Int J Clin Exp Pathol 2023;16(3):57-66
www.ijcep.com /ISSN:1936-2625/IJCEP0147566

*Original Article*

# MiR-199a-3p promotes repair of myocardial infarction by targeting NACC2

Xue-Zheng Wang[1], Lei Chen[1], Hao Sun[1], Xiao-Qian Li[1], Hu Wang[1], Xiao-Peng Zhang[1], Jiang-Bin Sun[1], Hai-Yong Wang[2]

*[1]Heart Center Third Ward, Guilin People's Hospital/The Fifth Clinical Medical College, Guilin Medical University, Guilin, Guangxi, P. R. China; [2]Department of Cardiovascular Surgery, Affiliated Hospital of Guilin Medical University, Guilin, Guangxi, P. R. China*

Received November 7, 2022; Accepted February 13, 2023; Epub March 15, 2023; Published March 30, 2023

**Abstract:** Objective: Myocardial infarction (MI) has gained widespread interest due to its high death and disability rate worldwide. Some miRNAs are markers of heart disease. Therefore, it is necessary to understand the mechanism for repairing MI injury. Methods: Here, we evaluated the relative expression levels of miR-199a-3p in mouse and human myocardial cell models of injury, and its effect on myocardial cells viability using Cell Counting Kit-8 (CCK-8) assay, 5-ethynyl-2'-deoxyuridline (EdU) assay, and flow cytometry assay as well as western blot in vitro. Furthermore, we performed bioinformatic online analysis to investigate the role that miR-199a-3p plays in cardiomyocyte injury, measured by dual-luciferase reporter assay. Results: The results showed that miR-199a-3p significantly increased the growth rate of cardiomyocytes after treating them with hydrogen peroxide ($H_2O_2$). miR-199a-3p also acted as an inhibitor that directly targeted NACC2, resulting in a higher NACC2 expression level in the injury model of cardiomyocytes than normal myocardial cells and thus preventing miR-199a-3p-induced proliferation promotion in model cardiomyocytes. Conclusion: Our results demonstrate that miR-199a-3p may be a prognostic biomarker in myocardial injury.

**Keywords:** Myocardial infarction, miR-199a-3p, NACC2, cell viability, mouse primary myocardial cell, AC16

## Introduction

Myocardial infarction (MI), mainly resulting from coronary artery disease, has been a leading cause of death in industrialized countries. Although a series of effective treatments for myocardial infarction have been developed, the high mortality rate and complications, such as arrhythmias and myocardial damage are still inevitable. On the other hand, the increase in long-term survivors of AMI means that the burden of disease to society and families has generally increased [1].

miRNAs, a type of small non-coding single-stranded RNA, inhibit the translation of mRNAs or degrade them at post-transcriptional level by directly binding to their 3'UTR [2-4]. miRNAs are commonly considered as significant regulatory factors of gene expression in numerous biological and pathological processes, e.g. cell growth, apoptosis, and stress response [5, 6]. Recently, miRNA in the pathophysiological process of cardiovascular disease has aroused tremendous interest. For example, Pasquale et al. showed a significant change in miRNAs expression in cardiac myocytes, providing potential therapeutic targets against heart disease, followed by acute ischemic diseases [7]; Meder and his coworker also showed that some miRNAs were markers of heart disease [8].

Notably, it has been identified that expression of specific miRNA is associated with the recovery activity of myocardial cells after MI in which enhanced or reduced miRNA expression result in cardiac repair in pigs [9]. Hathaway et al. found that miRNA-378a played a pivotal role in cardiovascular health [10]. MiRNA-21 has been proven to effectively repair cardiac function after localized injection in myocardial infarction [11]. miR-199a-3p is one of the most well-known miRNAs that are thought to play a beneficial role in improving cardiac function [12].

However, the precise mechanism of miR-199a-3p in human myocardial infarction is still unclear.

The protein NACC2, containing BTB and BEN domains, acts as a transcriptional regulator and induces apoptosis by sensitizing cells to DNA damage and suppressing cell proliferation [13]. The BTB domain, a conserved protein-protein interaction motif, is widely involved in critical biologic and pathologic processes, including development, apoptosis, and oncogenesis [14-16]. For example, in chromatin organization and transcription steps, the BEN motif regulates protein-DNA and protein-protein interactions [17].

Here, we explain the role that miR-199a-3p plays in myocardial damageing to provide novel insight into mechanisms and clues in diagnosis and thus new therapeutic methods in MI. We explore the roles of miR-199a-3p in MI and further investigate the mechanism by which miR-199a-3p may be a regulator of myocardial cell growth by targeting the NACC2 gene.

## Materials and methods

### *Cell lines and culture*

Suckling mice (Guangdong Medical Laboratory Animal Center, China) were anesthetized with sodium pentobarbital (80 mg/kg). Mice were killed by cervical dislocation. The hearts were quickly dissected and washed with D-Hanks solution. The preserved apex of the heart was digested using trypsin (Gibco, USA) and then treated with an enzyme solution containing collagenase II (Gibco, USA). Repeated digestion and collection of supernatant was performed to isolate single myocardial cells [18]. AC16 (Human cardiomyocytes) was purchased from ATCC (Rockville, MD) and incubated in DMEM/F-12 medium (Gibco, USA) supplemented with 10% fetal calf serum (FBS, Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA). Cells were cultured in an incubator at 37°C, containing 5% $CO_2$.

### *Cell transfection*

miRNA-mimic, miRNA-inhibitor, siRNA, and plasmid transfection were performed using Lipofectamine 3000 transfection reagent (ThermoFisher, USA). Mouse primary myocardial cells and AC16 cells were seeded into a 6-well plate at an appropriate quantity per well. After 12 hours, cells were transfected with miRNA-mimic, miRNA-inhibitor, siRNA, or plasmid respectively by the manufacturer's instructions. After 24-48 hours, cells were used for the next experiments.

### *Total RNA extraction and real-time quantitative PCR (qPCR)*

RNA was extracted from mouse primary myocardial cells and AC16 cells using TRIzol reagent (Invitrogen, USA). The extracted total RNA was reversely transcribed into cDNA by using the M-MLV Reverse Transcriptase (Promega, USA). RT-qPCR analysis was performed in triplicate for each sample with SYBR Green Master Mix (Vazyme, China). The qPCR cycling sections were used as follows: 95°C for 5 min, 40 cycles of 95°C for 10 s, and 60°C for 30 s. GAPDH served as the endogenous control for target mRNA, and U6 RNA served as the endogenous control for detecting miRNA. Primers were as follows: miR-199a-3p RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTAACCA, U6-F: CTCGCTTCGGCAGCACA, U6-R: AACGCTTCACGAATTTGCGT, miR-199a-3p-F: GTCACAGTAGTCTGCACAT, miRNA Universal-R: GTGCAGGGTCCGAGGT, H-GAPDH-F: CCCACTCCTCCACCTTTGAC, H-GAPDH-R: CATACCAGGAAATGAGCTTGACAA, H-NACC2-F: ACGCTGTGAAATTGTACTGTC, H-NACC2-R: CAGCATGGACTTGATCTTGG, Mus-NACC2-F: CTGCACATCGAGATCCCCAA, Mus-NACC2-R: GGCAGCTCGAATGCACTCTT, Mus-GAPDH-F: GGAGAGTGTTTCCTCGTCCC, Mus-GAPDH-R: ACTGTGCCGTTGAATTTGCC.

### *CCK-8 assay*

Approximately $2\times10^3$ cardiomyocytes/well were seeded in a 96-well plate, and 100 μL corresponding culture medium was added into each well. The plate was placed in the incubator at 37°C, 5% $CO_2$ overnight. The treatment was accomplished in the following days. 10 μL CCK-8 (Dojindo, Kyushu, Japan) solution was added to the plates and the absorption of the cardiomyocytes was detected according to the manufacturer's instruction. All experiments were repeated three times.

### *EdU assays*

Cells were incubated in 24-well plates and treated with the corresponding reagent. Ten μM EdU reagent was then added per well at room

temperature for 6 h. After that, it was fixed with 4% paraformaldehyde, followed by permeabilizing with 0.3% TritonX-100 at room temperature for 20 min. Next, cells were washed with 1× PBS, and incorporated EdU was explored with the kFluor488-EdU reagent (KeyGEN, China) at room temperature for 30 min. Subsequently, Hoechst-33342 was used to stain for 20 min, and images were captured by fluorescence microscope.

*Flow cytometry*

Cell apoptosis was assessed using the Cell Apoptosis Flow Cytometry Analysis Kit (Beyotime, China) according to the manufacturer's instructions.

*Western blot analysis*

Total proteins were isolated from mouse primary myocardial cells and AC16 cells using RIPA lysis buffer supplemented with PMSF and proteinase inhibitor. The concentration of all proteins was detected by the BCA protein assay kit (Beyotime, China). Total proteins were separated by SDS-polyacrylamide gel (4%-15%) electrophoresis and transferred to PVDF membranes (Millipore, USA). PVDF membranes were then blocked in 5% BSA (BioFroxx, Germany) and incubated with primary detection antibodies against NACC2 (1:1000, ThermoFisher, PA5-40941, USA), or GAPDH (1:10000, ab8245, Abcam, USA) at 4°C overnight and were washed in TBST three times. After that, specimens were incubated with anti-mouse or anti-rabbit secondary antibodies for 1 hour at room temperature, and the expression of proteins was measured using BeyoECLPlus (Beyotime, Jiangsu, China). GAPDH was used as a loading control.

*Luciferase reporter assay*

The NACC2 3'UTR, containing wild-type or mutant fragment of the predicted binding sites of miR-199a-3p, was subcloned in the dual-luciferase vector psiCHECK2 (Promega, USA). The luciferase reporter plasmids were transfected into mouse primary myocardial cells and AC16 cells with miR-199a-3p mimics or control mimics. The relative luciferase activity was detected using the Dual-Luciferase Reporter Assay System (Promega, USA) after 24 hours.

*Statistical analysis*

Statistical analysis was performed using the appropriate statistical methods, including Student's t-test (between two groups), one-way ANOVA (more than three groups), and chi-square test in SPSS software (version 16.0). Data were shown as mean ± SD of three separate experiments with GraphPad Prism (version 8.0) and normalized to the control group. Significance was set at *P < 0.05, **P < 0.01, ***P < 0.001.

## Results

*miR-199a-3p attenuated myocardial cell viability in vitro*

To explore the function of miR-199a-3p in regulating MI, we first established a myocardial infarction model in vitro after treating MPC and AC16 cells with $H_2O_2$ in different doses or times, respectively. CCK8 assay was applied to verify the cell viability of MPC and AC16 cells during treatment with $H_2O_2$ at different doses (**Figure 1A**) or times (**Figure 1B**). We demonstrated that viability of MPC and AC16 cells decreased within 24 h after $H_2O_2$ treatment, with a peak at the dose of 100 μM and at 24 h. We also detected the proliferation of myocardial cells using an EdU assay (**Figure 1C**). When compared to the control treatment, cell viability significantly decreased in $H_2O_2$-induced cell lines. These data indicated that 100 μM $H_2O_2$ effectively downregulated viability in MPC and AC16 cells within 24 hours.

qPCR was used to confirm RNA expression levels of miR-199a-3p after treatment with different doses of $H_2O_2$ (**Figure 2A**) and at different time points (**Figure 2B**). The results showed that the relative expression of miR-199a-3p was gradually downregulated with the progression of myocardial infarction in vitro (**Figure 2A**, **2B**). Then, we transfected miR-199a-3p-mimics or miR-199a-3p-inhibitor into MPC and AC16 cells. qPCR was used to verify the transfection efficiency of miR-199a-3p. The result showed that miR-199a-3p-mimics significantly increased the expression of miR-199a-3p in MPC and AC16 cells (**Figure 2C**), while miR-199a-3p-inhibitor decreased the expression of miR-199a-3p in MPC and AC16 cells (**Figure 2D**). The effects of miR-199a-3p on MPC and AC16 cell viability were evaluated with CCK8

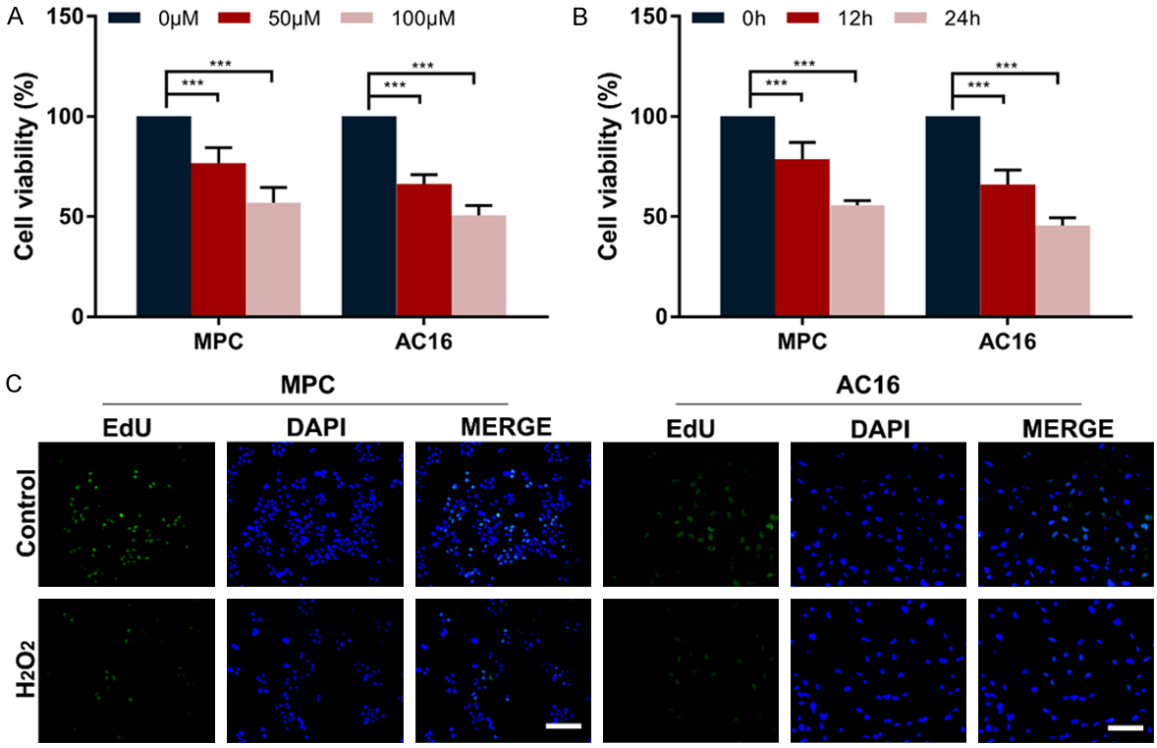


**Figure 1.** Construction of myocardial infarction model in mouse primary cardiomyocytes (MPC) and human cardiomyocytes (AC16). A. MPC and AC16 cells were treated with different doses of $H_2O_2$ for 24 h and relative cell viability was detected by CCK8 assay. B. MPC and AC16 cells were treated with 100 μM $H_2O_2$ and relative cell viability was detected at different points (12 h and 24 h) using CCK8 assay. C. MPC and AC16 cells were treated with 100 μM $H_2O_2$ for 24 h. EdU assay was performed to measure the cell viability. Magnification is 40 ×. Scale bar = 100 μm. ***P < 0.001.

assay. As shown in **Figure 2D**, the overexpression of miR-199a-3p significantly reduced cell viability of MPC and AC16 cells, while co-treatment of both miR-199a-3p and $H_2O_2$ counteracted their effects. These trends were further confirmed by the relative expression level of increased anti-apoptosis proteinmarker BCL-2 and decreased expression of pro-apoptosis markers, BAX and c-Caspase 3 by miR-199a-3p in the $H_2O_2$-induced MPC and AC16 cells (**Figure 2E**). These results showed that miR-199a-3p could reduce the damage to MPC and AC16 cells.

*miR-199a-3p downregulated NACC2 expression*

To evaluate how miR-199a-3p regulates myocardial infarction in vitro, we performed online bioinformatic analysis to screen underlying target genes of miR-199a-3p. NACC2, an important transcriptional regulator for cell proliferation [19], was found to conjugate with miR-199a-3p. The predicted binding sequences are shown in **Figure 3A**. To further confirm the predicted binding site, we performed the dual-luciferase reporter assay. As expected, miR-199a-3p-mimics transfection significantly reduced the reporter activity of wild-type NACC2, but did not markedly decrease the luciferase activity of vector containing the mutant NACC2 in both MPC and AC16 cells (**Figure 3D**). Furthermore, qPCR and western blot confirmed that miR-199a-3p down-regulated NACC2 expression both in mRNA (**Figure 3C**) and protein (**Figure 3B**) levels of NACC2 and in MPC and AC16 cells. These results suggested that miR-199a-3p significantly reduced the expression of NACC2.

*Up-regulation of NACC2 suppresses myocardial cell growth*

As NACC2 expression was significantly inhibited by miR-199a-3p over-expression, we further investigated its function on $H_2O_2$-treated MPC and AC16 cells. We treated MPC and AC16 cells with different doses of $H_2O_2$ for 24 h or with

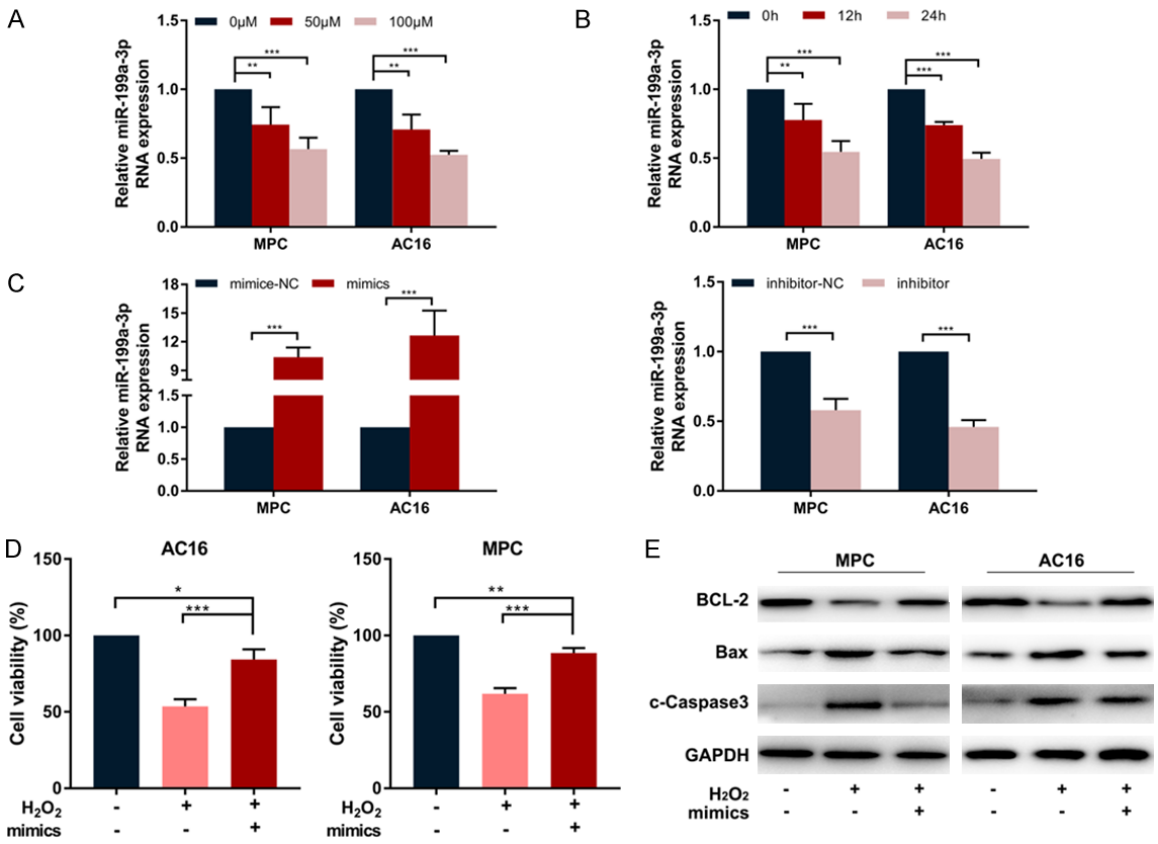


**Figure 2.** Quantitative data analysis for miR-199a-3p expression in different models. A. Relative miR-199a-3p expression level in MPC and AC16 cells was detected using real-time PCR after treatment with different doses of $H_2O_2$. B. Relative miR-199a-3p expression level in MPC and AC16 cells was detected using real-time PCR after treatment with 100 μM $H_2O_2$ at different points. C. miR-199a-3p expression level in miR-199a-3p mimic-transfected or inhibitor-transfected MPC and AC16 cells was evaluated by qPCR. D. The relative cell viability in MPC and AC16 cells treated with $H_2O_2$ was measured by CCK8 assay. E. Relative protein level of BCL-2, Bax, and c-Caspase 3 in MPC and AC16 cells. n = 3 per group. * P < 0.05, **P < 0.01, ***P < 0.001.

$H_2O_2$ for different durations. Western blot results revealed that the relative expression of NACC2 was gradually up-regulated with the progression of myocardial infarction in vitro (**Figure 4A**, **4B**). The expression of NACC2 in MPC and AC16 cells after transfection with si-NACC2 was detected by western blot. We found that the protein level of NACC2 was effectively reduced by transfection with si-NACC2 (**Figure 4C**). The CCK8 assay demonstrated that NACC2 knockdown significantly reversed the impaired MPC and AC16 cell viability, that resulted from $H_2O_2$ conditions (**Figure 4D**). These findings were further verified by the relative expression level of apoptosis protein markers BCL-2, BAX, and c-Caspase3 (**Figure 4E**). Additionally, flow cytometry of Annexin V/PI staining assay was used to evaluate apoptosis in MPC and AC16 treated with $H_2O_2$. Knockdown of NACC2 using siRNA markedly reversed the cell apoptosis caused by $H_2O_2$ (**Figure 4F**). These findings suggested that $H_2O_2$-induced MPC and AC16 cell injury was attenuated by NACC2 knockdown in these cells.

*Viability of myocardial cells was promoted by miR-199a-3p by down-regulating NACC2*

Although the CCK8 assay (**Figure 5**) confirmed that the relative cell viability of MPC and AC16 cells in the MI model decreased after treatment with NACC2 overexpression (OE-NACC2), this was reversed after cells were co-transfected with OE-NACC2 and miR-199a-3p-mimics (**Figure 5**). The data showed that miR-199a-3p benefited cardiomyocyte repair after treatment with $H_2O_2$ through directly suppressing NACC2 expression.

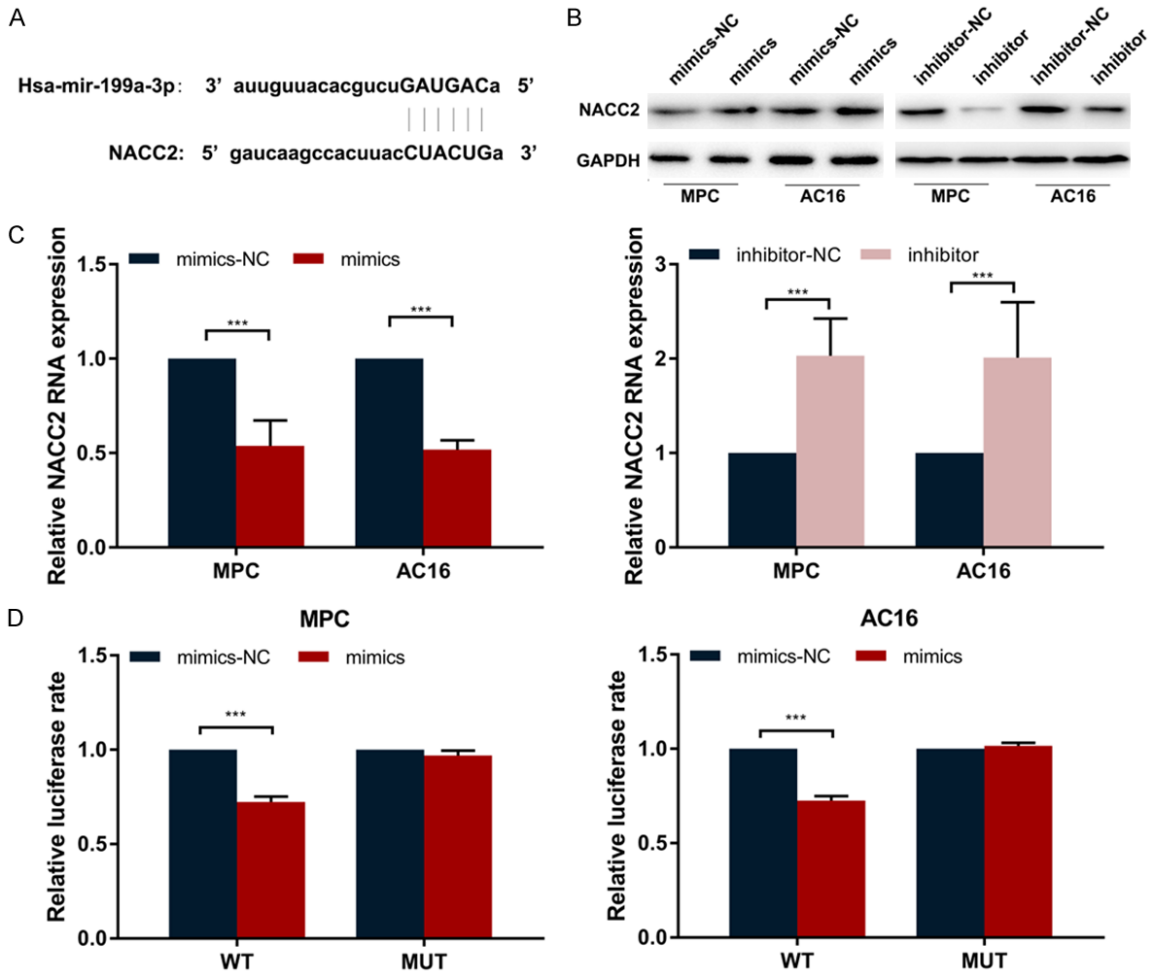


**Figure 3.** NACC2 expression downregulated by miR-199a-3p. A. Sequence alignment of NACC2 3'-UTR with wild-type (WT) and mutant miR-199a-3p targeting sites was showed using bioinformatic online databases, Targetscan. B, C. Relative NACC2 expression in miR-199a-3p mimic-transfected MPC, AC16 cells, or inhibitor-transfected MPC and AC16 cells evaluated by western blot and qPCR. D. The dual-luciferase reporter assay was performed to detect the luciferase activity. Firefly luciferase activity was normalized based on activity of renilla luciferase. *P < 0.05, **P < 0.01, ***P < 0.001.

## Discussion

Acute myocardial infarction is a growing problem [19]. However, major risk elements, diagnosis, and therapeutic options in MI patients remain to be studied [20, 21]. Therefore, a better understanding of the molecular mechanism of MI may help the exploration of diagnostic and therapeutic methods. With the continuous improvement of high-throughput databases, we could effectively identify key genes that cause epigenetic change in myocardial damage [18, 22]. Among them, microRNAs and their downstream biomolecules are considered as possible targets for developing diagnostic biomarkers and therapeutic agents for MI. Indeed, studies have shown that the control of miRNAs and mRNAs plays an essential role in the repair of cardiomyocytes [23].

miR-199a-3p is differentially expressed during the MI process and acts as a key regulator of cardiac function. A previous study has revealed that miR-199a-3p modulates tumor growth by regulating mTOR and PAK4 pathways in a transgenic mouse model of hepatocellular carcinoma [24]. Also, it inhibits the expression of MEF2C, which involves the progress by which stem cells differentiate into cardiomyocytes [12]. During ischemia/reperfusion experiments, carvedilol inhibits miR-199a-3p expression and further lowers the level of ddit4 and

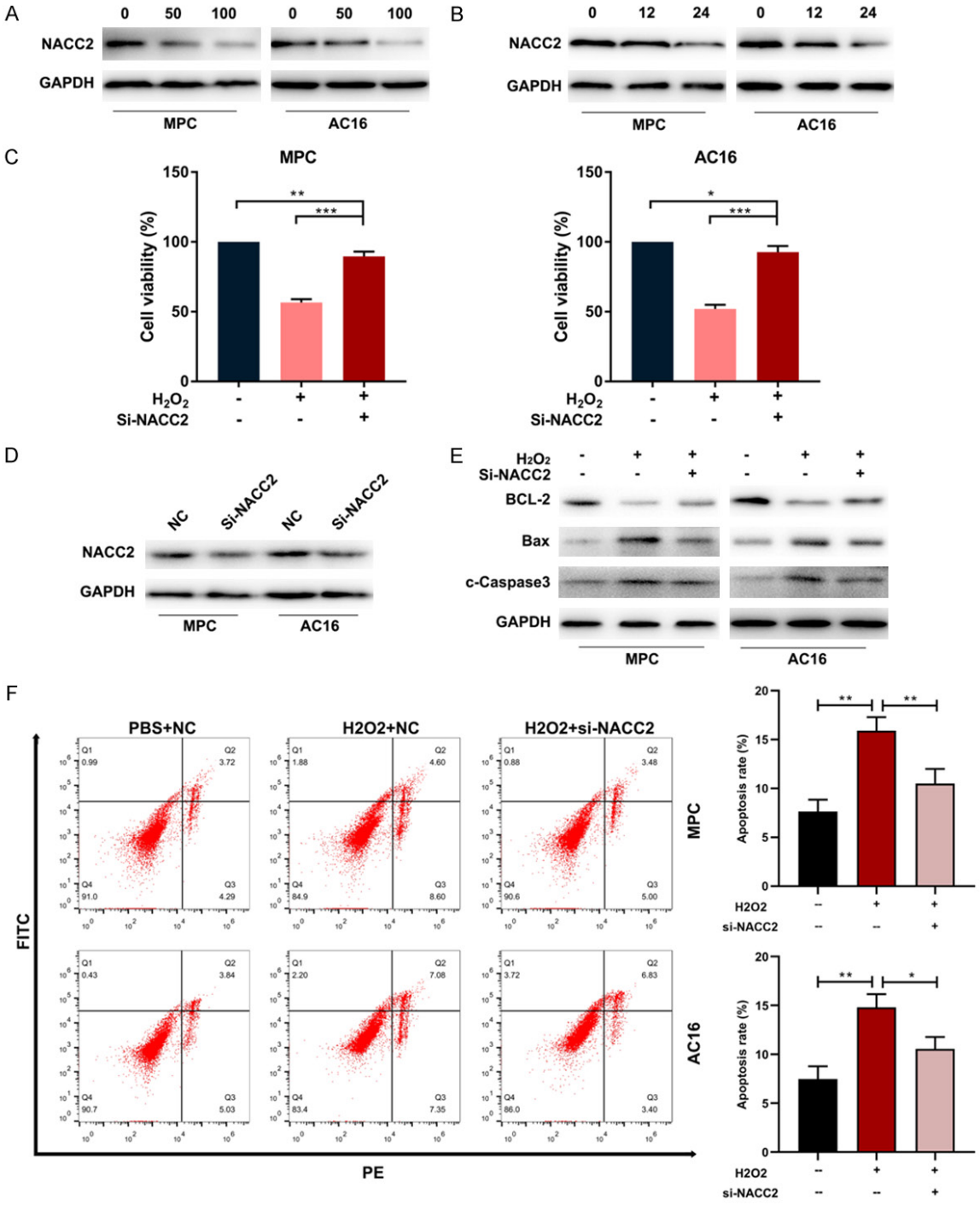


**Figure 4.** Downregulation of NACC2 suppresses MPC and AC16 cell growth. A. Relative NACC2 protein expression level in MPC and AC16 cells was detected using western blot after treatment with different doses of $H_2O_2$. B. Relative NACC2 protein expression level in MPC and AC16 cells was detected using western blot following treatment with 100 μM $H_2O_2$ at different points. C. MPC and AC16 cells following $H_2O_2$ injury were transfected with si-NACC2, and the relative cell viability was measured by CCK8 assay. D. Western blot showing the knockdown efficiency of NACC2 in cells transfected with si-NACC2. E. Western blot measuring the relative protein levels of BCL-2, Bax, and c-Caspase3 in MPC and AC16 cells following $H_2O_2$ injury and being transfected with si-NACC2. F. Flow cytometry measuring cell apoptosis in MPC and AC16 cells following $H_2O_2$ injury after being transfected with si-NACC2. Data are expressed as mean ± SD of three separate experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

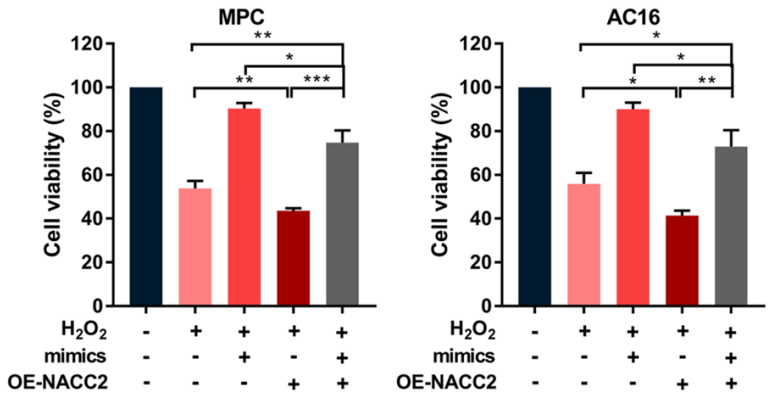


**Figure 5.** Myocardial cell viability was promoted by miR-199a-3p by down-regulating NACC2. MPC and AC16 cells following $H_2O_2$ injury were transfected with OE-NACC2, miR-199a-3p-mimics, or OE-NACC2 and miR-199a-3p-mimics together, and the relative cell viability was measured by CCK8 assay. Data are expressed as mean ± SD of three separate experiments. *P < 0.05, **P < 0.01, ***P < 0.001.

ing4 to protect myocardial cells against apoptosis [13]. miR-199a-3p is of interest because of its regulatory function in myocardial cells. In this study, we showed that the expression level of miR-199a-3p is lower in damaged cardiomyocytes. By using a $H_2O_2$ in vitro model, we revealed that miR-199a-3p promotes cell viability, which may contribute to myocardial cells' survival resistance to injury.

Most miRNAs exert their effects by direct binding to their 3'UTR, resulting in the degradation of mRNAs or translational inhibition of downstream target genes [3, 25]. Previous studies have revealed some of the targets of miR-199a-3p, including mTOR, c-Met [26], CD44 [27], ZHX1 [28], and TFAM [29] in cancer cells and ddit4 [13], MEF2C [12], and Cd151 [30] in heart cells. However, other targets of miR-199a-3p regarding MI are largely unknown. In this study, we showed a new target of miR-199a-3p. miR-199a-3p inhibited cell viability by binding to NACC2.

Nucleus accumbens-1 (NACC1), in the BTB/POZ gene family, plays an essential role in cell proliferation and growth [31]. NACC2 also belongs to the BTB gene family. Evidence has shown it acts as a negative transcriptional regulator in cell proliferation and has a positive role in inducing apoptosis [14]. NACC2 attracts our attention because it encodes proteins that contain BTB and BEN domains. In a previous study, NACC2 was shown to be upregulated in very severe obstructive sleep apnea patients with long-term continuous positive airway pressure [32]. Existing knowledge of its function is relevant to the development of pediatric solid cancer [33]. However, its function in MI injury remains unknown. Considering its potential role in cancer development and the function of NACC1 in tumor growth, we suggested a function of NACC2 in cell survival during MI injury. We demonstrated that the NACC2 expression is affected by miR-199a-3p and that it decreases the expression of NACC2. Moreover, we suggested that the decrease in NACC2 expression was at least partially caused by the binding of miR-199a-3p to its 3'UTR. We then verified the function of NACC2 in cardiac cell proliferation. CCK8 and the flow cytometry of Annexin V/PI staining assay showed that NACC2 could strongly inhibit the viability of the myocardial cells. Our results extend the knowledge of the miR-199a-3p downstream target relevant to cell proliferation in MI. These effects may promote the repair of cardiomyocyte damage by targeting NACC2.

Thus, we demonstrate that miR-199a-3p promotes MPC and AC16 cell proliferation by targeting the 3'UTR of NACC2 mRNA. Our study provides novel insight on the mechanisms of MI involving miR-199a-3p, and these findings might provide new methods against cardiomyocyte damage for patients.

## Acknowledgements

This study was supported by grants from the Health Department Self-raised Funds of Guangxi province (z20190838).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Jiang-Bin Sun, Heart Center Third Ward, Guilin People's Hospital/The Fifth Clinical Medical College, Guilin Medical University, 12 Wen Ming Road, Xiangshan, Guilin 541002, Guangxi, P. R. China. Tel: +86-0773-2827626; Fax: +86-0773-2827626; E-mail: 494-817853@qq.com; Hai-Yong Wang, Department of

Cardiovascular Surgery, Affiliated Hospital of Guilin Medical University, 15 Le Qun Road, Linxiufeng, Guilin 541002, Guangxi, P. R. China. Tel: +86-0773-2827626; Fax: +86-0773-2827626; E-mail: docwanghy@foxmail.com

## References

[1] Johansson S, Rosengren A, Young K and Jennings E. Mortality and morbidity trends after the first year in survivors of acute myocardial infarction: a systematic review. BMC Cardiovasc Disord 2017; 17: 53.

[2] Du P, Pirouz M, Choi J, Huebner AJ, Clement K, Meissner A, Hochedlinger K and Gregory RI. An intermediate pluripotent state controlled by microRNAs is required for the naive-to-primed stem cell transition. Cell Stem Cell 2018; 22: 851-864, e5.

[3] Wang F, Harel-Sterling L, Cohen S, Liu A, Brophy JM, Paradis G and Marelli AJ. Heart failure risk predictions in adult patients with congenital heart disease: a systematic review. Heart 2019; 105: 1661-1669.

[4] Li J, Cai SX, He Q, Zhang H, Friedberg D, Wang F and Redington AN. Intravenous miR-144 reduces left ventricular remodeling after myocardial infarction. Basic Res Cardiol 2018; 113: 36.

[5] Fasanaro P, Greco S, Ivan M, Capogrossi MC and Martelli F. microRNA: emerging therapeutic targets in acute ischemic diseases. Pharmacol Ther 2010; 125: 92-104.

[6] Meder B, Keller A, Vogel B, Haas J, Sedaghat-Hamedani F, Kayvanpour E, Just S, Borries A, Rudloff J, Leidinger P, Meese E, Katus HA and Rottbauer W. MicroRNA signatures in total peripheral blood as novel biomarkers for acute myocardial infarction. Basic Res Cardiol 2011; 106: 13-23.

[7] Qipshidze Kelm N, Piell KM, Wang E and Cole MP. MicroRNAs as predictive biomarkers for myocardial injury in aged mice following myocardial infarction. J Cell Physiol 2018; 233: 5214-5221.

[8] el Azzouzi H, Leptidis S, Dirkx E, Hoeks J, van Bree B, Brand K, McClellan EA, Poels E, Sluimer JC, van den Hoogenhof MM, Armand AS, Yin X, Langley S, Bourajjaj M, Olieslagers S, Krishnan J, Vooijs M, Kurihara H, Stubbs A, Pinto YM, Krek W, Mayr M, da Costa Martins PA, Schrauwen P and De Windt LJ. The hypoxia-inducible microRNA cluster miR-199a-214 targets myocardial PPARδ and impairs mitochondrial fatty acid oxidation. Cell Metab 2013; 18: 341-354.

[9] Gabisonia K, Prosdocimo G, Aquaro GD, Carlucci L, Zentilin L, Secco I, Ali H, Braga L, Gorgodze N, Bernini F, Burchielli S, Collesi C, Zandonà L, Sinagra G, Piacenti M, Zacchigna S, Bussani R, Recchia FA and Giacca M. MicroRNA therapy stimulates uncontrolled cardiac repair after myocardial infarction in pigs. Nature 2019; 569: 418-422.

[10] Hathaway QA, Durr AJ, Shepherd DL, Pinti MV, Brandebura AN, Nichols CE, Kunovac A, Goldsmith WT, Friend SA, Abukabda AB, Fink GK, Nurkiewicz TR and Hollander JM. miRNA-378a as a key regulator of cardiovascular health following engineered nanomaterial inhalation exposure. Nanotoxicology 2019; 13: 644-663.

[11] Song Y, Zhang C, Zhang J, Jiao Z, Dong N, Wang G, Wang Z and Wang L. Localized injection of miRNA-21-enriched extracellular vesicles effectively restores cardiac function after myocardial infarction. Theranostics 2019; 9: 2346-2360.

[12] Chen HP, Wen J, Tan SR, Kang LM and Zhu GC. MiR-199a-3p inhibition facilitates cardiomyocyte differentiation of embryonic stem cell through promotion of MEF2C. J Cell Physiol 2019; 234: 23315-23325.

[13] Park KM, Teoh JP, Wang Y, Broskova Z, Bayoumi AS, Tang Y, Su H, Weintraub NL and Kim IM. Carvedilol-responsive microRNAs, miR-199a-3p and -214 protect cardiomyocytes from simulated ischemia-reperfusion injury. Am J Physiol Heart Circ Physiol 2016; 311: H371-383.

[14] Xuan C, Wang Q, Han X, Duan Y, Li L, Shi L, Wang Y, Shan L, Yao Z and Shang Y. RBB, a novel transcription repressor, represses the transcription of HDM2 oncogene. Oncogene 2013; 32: 3711-3721.

[15] Shivakumar M, Lee Y, Bang L, Garg T, Sohn KA and Kim D. Identification of epigenetic interactions between miRNA and DNA methylation associated with gene expression as potential prognostic markers in bladder cancer. BMC Med Genomics 2017; 10 Suppl 1: 30.

[16] Zhang Y, Ren YJ, Guo LC, Ji C, Hu J, Zhang HH, Xu QH, Zhu WD, Ming ZJ, Yuan YS, Ren X, Song J and Yang JM. Nucleus accumbens-associated protein-1 promotes glycolysis and survival of hypoxic tumor cells via the HDAC4-HIF-1α axis. Oncogene 2017; 36: 4171-4181.

[17] Abhiman S, Iyer LM and Aravind L. BEN: a novel domain in chromatin factors and DNA viral proteins. Bioinformatics 2008; 24: 458-461.

[18] Lv G, Shao S, Dong H, Bian X, Yang X and Dong S. MicroRNA-214 protects cardiac myocytes against H2O2-induced injury. J Cell Biochem 2014; 115: 93-101.

[19] Bui AL, Horwich TB and Fonarow GC. Epidemiology and risk profile of heart failure. Nat Rev Cardiol 2011; 8: 30-41.

[20] Goretti E, Wagner DR and Devaux Y. miRNAs as biomarkers of myocardial infarction: a step forward towards personalized medicine? Trends Mol Med 2014; 20: 716-725.

[21] Matsumoto S, Sakata Y, Suna S, Nakatani D, Usami M, Hara M, Kitamura T, Hamasaki T, Nanto S, Kawahara Y and Komuro I. Circulating p53-responsive microRNAs are predictive indicators of heart failure after acute myocardial infarction. Circ Res 2013; 113: 322-326.

[22] Jayawardena TM, Finch EA, Zhang L, Zhang H, Hodgkinson CP, Pratt RE, Rosenberg PB, Mirotsou M and Dzau VJ. MicroRNA induced cardiac reprogramming in vivo: evidence for mature cardiac myocytes and improved cardiac function. Circ Res 2015; 116: 418-424.

[23] Halushka PV, Goodwin AJ and Halushka MK. Opportunities for microRNAs in the crowded field of cardiovascular biomarkers. Annu Rev Pathol 2019; 14: 211-238.

[24] Callegari E, D'Abundo L, Guerriero P, Simioni C, Elamin BK, Russo M, Cani A, Bassi C, Zagatti B, Giacomelli L, Blandamura S, Moshiri F, Ultimo S, Frassoldati A, Altavilla G, Gramantieri L, Neri LM, Sabbioni S and Negrini M. miR-199a-3p modulates MTOR and PAK4 pathways and inhibits tumor growth in a hepatocellular carcinoma transgenic mouse model. Mol Ther Nucleic Acids 2018; 11: 485-493.

[25] Boon RA and Dimmeler S. MicroRNAs in myocardial infarction. Nat Rev Cardiol 2015; 12: 135-142.

[26] Fornari F, Milazzo M, Chieco P, Negrini M, Calin GA, Grazi GL, Pollutri D, Croce CM, Bolondi L and Gramantieri L. MiR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2010; 70: 5184-5193.

[27] Henry JC, Park JK, Jiang J, Kim JH, Nagorney DM, Roberts LR, Banerjee S and Schmittgen TD. miR-199a-3p targets CD44 and reduces proliferation of CD44 positive hepatocellular carcinoma cell lines. Biochem Biophys Res Commun 2010; 403: 120-125.

[28] Wang Z, Ma X, Cai Q, Wang X, Yu B, Cai Q, Liu B, Zhu Z and Li C. MiR-199a-3p promotes gastric cancer progression by targeting ZHX1. FEBS Lett 2014; 588: 4504-4512.

[29] Fan X, Zhou S, Zheng M, Deng X, Yi Y and Huang T. MiR-199a-3p enhances breast cancer cell sensitivity to cisplatin by downregulating TFAM (TFAM). Biomed Pharmacother 2017; 88: 507-514.

[30] Tao Y, Zhang H, Huang S, Pei L, Feng M, Zhao X, Ouyang Z, Yao S, Jiang R and Wei K. miR-199a-3p promotes cardiomyocyte proliferation by inhibiting CD151 expression. Biochem Biophys Res Commun 2019; 516: 28-36.

[31] Morita K, Fujii T, Itami H, Uchiyama T, Nakai T, Hatakeyama K, Sugimoto A, Miyake M, Nakai Y, Tanaka N, Shimada K, Yamazaki M, Fujimoto K and Ohbayashi C. NACC1, as a target of microRNA-331-3p, regulates cell proliferation in urothelial carcinoma cells. Cancers (Basel) 2018; 10: 347.

[32] Yap KL, Fraley SI, Thiaville MM, Jinawath N, Nakayama K, Wang J, Wang TL, Wirtz D and Shih Ie M. NAC1 is an actin-binding protein that is essential for effective cytokinesis in cancer cells. Cancer Res 2012; 72: 4085-4096.

[33] Levine, Shen Y, Mungall K, Serrano J, Snuderl M, Pleasance E, Jones S, Laskin J, Marra MA, Rassekh R, Deyell R, Yip S, Cheng S and Dunham C. NTRK2 fusion driven pediatric glioblastoma: identification of key molecular drivers by personalized oncology. Can J Neu Rol Sci 2019; 46: S64.