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Combination of three microRNAs as serum diagnostic panel for bladder urothelial carcinoma

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Abstract

OBJECTIVE

Bladder carcinoma (BC) is a malignant tumor that is formed in the bladder of the genitourinary system. The diagnosis at an early stage is directly associated with the improved overall survival of BC patients because a later stage usually means a poorer prognosis. Current methods to diagnose BC have various limitations, thus urologists call for novel effective non-invasive diagnostic markers. Herein, we identified miRNAs which can be used for the diagnosis of BC.

MATERIALS AND METHODS

Patients with BC (n = 112) and healthy individuals (n = 112) were recruited and enrolled in this study. The quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was carried out for the measurement of miRNAs expression in serum. A two-phase test was proceeded for the identification, selection, and confirmation of the miRNAs that could be used for BC diagnosis. A backward stepwise logistic regression (BSLR) was conducted to establish a model containing these miRNAs with superior diagnostic performance. In addition, bioinformatics and survival analysis was exerted by analyzing database in Mirwalk, Enrichr, and OncoLnc.

RESULTS

Five significantly aberrant miRNAs with good diagnostic value were validated, namely miR-129-2-3p, miR-29c-3p, miR-149-3p, miR-138-5p, and miR-194-5p. Then three of them (miR-129-2-3p, miR-29c-3p, and miR-149-3p) were used to establish a diagnostic panel, in which the area under the curve (AUC) was 0.927 (95% CI: 0.876 to 0.962), providing both high sensitivity (92.68%) and specificity (80.49%).

CONCLUSION

In this study, a panel of three miRNAs (miR-129-2-3p, miR-29c-3p, and miR-149-3p) was developed, which could be used for the diagnosis of BC sensitively and specifically.

1.Introduction

Bladder carcinoma (BC), also named bladder cancer or urothelial carcinoma, is a malignant tumor that is formed in the bladder of the genitourinary system. According to recent evidence, the crude, age-standardized by China standard population and by world standard population rates were $5.80/10^5$, $3.60/10^5$ and $3.57/10^5$ for incidence, and $2.37/10^5$, $1.31/10^5$ and $1.32/10^5$ for mortality, respectively, ranking the 13(th) most common cancer in China and 7(th) in male[1]. Generally, BC can be subdivided as

the muscle-invasive BC (MIBC) and non-muscle-invasive BC (NMIBC). About 75% of BC patients are initially diagnosed with NMIBC with a 5-year survival rate that is as high as 80% [2]. However, 50–70% of them are still projected to experience recurrence, and 10–20% of them even will progress to MIBC [3]. MIBC patients often present high mortality (nearly 40% in 5 years) despite systemic therapies, including radical surgery [4]. Therefore, early diagnosis is essential to improve treatment outcomes for BC patients.

For decades, the gold standard for BC diagnosis has been cystoscopy, which is very reliable and sensitive. However, cystoscopy may be inconclusive under many circumstances and is limited by the experience of the technician. Moreover, it is an invasive procedure and causes significant pain, stress, and anxiety to patients. As an important supplement to cystoscopy, voided urine cytology is also commonly used as a tool to detect BC. Though cytology presents a high specificity of 90–95%, due to the pain caused by the diagnosis with cystoscopy and the poor sensitivity for low-grade BC (30–40%), most of the BC patients do not like to subject to this diagnostic method [5, 6]. Several other markers based on the non-invasive urine, including the fluorescence in situ hybridization (FISH), bladder tumor antigen (BTA), and the nuclear matrix protein 22 (NMP22), have also been developed to diagnose BC [7], but none of them could be compared to cystoscopy because of their relatively low sensitivity or specificity [8, 9]. The ideal screening test should be non-invasive, rapid, easy to conduct, and at the same time, have high sensitivity and specificity. For urologists, identification of this kind of markers for effective BC diagnosis is urgently needed.

MicroRNAs (miRNAs) are a non-coding small single-stranded RNAs that are vital for regulation of gene expression at the post-transcriptional level [10]. Growing evidence has demonstrated that miRNAs are aberrantly expressed in many malignancies and participate in almost all of the known hallmarks of tumorigenesis [11–14]. Due to the easy detection in plasma and serum, miRNAs might be used as biomarkers for the diagnosis of cancers [15]. In fact, growing evidence have shown that, in comparison to healthy controls, BC patients exhibit dramatically abnormal expression of some miRNAs [16–20], suggesting the important value of miRNAs for the diagnosis of BC. There are also few reports about serum miRNAs in BC diagnosis, post-operation surveillance or prognosis prediction [21–24], but many limitations, such as lack of validation, narrow coverage, and small sample size, are among these studies. Therefore, further explorations about the association of serum miRNAs and BC diagnosis should be done.

In this study, a two-phase work was carried out for the identification of the miRNAs which are closely involved in the BC diagnosis. We identified 9 miRNAs and revealed their diagnostic values for BC. After initial testing in 30 BC and 30 healthy controls (HCs) serum samples, we selected 5 miRNAs and further identified them in more samples in the following validation stage. Next, 3 of the 5 miRNAs were applied for the construction of the final logistic regression model, with an ideal area under the curve (AUC), which lead to a model of miRNA panel to diagnose BC with significant accuracy.

2.Materials and methods

2.1 Control subjects and Patients

All procedures and protocols used in this study were reviewed and approved by the Peking University Shenzhen Hospital Ethics Committee (No. 2021016). Patients (n = 112) diagnosed with BC based on histopathology from November 2017 to January 2021 were enrolled in this study. Another 112 individuals without any cancer or other diseases were also enrolled as healthy controls (HC). Informed consent form was signed by each participant. No administrations, such as radiotherapy or chemotherapy were given to enrolled BC patients, nor these patients were subjected to surgery, before serum sample collection. Based on the tumor-node-metastasis (TNM) classification system, we confirmed the stages of all enrolled BC patients based on histopathology. Clinical characteristics of each participants were collected, including age, gender and histologic grade. During our two-phase testing, we included 30 HCs and 30 BC patients in our first test phase and included the remaining HCs and BC patients (n = 82 of each group) in the validation phase.

2.2 Collection and preparation of serum samples

After collection of venous blood (5 mL), 10-minute centrifugation at 3,000 rpm, and another 15-minute centrifugation at 12,000 rpm, supernatants of each blood sample were collected and stored at -80°C for future examinations.

2.3 Study guideline

In this study, a two-phase procedure was constructed for the identification of the diagnostic values of miRNAs in serum for BC (Fig. 1).

Testing phase

A total of 9 candidate miRNAs were obtained from microarray data published on the Gene Expression Omnibus database or reports on PubMed, with retrieval cues of ((“MicroRNAs” [Mesh]) OR miRNA) AND ((“Urinary Bladder Neoplasms” [Mesh]) OR [bladder cancer] OR [bladder tumor]). The criteria of selection included no assessment in blood samples at the same time and the abnormal expression verified by other studies in BC tissues. Expression level of the 9 selected miRNAs in serum collected from the BC patients and HCs (n = 30 or each group) were measured by qRT-PCR.

Validation phase

Serum samples from the remaining patients and HCs (n = 82 of each) were used to validate the expression of the miRNAs selected in the validation phase. Also, for each miRNA, we drew their Receiver Operating Characteristic (ROC) curves. After the confirmation of these miRNAs’ diagnostic values (p-value < 0.05), these miRNAs were used for the construction of the panel for diagnosis.

Construction of the miRNA panel for BC diagnosis

To construct the panel model for BC diagnosis sensitively and specifically, we conducted a backward stepwise logistic regression (BSLR) using the validated miRNAs. Then, bioinformatics analysis of the

miRNAs in the final model were performed, including the prediction of target genes and the annotation of functional enrichment.

2.4 RNA isolation and RT-qPCR detection

A cel-miR-54-5p (2 μl) supplied by RiboBio (China) was added into each sample before the experiments for the normalization of changes caused by different efficiency of each experiment, such as qPCR detection, reverse transcription, and RNA extraction. RNA was extracted from serum using TRIzol (Invitrogen, CA, USA), then concentration and purification were examined by NanoDrop 2000c (Thermo Fisher Scientific, Waltham, MA, USA). Reverse transcription of RNA was applied with HG TaqMan miRNA Real-time PCR Kit (HaiGene, China), and the expression of miRNAs was determined using SYBR Pre-mix Ex Taq II (TaKaRa, Dalian, China). RT-qPCR thermocycling was conducted on the Roche Light Cycler 480 Real-Time PCR system (Roche Diagnostics, Mannheim, Germany), with the following conditions: 95°C for 15 min; 40 cycles of 94°C for 15 sec; 55°C for 30 sec; and 72°C for 30 sec. We analyzed the relative expression level of miRNAs using the $2\text{-}\Delta\Delta Cq$ method [25].

2.5 Statistics

All statistical analyses of this study were performed with SPSS 20.0 (IBM SPSS) and p-values less than 0.05 was set as significant difference. Data are presented as number and percentage or mean ± standard deviation (SD). The demographic and clinical manifestation (gender, age and pathological grade), as well as difference between the miRNA expression levels within BCs and HCs, were assessed using Student's *t*-test or Wilcoxon-Mann Whitney test. Through the analysis of the ROC curve, we obtained the AUC and used these AUC values for the evaluation of cutoff values, specificity, and sensitivity of each miRNA. A good agreement of the golden standard with the miRNAs was identified through the conduction a consistency check. In addition, the analysis of survival was conducted with data from OncoLnc dataset, using Kaplan–Meier curve and two-sided log-rank test. MiRWalk 3.0 database were used to predict target gene candidates for each miRNA. And finally, the optimal panel that could distinguish BC from healthy controls with the highest sensitivity and specificity was established by BSLR.

3.Results

A total of 112 BC patients and 112 HCs were enrolled in this study. Table 1 shows the collected clinical characteristics from patients, including age, gender and histologic grade. Through comparison analysis, we confirmed that the differences of age and gender distribution were not significant between two phases of either group ($p > 0.05$).

Table 1
Demographic and clinical manifestation of 224 participants (BC and HC)

	Testing Phase (n = 60)			**Validation Phase** (n = 164)		
	BC	HC		BC	HC	
Total Number	30	30		82	82	
Age at diagnosis	63.3 ± 13.4	63.6 ± 10.6	p = 0.92	61.6 ± 13.5	63.4 ± 9.9	p = 0.35
Gender Male	16(53.3%)	14(46.7%)	p = 0.61	46(56.1%)	44(53.7%)	p = 0.76
Female	14(46.7%)	16(53.3%)		36(43.9%)	38(46.3%)	
Histologic grade						
High	19(63.3%)			40(48.8%)		
Low	11(36.7%)			42(51.2%)		

Among three stages, there was no significant difference between BC and HCs in age and gender. Parameters were shown as number (percentage). Statistical contrast was exerted through the Wilcoxon-Mann Whitney test.

3.1 Testing phase

In this phase, we identified and selected 9 miRNAs by searching in the literature, and confirmed them in the serum of the 30 BC patients and 30 HCs. As shown in Fig. 2, 5 miRNAs with dramatically abnormal expression ($p < 0.05$) were identified, namely miR-129-2-3p, miR-29c-3p, miR-138-5p, miR-149-3p, and miR-194-5p. Among these 5 miRNAs, BC patients exhibited dramatically increased miR-129-2-3p and reduced miR-29c-3p, miR-138-5p, miR-149-3p, and miR-194-5p expression compared to those in HCs.

3.2 Validating phase

In this phase, the remaining BC patients (n = 82) and HCs (n = 82) samples were used for the examination of the 5 identified miRNAs expression to further confirm our results obtained before. All the five miRNAs showed dramatically different expression, and their up-regulation or down-regulation were similar to the testing phase. Then, ROC curves were drawn for each miRNA for further examination of their diagnostic value, and results are shown in Table 2 and Fig. 3. Again, all these 5 miRNAs showed good diagnostic ability ($p < 0.05$) and none was excluded. Then, a final panel for BC diagnosis was constructed using these selected miRNAs.

Table 2
Outcomes of ROC analysis and Youden index for 5 candidate miRNAs and the three-miRNA panel.

	AUC	P value	95% CI	Associated criterion	Sensitivity (%)	Specificity (%)
miR-129-2-3p	0.791	< 0.001	0.721–0.850	> 1.08	82.93	68.29
miR-29c-3p	0.715	< 0.001	0.639–0.783	≤ 0.98	79.27	56.1
miR-138-5p	0.626	0.0037	0.548–0.701	≤ 1.08	87.8	36.59
miR-149-3p	0.824	< 0.001	0.757–0.879	≤ 0.79	73.17	80.49
miR-194-5p	0.709	< 0.001	0.633–0.777	≤ 1.0	84.15	51.22
three-miRNA panel	0.927	< 0.001	0.876–0.962	> 0.44671	92.68	80.49

AUC: area under curve; CI: confidence interval.

3.4 Construction of the miRNA panel for diagnosis

To construct a final diagnostic panel, BSLR was conducted based on these 5 miRNAs selected on the testing phase. Finally, we incorporated 3 miRNAs, including miR-129-2-3p, miR-29c-3p, and miR-149-3p, in our miRNAs panel model. MiR-138-5p and miR-194-5p were removed from the final regression model, to obtain the best panel to screening BC. The formula constructed the miRNA panel was:

Logit (P) = 3.226 + 6.373 × miR-129-2-3p − 5.905 × miR-29c-3p − 6.052 × miR-149-3p.

We also drew the final ROC curve which is shown in Fig. 4. The AUC value was 0.927 (95% CI: 0.876 to 0.962; sensitivity = 92.68%, specificity = 80.49%), which may be used as diagnostic tool.

3.5 Bioinformatic analysis of candidate miRNAs

We predicted target gene candidates for each miRNA using miRWalk 3.0 database. A total of 1393 genes were elected as target genes with the criteria of gene prediction in more than 2 miRNAs (Fig. 5A). When gene prediction was required in all the 3 miRNAs, 50 target genes were finally chosen, and then searched in GEPIA database. There was the relative expression of genes in 404 BC patients and 28 HCs. Then with the criteria of $|log2FC| > 1$ and $p < 0.01$, results from GEPIA database discovered six differently expressed genes in BC patients compared to HCs, which were CREBRF, NRG2, PRICKLE2, STXBP6, SYNPO2, and ZBTB47 (Fig. 5B-G). They could be regarded as the latent target genes for the three-miRNAs panel.

Furthermore, we predicted the target genes using our constructed model based on the 3 miRNAs and conducted the enrichment analysis of Kyoto encyclopedia of genes and genomes (KEGG) pathways and the annotation of Gene Ontology (GO) including Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). Figure 6 shows the top 10 rankings of GO annotation and KEGG pathways.

3.6 Survival analysis of candidate miRNAs

We searched the data of 404 BC patients from OncoLnc database and compared BC survival of patients with differently expressed miRNAs using Kaplan–Meier curve and two-sided log-rank test. As the data of miR-129-2-3p was unavailable from the database, we merely analyzed data of miR-29c-3p and miR-149-3p. Results manifested significant association between miR-29c-3p and the survival rate of BC. Patients with lower miR-29c-3p expression level tended to have worse prognosis (Fig. 7, $p = 0.0285$). But the result of miR-149-3p was negative, that is, BCs with lower miR-149-3p expression showed no significant different prognosis compared to higher expression ($p = 0.163$).

4.Discussion

BC remains a severe threat to human health because of its high morbidity and mortality[1]. Muscle invasion and metastasis are related to poor diagnosis, making early diagnosis of BC extremely important. For decades, the golden standard of BC diagnosis has been the cystoscopy, a painful procedure which is limited by the experience of technician[2]. Non-invasive markers for BC diagnosis are urgently needed. Due to the significant connections of miRNAs with tumorigenesis, miRNAs were considered as potential candidates for the BC diagnosis[26]. They may modulate protein synthesis by inhibiting or promoting the transcription of messenger RNA, thus acting as an oncogene or a suppressor gene [27–29]. Due to the stable existence and abundance in serum, miRNAs can be used as biomarkers for disease diagnosis with low invasiveness and high efficiency [15]. Remarkable observations and achievements were obtained by previous studies in this field, however the use of miRNAs in BC diagnosis is not clearly explored. Herein, a panel model containing 3 miRNAs was constructed for the effectively and specifically diagnosis of BC, suggesting that they can be used as biomarkers in the clinic.

By literature search, we initially selected 9 miRNAs, all of which have been proved, by previous studies, to play significant roles in the occurrence and progression of BC. Each of them was differently expressed in BC tissues, but there was no evidence of their relevance in blood, until now. In this study, we tested these miRNAs for their potential value as diagnostic serum markers step by step. Five of them were validated to have a good diagnostic ability. However, it was not easy to find a single miRNA with both good sensitivity and specificity, as shown in Table 2. To overcome this problem, some previous studies developed a novel method of miRNAs combination, and the results seemed satisfying [30, 31]. Thus, we tried a BSLR with the 5 validated miRNAs and successfully built a model with 3 of them. The AUC value of this panel was 0.927, and the sensitivity and specificity were 92.68% and 80.49%, respectively, for BC diagnosis. Due to the limitations of the mentioned cytology and urinary markers for BC diagnosis [8], this panel model constructed in our study has potential and important values for BC diagnosis compared to the above

existing methods. Although this study disclosed some new promising findings, there were several limitations. First, because of the same source of samples, possible bias of selection might be formed. Second, the potential target genes of these miRNAs and the underlying mechanisms are not clearly explored directly. Finally, only 9 miRNAs were initially included in this study, which may not cover many of the potential miRNAs. More serum miRNAs should be adopted in our future studies.

As one of the miR-129 family members, miR-129-2-3p significantly participates in the regulation of cell growth in various cancers, and usually together with other family members, including miR-129-1-3p, miR-129-2-3p, and miR-129-5p [32–34]. Yu *et al.* study observed the dramatically reduced expression of miR-129-2-3p in gastric cancer cells compared to normal gastric epithelial cells [33]. In breast cancer, a study indicated that by sponging miR-129-2-3p, Circ-TFF1 regulated the expression of IRAK1, thereby promoting the development of breast cancer [35]. The suppressive effect of miR-129-2-3p on the pathogenesis of intrahepatic cholangiocarcinoma by direct interaction with Wip was identified by Chen *et al*. [36]. As to bladder cancer, a previous study showed that, due to the abnormal expression of miR-125b, miR-99a, miR-214, miR-205, miR-200a, and miR-129, they can be used as prognostic and diagnostic biomarkers in urine for BC. This study proclaimed serum miR-129-2-3p may be used as a diagnostic biomarker for BC.

As one of the panel miRNAs, miR-29c-3p has been previously reported to play important roles in tumorigenesis and development of several cancers, including esophageal cancer [37], hepatocellular cancer [38], ovarian cancer [39], as well as bladder cancer [40]. Mostly, miR-29c-3p acts as a tumor suppressor, inhibiting cell proliferation, migration, and invasion via several pathways. In tissues from patients with bladder cancer, the mir-29c-3p expression was down-regulated and TUG1 was significantly up-regulated with a negative correlation between their expressions. Also mir-29c-3p proved to target TUG1, affecting the migration and invasion of bladder cancer cells by regulating the expression of CAPN7 [40]. Because of the stable expression in urine of the BC patients and HCs, miR-29c-3p can be used as a inter reference for further comparison of other miRNAs in urine of BC patients and HCs [41]. Interestingly, a previous report proved that miR-29c-3p is one of the 9 identified miRNAs dysregulated in clinical samples. They also exhibited significantly prognostic and predictive potentials in patients with BC [42]. Yet, diagnostic values of this panel are not confirmed. Regardless of these findings, no study about the evaluation of miR-29c-3p expression in serum from the BC patients were conducted before. Thus, this is the first study to clarify the association of miR-29c-3p in serum with BC. In addition, we also found some interesting results about miR-29c-3p and BC prognosis. Data from OncoLnc showed that BCs with lower miR-29c-3p expression level tended to have worse prognosis ($p = 0.0285$), suggesting that studies for its prognostic values in BC patient are also needed.

Another member included in the panel was miR-149-3p. Similar to miR-29c-3p, this is also a tumor suppressor, according to some previous studies[43, 44]. The RNA sequencing analysis also demonstrated that miR-149-3p and some other passenger miRNAs (miR-144-5p, miR-145-3p, miR-199a-3p, and miR-150-3p) have potentially tumor-suppressive effect in many kinds of cancer cells [45]. In BC tissue, a study proved that miR-149-3p could effectively reverse the promotion of cell invasion, migration, and growth caused by S100A4 [43]. Though downregulated in BC tissue as a tumor suppressor, it was unclear

whether serum miR-149-3p would show low expression. Now this question seems be preliminary answered by our study.

MiR-138-5p and miR-194-5p were also among the 5 validated miRNAs. Yang *et al*. found that miR-138-5p is significantly connected to the processes of cell invasion and growth through direct regulation of survivin, playing a role as a tumor suppressor in bladder cancer by inhibiting BIRC5 translation [46]. Also, miR-138-5p was among a panel of 5 miRNAs which could be used as a putative diagnostic tool for muscle-invasive bladder cancer in tumor samples and urinary extracellular vesicles [47]. MiR-194-5p proved to directly target E2F3 and inhibits cell migration and invasion in BC [48]. The enhanced sensitivity of BC cells to cisplatin, impaired cell growth, and increased cell apoptosis, were observed after miR-194-5p overexpression or TUG1 suppression [49]. In our study, both serum miRNAs were validated to be relevant to BC diagnosis, but neither of them possessed satisfying specificity and sensitivity. In fact, their value of diagnosing BC was lower when compared to other 3 miRNAs (miR-129-2-3p, miR-29c-3p, and miR-149-3p), and they were removed when building the final regression model. Nevertheless, they may still display potential value in some other diagnostic or prognostic panels, as well as providing novel treatment targets in BC.

According to the results of GO functional annotation and KEGG pathway enrichment, we found that miR-129-2-3p, miR-29c-3p, and miR-149-3p may be significantly correlated to various pathways, for example, forkhead box class O (FoxO) signaling pathway. Many previous studies demonstrated that FOXOs had highly conserved forkhead DNA-binding domain[50], mediated a variety of cellular processes like apoptosis, proliferation, cell cycle progression and DNA damage, and were widely implicated in many cancers, including bladder cancer[51]. We also searched data of these 3 miRNAs using miRWalk 3.0 and Enrichr database and discovered six differently expressed genes, which were CREBRF, NRG2, PRICKLE2, STXBP6, SYNPO2 and ZBTB47. They could be regarded as the latent target genes for the three-miRNA panel. Of course, these results are still insufficient for understanding the molecular mechanisms of these miRNAs, but may offer novel clue and help for further research in the future.

5. Conclusion

We discovered 5 significantly aberrant miRNAs in serum with good value for BC diagnosis, namely miR-129-2-3p, miR-29c-3p, miR-149-3p, miR-138-5p, and miR-194-5p. Also, we constructed three miRNAs (miR-129-2-3p, miR-29c-3p, and miR-149-3p) containing panel model for the diagnosis of BC sensitively and specifically.

Declarations

Acknowledgements

Not applicable.

Author contributions

YL designed this study and performed manuscript review; RL and HL summarized and analyzed the results; XL, CL and ZG interpreted all the clinical and laboratory data; HL was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Statement of Ethics

All the procedures of this work were approved by the Ethics Committees of Peking University Shenzhen Hospital (decision reference number JCYJ2017001), and written informed consent for participate in the study was obtained from every participant.

Patient consent for publication

No identifying information of participants was included in the manuscript and written informed consent for publication was obtained from every participant.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Data Availability Statement

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Figures

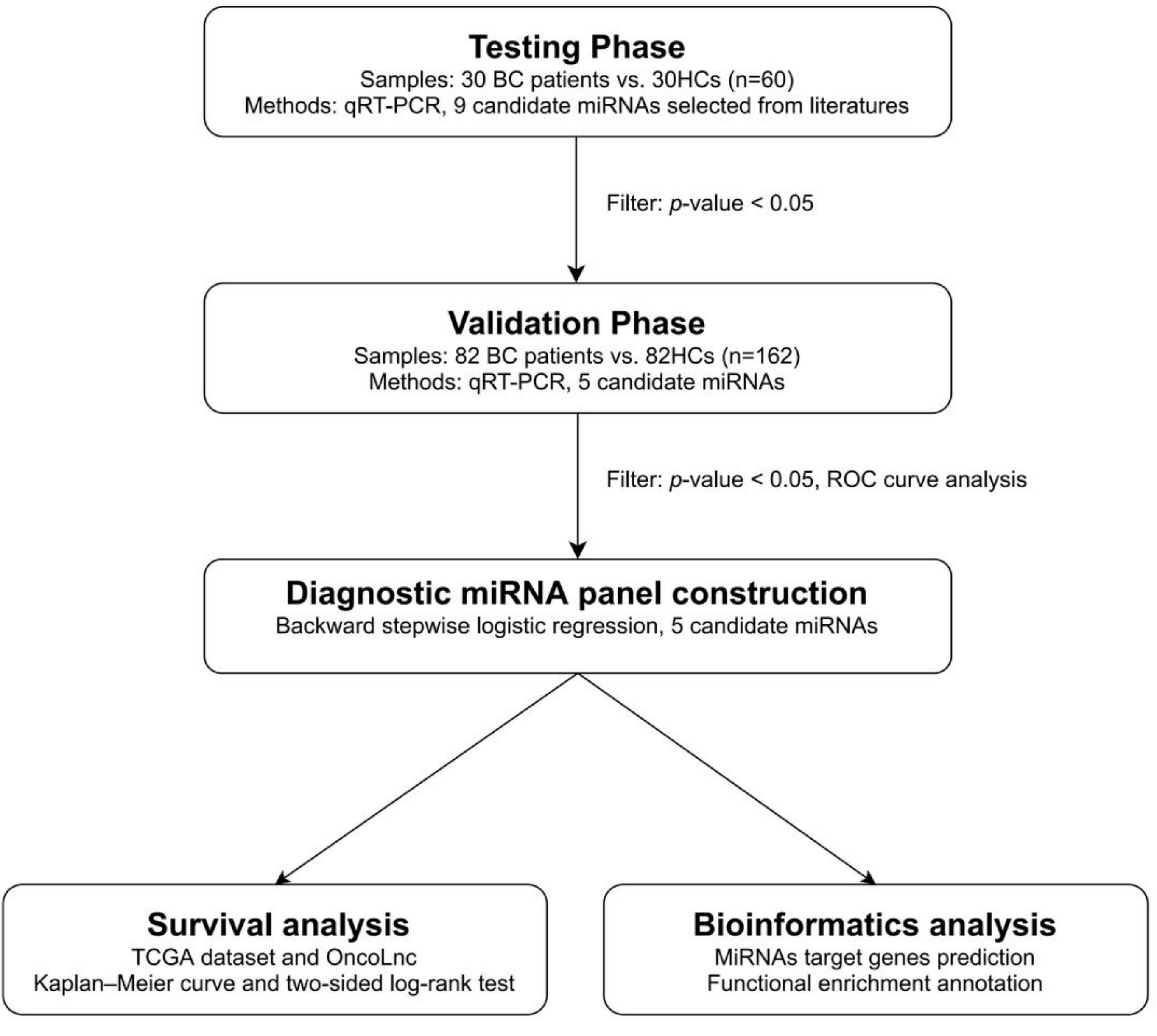

Figure 1

Overview of the study design. BC, bladder cancer; HC, healthy control.

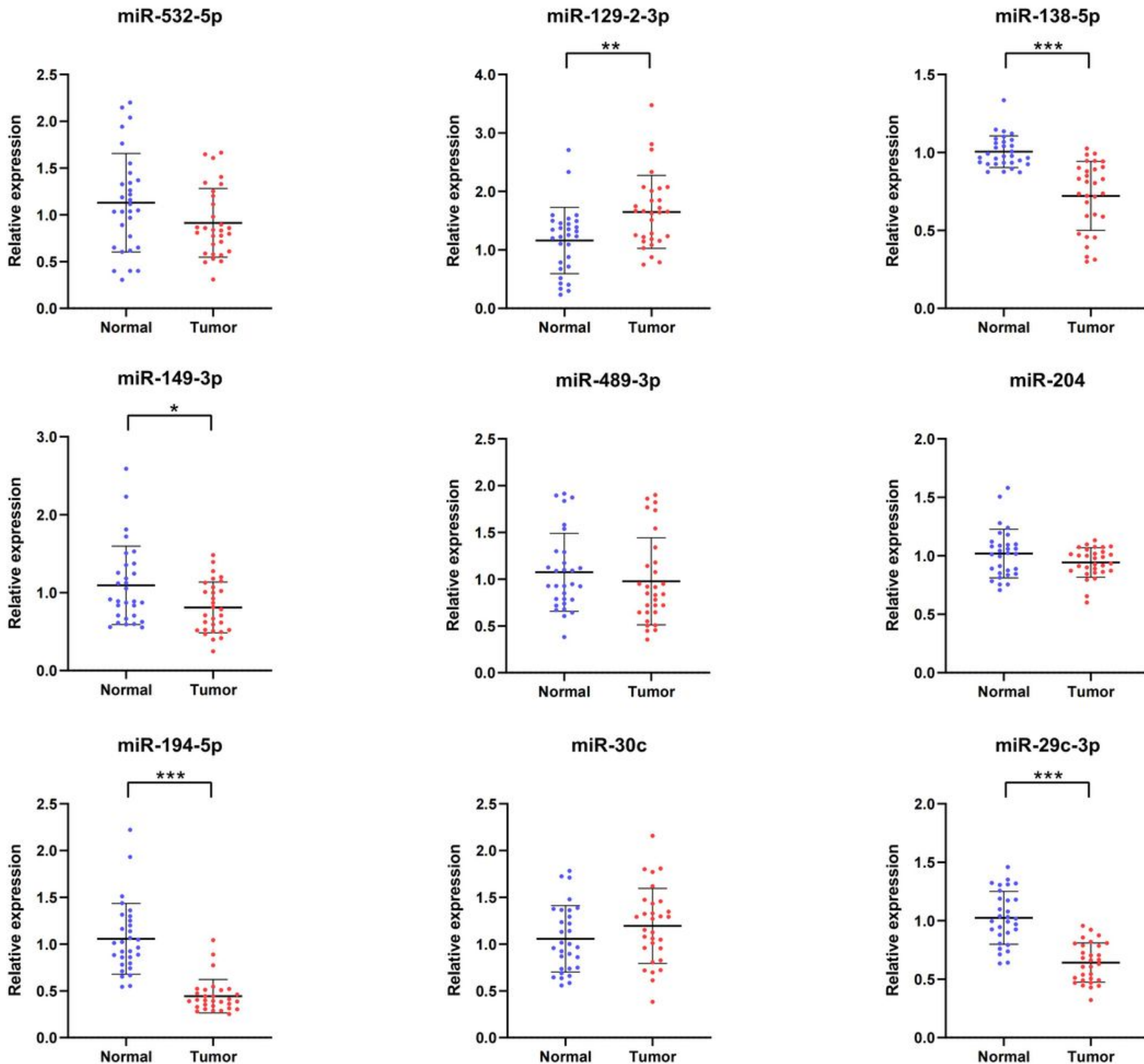

Figure 2

9 serum miRNAs relative expression level in the testing stage. Serum samples of 30 BC patients and 30 HCs were analyzed. 5 miRNAs with significantly different expression between BC patients and HCs were identified as candidate miRNAs.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

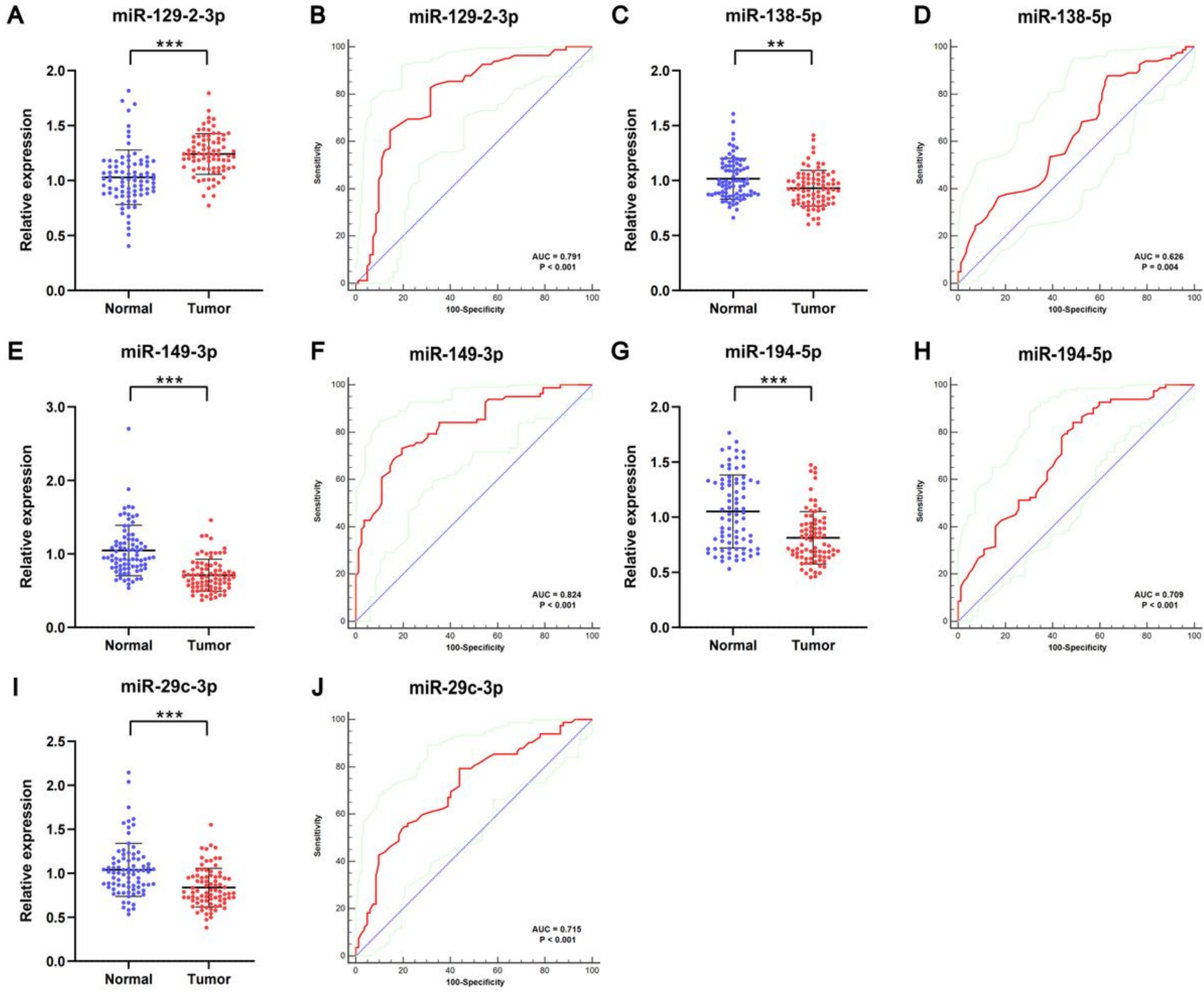

Figure 3

Serum expression level of 5 candidate miRNAs in HCs and BC samples in validation stage and their ROC curves, with 82 BC sample and 82 HCs. (A) expression of miR-129-2-3p, (B) ROC curve of miR-129-2-3p, (C) expression of miR-138-5p, (D) ROC curve of miR-138-5p, (E) expression of miR-149-3p, (F) ROC curve of miR-149-3p, (G) expression of miR-194-5p, (H) ROC curve of miR-194-5p, (I) expression of miR-29c-3p, (J) ROC curve of miR-29c-3p.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

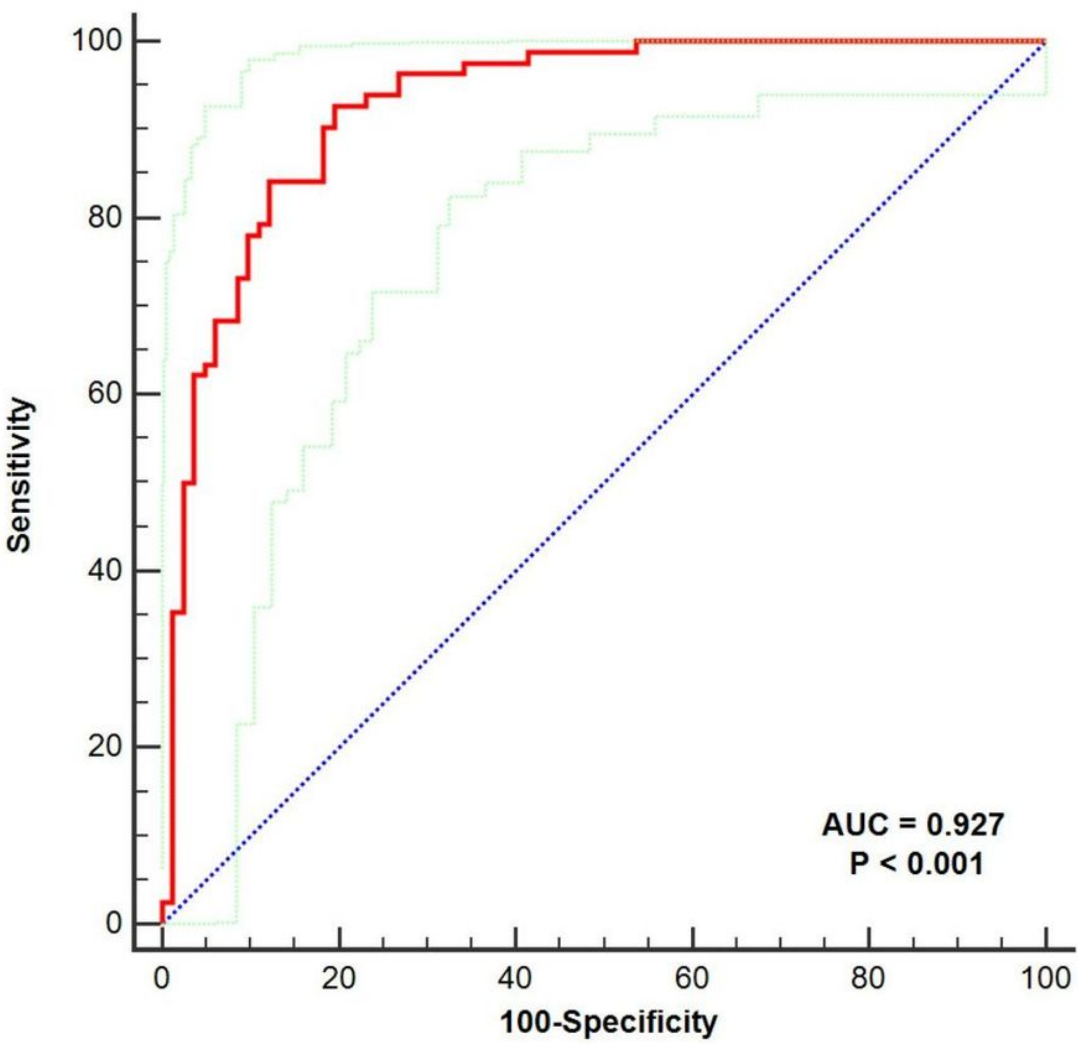

Figure 4

ROC curve analyses of the three-miRNA panel. AUC of the panel was 0.927 (95% CI: 0.876 to 0.962; sensitivity= 92.68%, specificity= 80.49%).

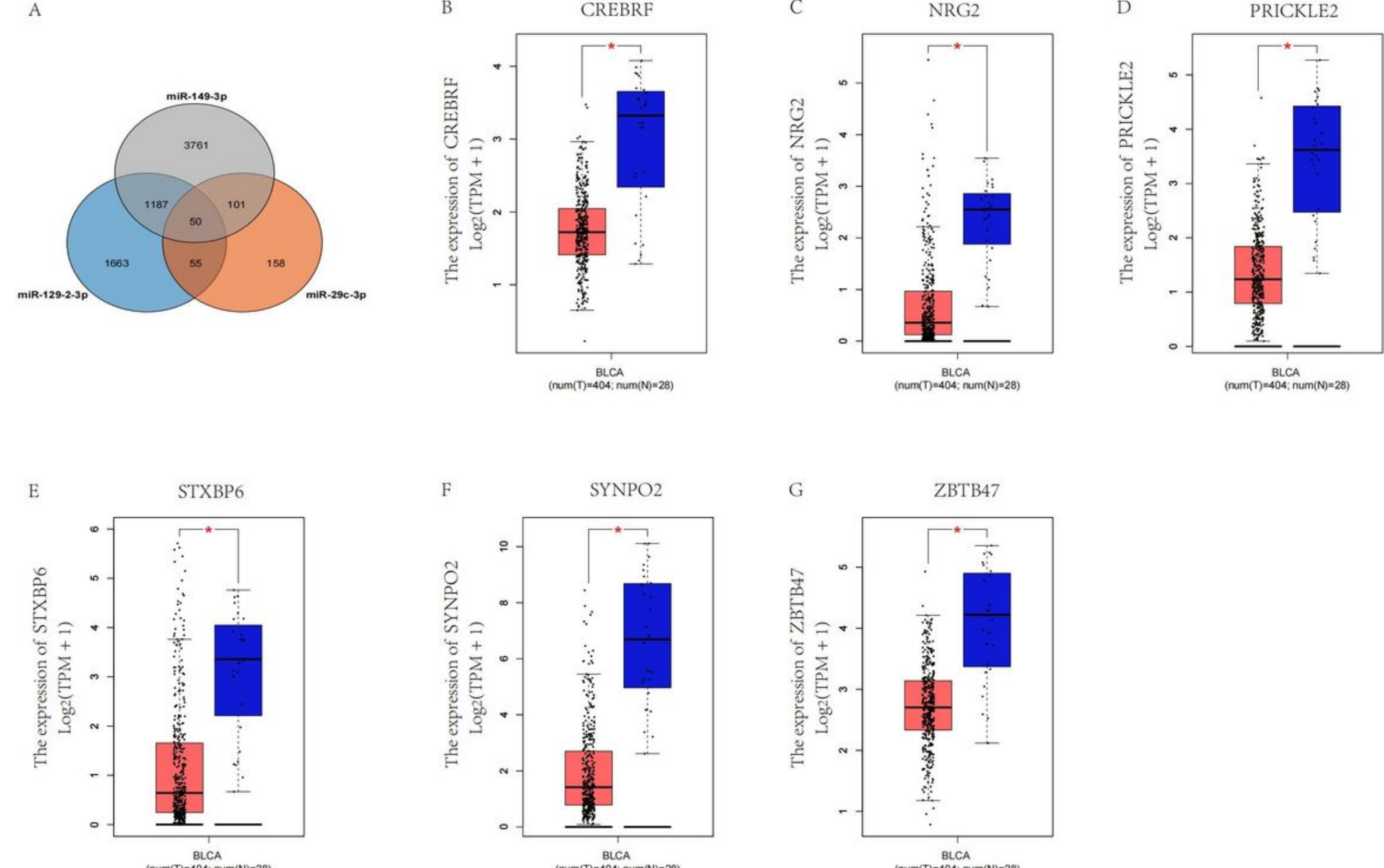

Figure 5

Target gene prediction of the 3 miRNAs and 6 differently expressed genes discovered from GEPIA database. (A) target genes elected with the criteria of gene prediction in more than 2 miRNAs, (B) expression of CREBRF from GEPIA database, (C) expression of NRG2, (D) expression of PRICKLE2, (E) expression of STXBP6, (F) expression of SYNPO2, (G) expression of ZBTB47.

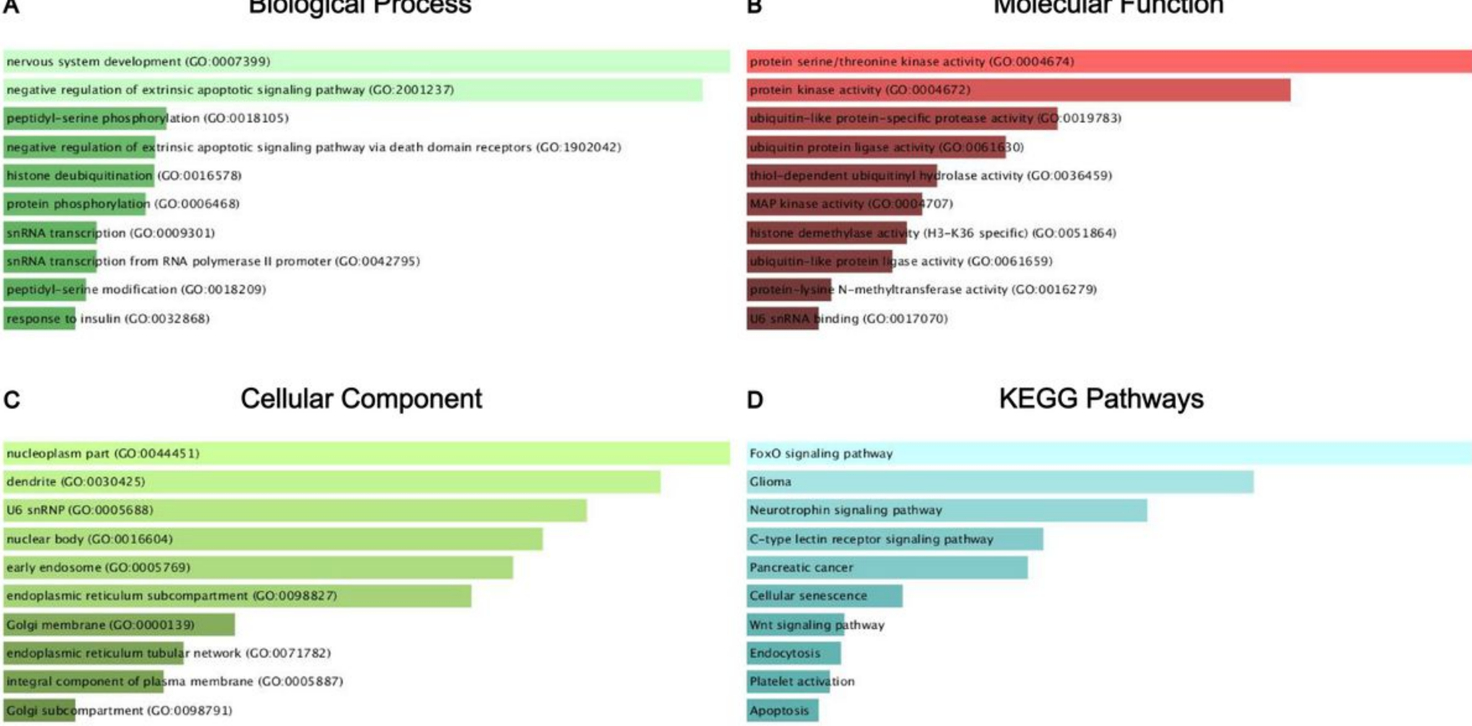

Figure 6

GO functional annotation and KEGG pathway enrichment of the target genes predicted by these three miRNAs. Top 10 of the biological process, cellular component, molecular function and KEGG pathways were shown. (A) Top 10 of biological process, (B) Top 10 of cellular component, (C) Top 10 of molecular function, (D) Top 10 of KEGG pathways.

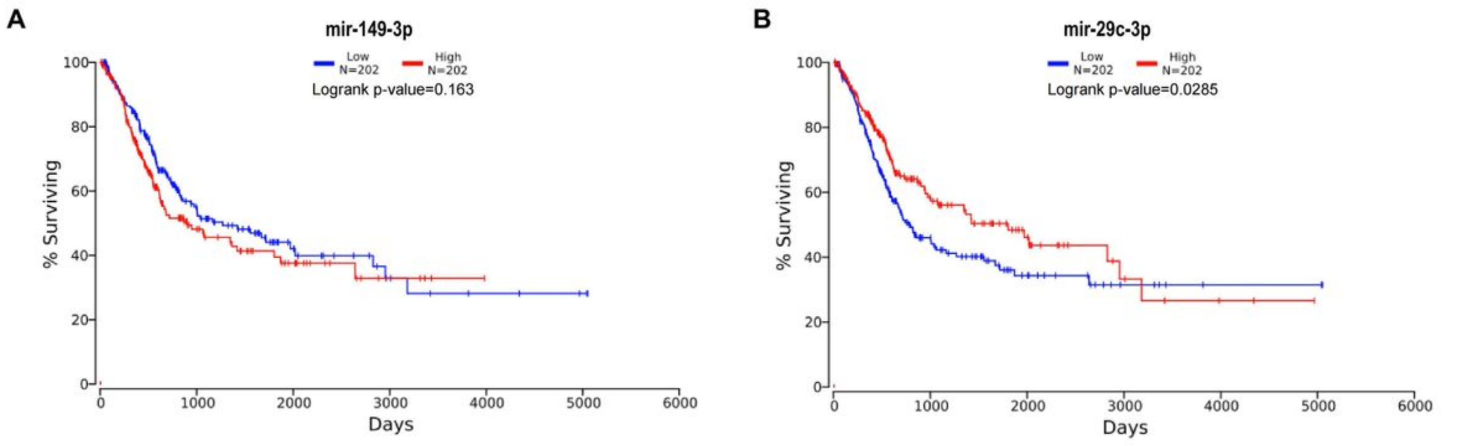

Figure 7

Surviving curve of miR-149-3p and miR-29c-3p from data of the OncoLnc database. (A) Surviving curve of miR-149-3p, (B) Surviving curve of miR-29c-3p.