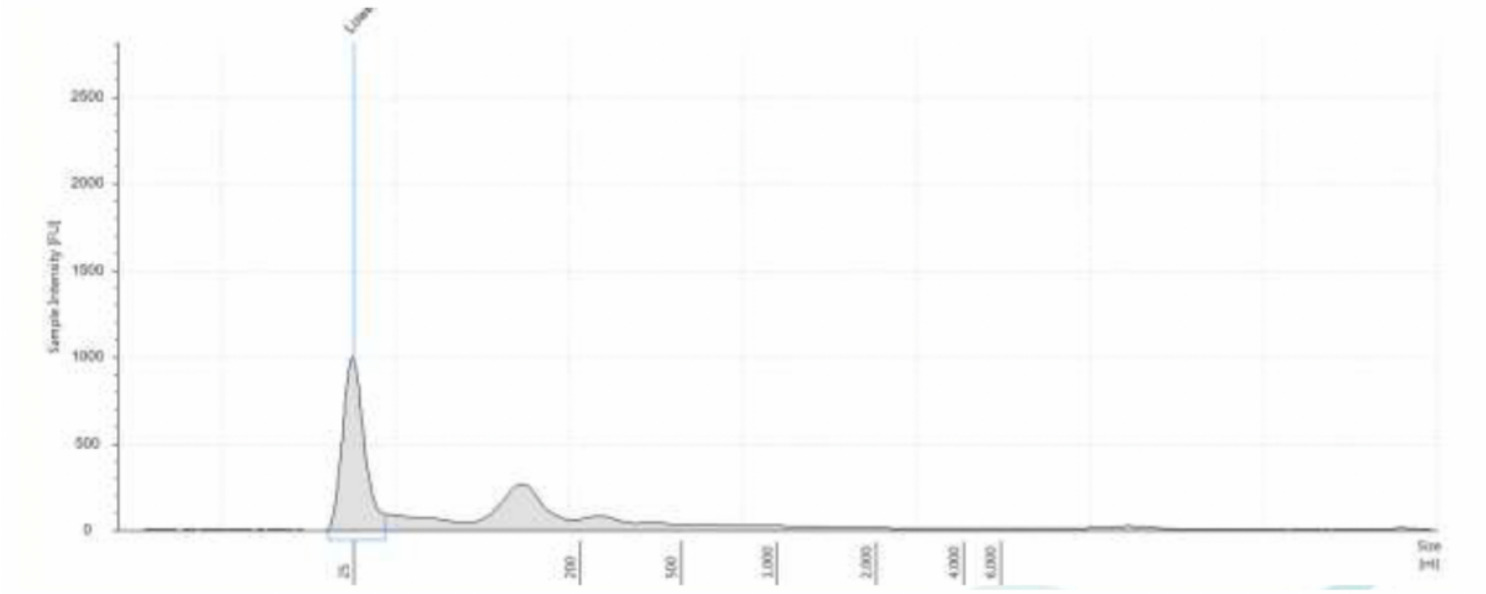

Figure 2

Sequence length distribution analysis of EVs by RNA-Seq.

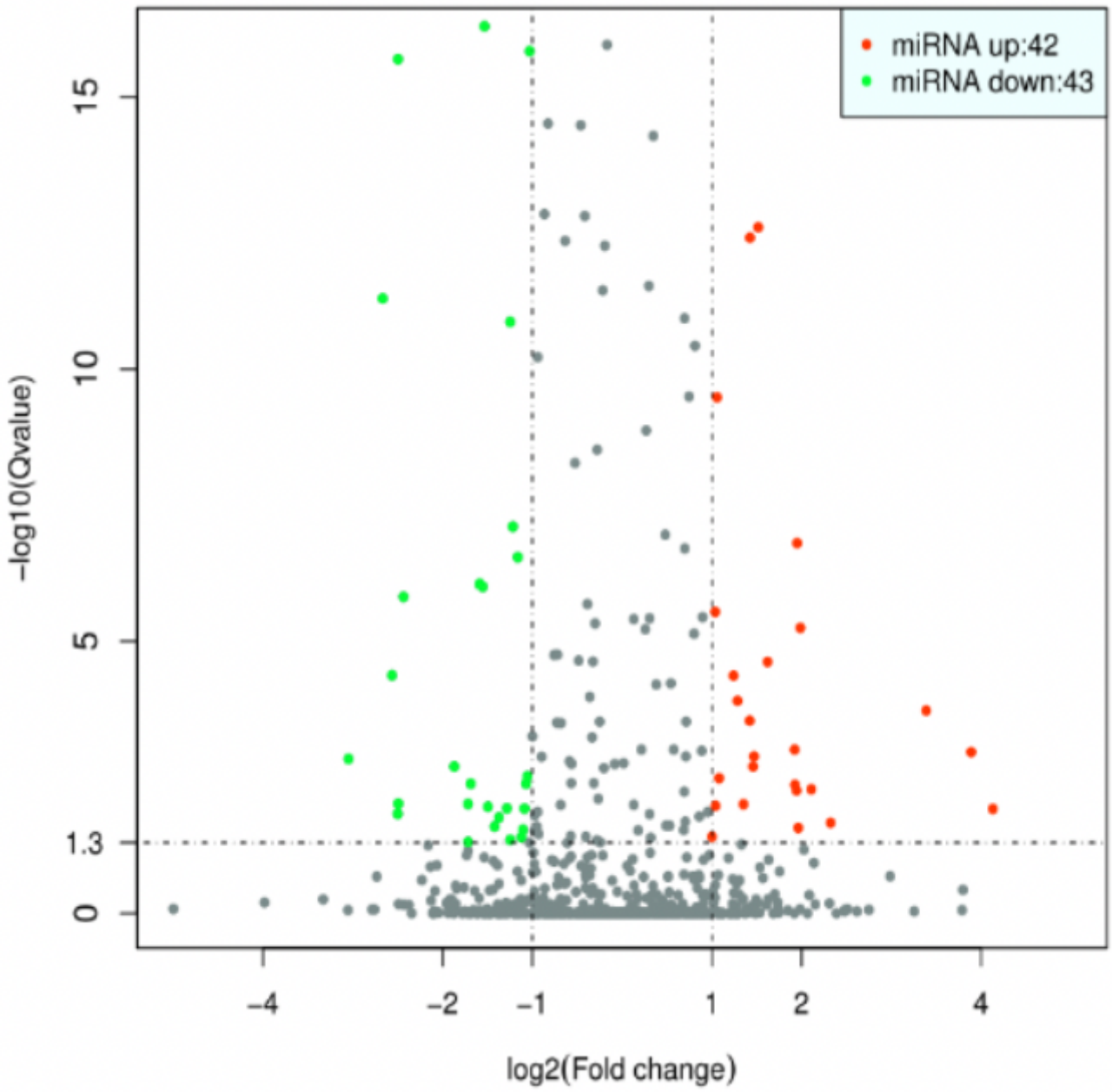

Figure 3

Volcanic diagram of miRNA differential expression analysis among samples. The horizontal line indicates P value of 0.05, the vertical lines correspond to 2-fold upregulation and downregulation.

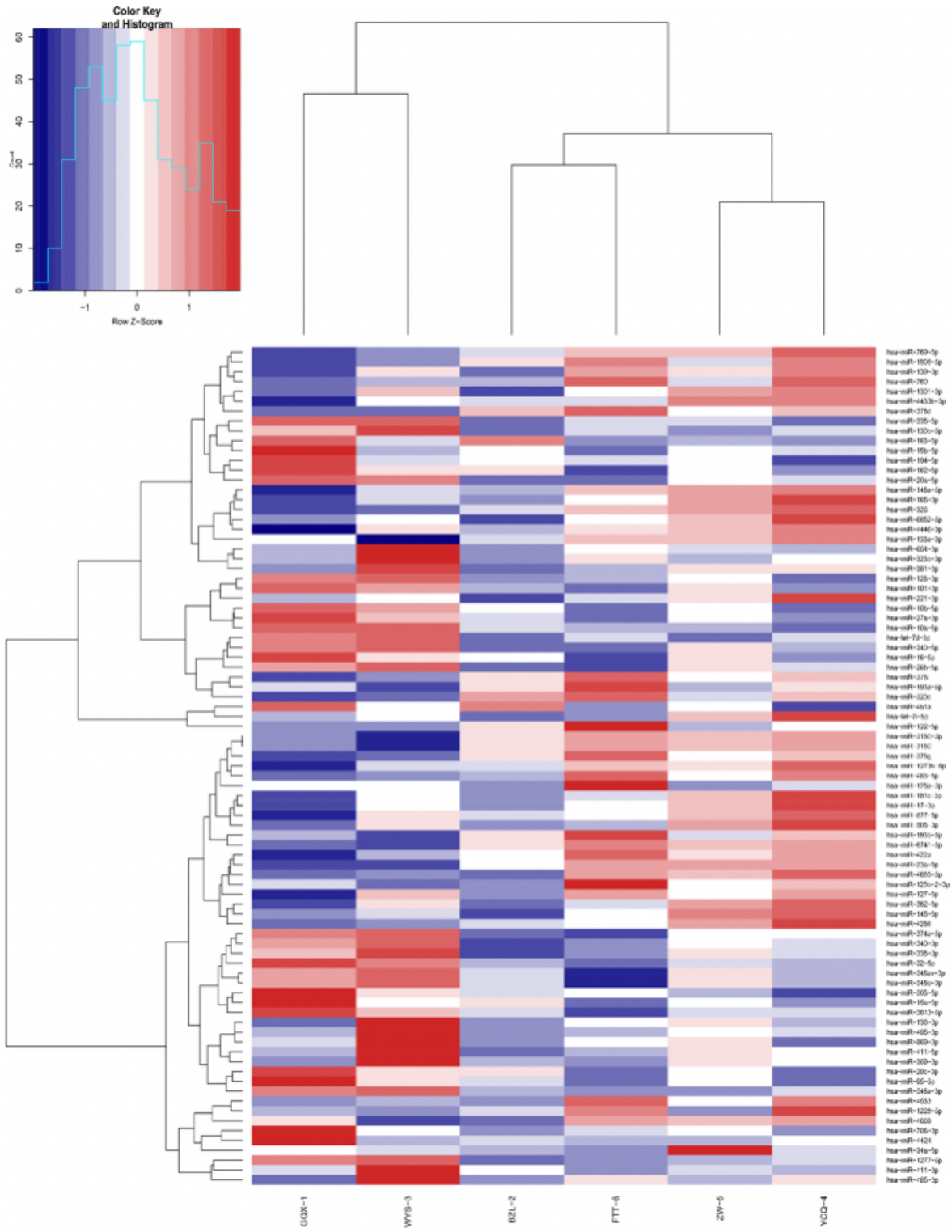

Figure 4

Heatmap showing 85 miRNAs with significantly different expression (Q < 0.05, P < 0.05, | log 2 (FoldChange) | ≥ 1) between AAA plasma EVs and the control.

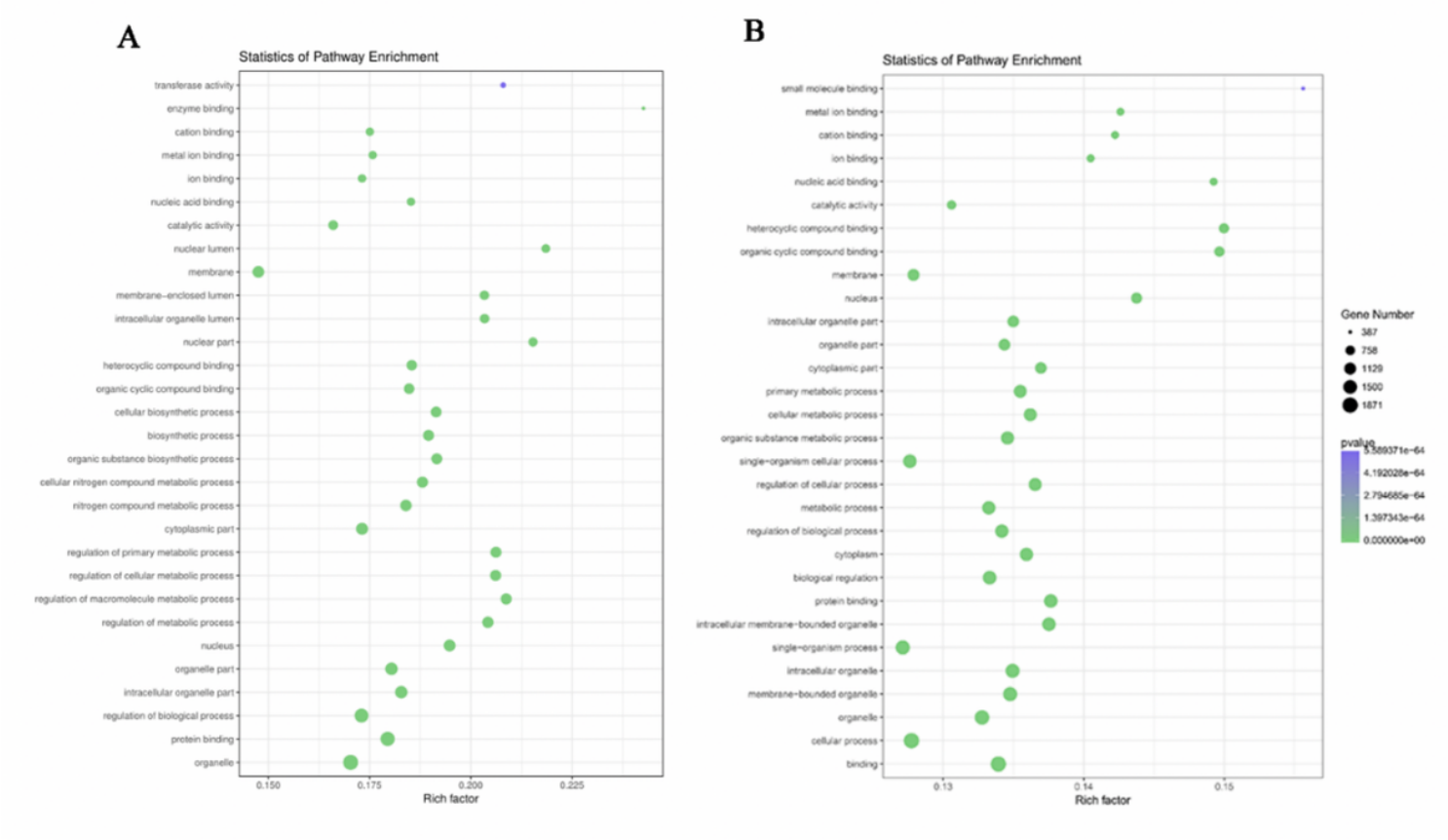

Figure 5

Function enrichment of the predicted genes in the GO. A Targets of down-regulated miRNAs GO enrichment analysis results. B Targets of up-regulated miRNAs GO enrichment analysis results.

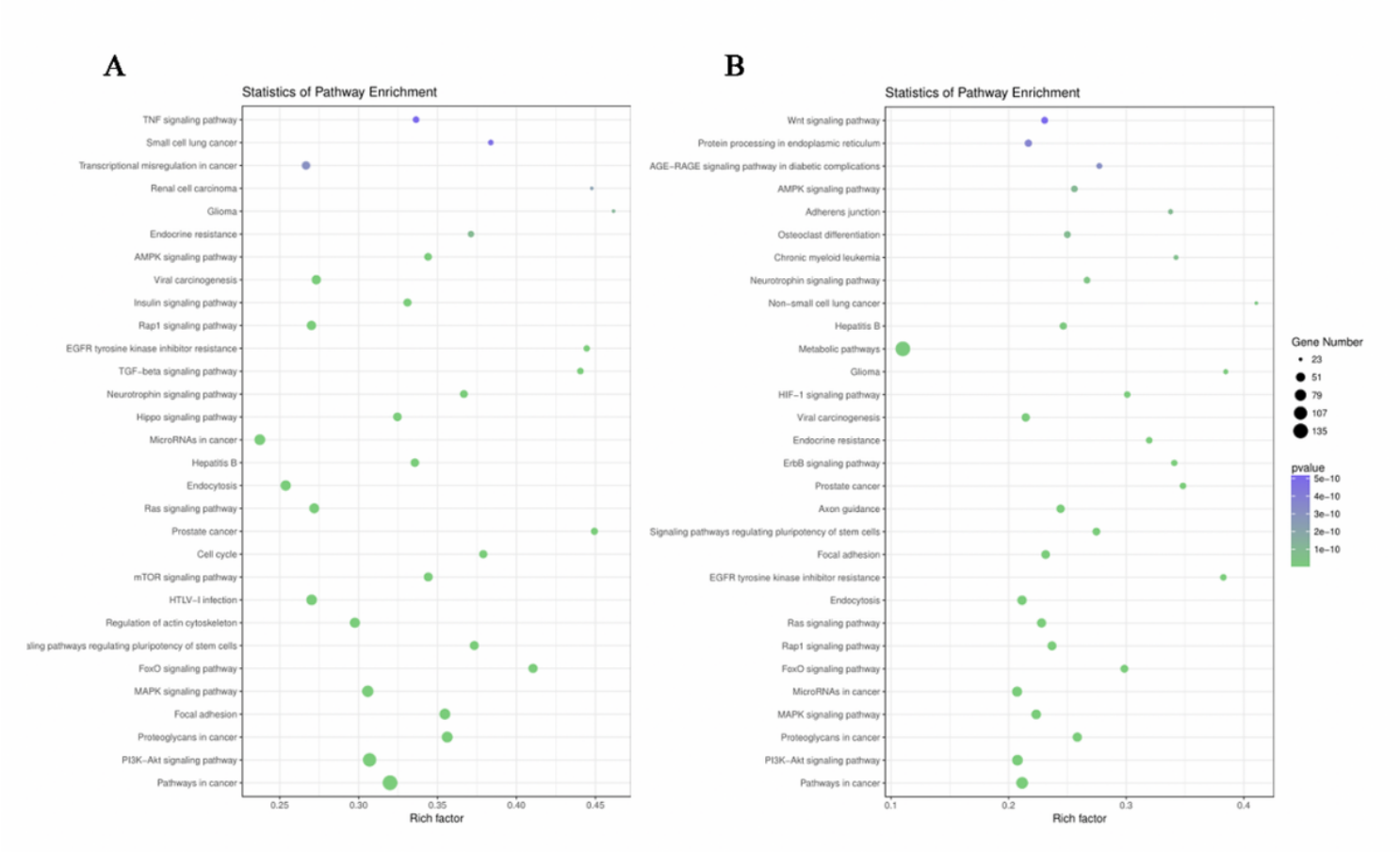
A
Statistics of Pathway Enrichment
TNF signaling pathway
Small cell lung cancer
Transcriptional misregulation in cancer
Renal cell carcinoma
Glioma
Endocrine resistance
AMPK signaling pathway
Viral carcinogenesis
Insulin signaling pathway
Rap1 signaling pathway
EGFR tyrosine kinase inhibitor resistance
TGF-beta signaling pathway
Neurotrophin signaling pathway
Hippo signaling pathway
MicroRNAs in cancer
Hepatitis B
Endocytosis
Ras signaling pathway
Prostate cancer
Cell cycle
mTOR signaling pathway
HTLV-I infection
Regulation of actin cytoskeleton
xling pathways regulating pluripotency of stem cells
FoxO signaling pathway
MAPK signaling pathway
Focal adhesion
Proteoglycans in cancer
PI3K-Akt signaling pathway
Pathways in cancer
0.25
0.30
0.35
0.40
0.45
Rich factor
B
Statistics of Pathway Enrichment
Wnt signaling pathway
Protein processing in endoplasmic reticulum
AGE-RAGE signaling pathway in diabetic complications
AMPK signaling pathway
Adherens junction
Osteoclast differentiation
Chronic myeloid leukemia
Neurotrophin signaling pathway
Non-small cell lung cancer
Hepatitis B
Metabolic pathways
Glioma
HIF-1 signaling pathway
Viral carcinogenesis
Endocrine resistance
ErbB signaling pathway
Prostate cancer
Axon guidance
Signaling pathways regulating pluripotency of stem cells
Focal adhesion
EGFR tyrosine kinase inhibitor resistance
Endocytosis
Ras signaling pathway
Rap1 signaling pathway
FoxO signaling pathway
MicroRNAs in cancer
MAPK signaling pathway
Proteoglycans in cancer
PI3K-Akt signaling pathway
Pathways in cancer
0.1
0.2
0.3
0.4
Rich factor
Gene Number
23
51
79
107
135
pvalue
5e-10
4e-10
3e-10
2e-10
1e-10

Figure 7