Original Article

MiR-197-3p regulates endothelial cell proliferation and migration by targeting IGF1R and BCL2 in Kawasaki disease

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Abstract: Background: Kawasaki disease (KD) is a multisystemic vasculitis syndrome. Accumulating evidences indicated that microRNAs play a critical role in KD. However, the mechanism was still not fully understood. The study aimed to research the functions of microRNA-197-3p (miR-197-3p) in the progression of KD.

Methods: Level of miR-197-3p was detected by quantitative polymerase chain reaction (qRT-PCR) in acute KD serum. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay was performed to determine cell proliferation in HCAECs. Cell apoptosis was evaluated by flow cytometry. In addition, transwell assay was used to identify the migration capacity of HCAECs in vitro. The expression of insulin-like growth factor type 1 receptor (IGF1R), B cell lymphoma 2 (BCL2), apoptosis-relative of Cleaved-caspase 3 (C-caspase 3) and Cleaved-PARP (C-PARP), as well as transition-relative of E-cadherin, N-cadherin, and Vimentin were measured by western blot assay. Lastly, dual-luciferase reporter assay was employed to verify the relationship between miR-197-3p and IGF1R or BCL2 in vitro. Results: Level of miR-197-3p was higher in Acute KD samples than that of healthy control and convalescent KD samples. Mechanistically, the role of miR-197-3p was exerted through directly targeting IGF1R and BCL2 in KD serum-induced HCAECs. Cell apoptosis was evaluated by flow cytometry. In addition, transwell assay was used to identify the migration capacity of HCAECs in vitro. The expression of insulin-like growth factor type 1 receptor (IGF1R), B cell lymphoma 2 (BCL2), apoptosis-relative of Cleaved-caspase 3 (C-caspase 3) and Cleaved-PARP (C-PARP), as well as transition-relative of E-cadherin, N-cadherin, and Vimentin were measured by western blot assay. Lastly, dual-luciferase reporter assay was employed to verify the relationship between miR-197-3p and IGF1R or BCL2 in vitro. Results: Level of miR-197-3p was higher in Acute KD samples than that of healthy control and convalescent KD samples. Mechanistically, the role of miR-197-3p was exerted through directly targeting IGF1R and BCL2 in KD serum-induced HCAECs. Functionally, the inhibiting effect on cell apoptosis as well as promoting effects on cell proliferation and migration of miR-197-3p deletion was abrogated by either si-IGF1R or si-BCL2. Conclusion: miR-197-3p modifies cell behaviors of proliferation, apoptosis and migration by targeting IGF1R and BCL2 in KD, providing a new perspective for the treatment of KD clinically.

Keywords: miR-197-3p, IGF1R, BCL2, KD, proliferation, apoptosis, migration

Introduction

Kawasaki disease (KD) is a characteristically multi-systemic vasculitis syndrome, most commonly occurring in infants and young children, along with vascular injury which acts as the major reason of its morbidity and mortality [1, 2]. Meanwhile, the probability of untreated children develops into coronary artery aneurysms (CAAs) or ectasia at a high range of 15 to 25%. This risk is decreased to 5% after therapy with immunoglobulin intravenously in acute KD patients [3, 4]. Nevertheless, the mechanism of KD-stimulated vascular endothelial injury remains largely unknown. Thus, there is a crying need to find a therapeutic target for KD treatment clinically.

MicroRNAs (miRNAs), are endogenous small noncoding RNAs with 20-24 nucleotides, which regulate biological functions and directly target over than 30% genes in cells post transcription and translation in the cardiovascular system [5, 6]. Surprisingly, miRNAs have been demonstrated to be explored in cells [7, 8] and were also found in circulating blood and urine. Increasing evidences suggested that miRNAs participated in the development of multiple human diseases, including KD [9]. For example, microRNA (miR)-200c and miR-371-5p levels were elevated in KD of children [10]. The expression of miR-145 was significantly elevated in children patients with KD [11]. MiR-93 may regulate vascular endothelial growth factor A (VEGFA) of circulating peripheral blood mono-
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Table 1. Clinical characteristics of KD patients and healthy control

<table>
<thead>
<tr>
<th>Groups</th>
<th>Healthy control</th>
<th>Acute KD</th>
<th>Convalescent KD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>18</td>
<td>32</td>
<td>20</td>
</tr>
<tr>
<td>Male</td>
<td>11 (61.11)</td>
<td>21 (65.63)</td>
<td>11 (55.00)</td>
</tr>
<tr>
<td>Female</td>
<td>7 (38.89)</td>
<td>11 (34.37)</td>
<td>9 (45.00)</td>
</tr>
<tr>
<td>Age (months)</td>
<td>38.65±18.80</td>
<td>46.28±32.14</td>
<td>32.16±24.35</td>
</tr>
<tr>
<td>WBC (×10^9/L)</td>
<td>6.52±3.65</td>
<td>18.96±6.85</td>
<td>7.69±3.39</td>
</tr>
<tr>
<td>PLT (×10^9)</td>
<td>352.35±84.52</td>
<td>408.26±94.06</td>
<td>487.21±102.05</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>/</td>
<td>102.36±66.41</td>
<td>4.05±2.65</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>18.26±13.02</td>
<td>84.36±62.05</td>
<td>78.69±65.57</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>39.65±30.35</td>
<td>41.03±35.89</td>
<td>44.23±29.67</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>/</td>
<td>947.0±1055.23</td>
<td>320.02±342.03</td>
</tr>
</tbody>
</table>

WBC: white blood cell; PLT: platelet; CRP: C-reactive protein; ALT: glutamic pyruvic transaminase; AST: glutamic oxaloacetic transaminase; NT-proBNP: N-terminal pro natriuretic peptide type B.

nuclear cells in children with acute KD [12]. Apart from this, miR-197-3p functioned as a prognostic marker, as well as suppressed cell invasion in hepatocellular carcinoma [13]. MiR197-3p also was a novel biomarker in primary biliary cirrhosis [14]. While less research was conducted on miR-197-3p in endothelial cells (ECs) injury and KD. The study paid attention to the role of miR-197-3p in the progression of KD.

Recently, like growth factor type 1 receptor (IGF1R) has been reported to be a direct target gene in vascular smooth muscle cells (VSMCs) [15], and a vital regulator of apoptosis in both VSMCs and ECs [10, 16]. What’s more, functions of IGF1R in endothelial cells were studied in transgenic mice [17]. It was also associated with the signaling of diabetes and insulin resistance [18], which were closely related with ECs function. Based on the relationship between IGF1R and functions of ECs, we speculated that IGF1R participated in the regulatory mechanism of ECs injury, further modified the progression of KD.

Emerging research showed that B cell lymphoma 2 (BCL2) was a critical gene of cell apoptosis [19]. Silencing BCL2 caused fulminant lymphoid apoptosis and polycystic kidneys, as well as hypopigmented hair [20]. Moreover, the regulation of cell apoptosis by BCL2 protein family supplied implications for physiology and therapy [21]. The role of BCL2 in inflammatory response aspects was also explored; it could induce autoimmune disease [22]. Taken together, BCL2, as an apoptosis-related and immune-related protein, may play an important role in human disease.

Herein, the study measured level of miR-197-3p in acute serum and Levels of miR-197-3p, IGF1R, and BCL2 were also detected in KD serum stimulated KD cell model. Furthermore, the relationship between miR-197-3p and IGF1R or BCL2 in the progression of KD cell model was identified in this study.

Materials and methods

KD patient sera and cell culture

The study used acute KD children sera (n=32) as the experimental specimens, and 38 other children sera as contrasts, including healthy control (n=18) and convalescent KD (n=20) control. All samples were gained from Department of Pediatric Cardiology, Chengdu Women’s and Children’s Central Hospital. What’s more, the clinical characteristics of investigated objects were presented in Table 1. All children participated in the research were diagnosed with KD through at least 5 days fever and met at least 4 in the 5 clinical criteria or 3 of 5 criteria along with coronary abnormalities. The KD patients did not undergo with globulin. Apart from this, all participators in the study afforded signed written informed consent for the serum samples to be applied for research. Moreover, ethical approval for the research was acquired from The Department of Pediatric Cardiology, Chengdu Women’s and Children’s Central Hospital.

For cell culture, firstly, human coronary arterial endothelial cells (HCAECs) which were obtained from Cell Bank of the Chinese Academy of Sciences (Chengdu, China) were cultured with 20% serum from either KD patients or healthy controls. Complete cell culture medium was used Roswell Park Memorial Institute-16 (RPMI-1640; Hyclone, Logan, UT, USA) supplemented serum, which was obtained from children, and 1× Penicillin & Streptomycin (Gibco, Carlsbad, CA, USA, 100 U/mL penicillin and 100 µg/mL...
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streptomycin). Subsequently, cells were placed in the damp condition with 5% CO₂ at 37°C for appropriate time.

Quantitative polymerase chain reaction (qRT-PCR)

The total RNA was harvested and extracted from cultured HCAECs using TRizol (Invitrogen, Carlsbad, CA, USA) accordingly at 48 h post-transfection. After that, lysates were incubated with RNase-free DNase I (Takara, Dalian, China), and then first-strand cDNA was reversely transcribed through PrimeScript 1st Strand cDNA synthesis kit (Invitrogen). Subsequently, levels of genes and miRNAs were detected using ABI 7500 (Applied Biosystems, Rockford, IL, USA) with reagent of the SYBR ExScript qRT-PCR kit (Takara). All reaction solutions were mixed in triplicate, and relative levels of miRNAs and genes were calculated using 2-ΔΔCt method utilizing glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 as internal controls. Primers used for the amplification of the miRNAs and genes were exhibited as follows: miR-197-3p: (forward: 5'-GTTCACCACCTTCTCCAC-3' and reverse: 5'-GTGCAGGGTCCGAGGT-3'); IGF1R: (forward: 5'-GGACAGGTCAGAGGGTTTC-3' and reverse: 5'-CTCGTAACTCTTCTTGACC-3'); BCL2: (forward: 5'-CGCCCTGTGAGTAGCATGAGT-3' and reverse: 5'-CTCGTAACTCTTCCTGTGCC-3'); BCL2 (si-BCL2), and scramble (si-NC). Transient transfection was implemented by using lipofectamine 2000 transfection reagent (Invitrogen) and RPMI-1640 with serum detection according to manufacturer’s manuals. Lastly, cell proliferation was visually assessed via a microplate reader (Bio-Rad, Hercules, CA, USA).

Flow cytometry

Apoptosis of transfected HCAECs was determined using a fluorescein isothiocyanate (FITC)-labeled annexin V (annexin V-FITC) apoptosis detection kit and identified by flow cytometry. Briefly, HCAECs were harvested and washed at 48 h post-transfection. Then cells were re-suspended in 5 µL of binding buffer conducting annexin V-FITC and 5 µL propidium iodide (PI) staining Solution for 10 min in the dark. After this, apoptotic cells were analyzed by FACS Caliber flow cytometer (BD Biosciences, San Jose, CA, USA).

Transwell assay for cell migration

After HCAECs were stimulated accordingly, cells were seeded into the upper chambers of transwell chambers (8-µm pore size; Corning, NY, USA) with serum-free medium and then the complete cell culture medium was added into the lower chambers. Subsequently, cells migrated through the membrane were stained using 0.1% crystal violet (Solarbio, Beijing, China) for 20 min after HCAECs were incubated for 24 h in chambers at 37°C. The number of migrating cells was observed and counted under a microscope in 5 randomly selected fields.

Western blot assay

Western blot was carried out by using specific primary antibodies against E-cadherin (1:1000, ab76055, Abcam, Cambridge, MA, USA), Vimentin (1:1000, ab8979, Abcam), N-cadherin (1:500, ab18203, Abcam), Cleaved-caspase 3 (C-caspase 3; 1:500, ab13847, Abcam) IGF1R (1:800, ab39675, Abcam) or Cleaved-PARP (C-PARP; 1:1000, ab32064, Cell Signaling Technology, Boston, MA, USA), and GAPDH (1:1000, ab8245, Abcam) was used as internal control.
In short, after transfected HCAECs were washed with ice-cold phosphate buffer saline (PBS; Hyclone) for 3 times, they were lysed using RIPA lysis buffer (Millipore, Bedford, MA, USA) containing protease inhibitors (Sigma, St. Louis, MO, USA), homogenized on ice for 30 min, and then centrifuged at 12000 rpm at 4°C for 10 min. The supernatant was collected in clean tubes and saved in -80°C or proceed directly to the next experiment. Protein concentration was measured using Bradford assay (Bio-Rad) and equal protein (20 µg) was loaded onto 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels. Subsequently, isolated proteins were then transferred onto nitrocellulose membranes (Thermo Fisher Scientific, Rockford, IL, USA), then membranes containing separated proteins were blocked with 5% (w/v) non-fat milk powder which was dissolved in Tris-Buffered Saline Tween-20 (TBST; Solarbio) for 2 h, and washed using 1×TBST for thrice. Afterwards, membranes were incubated with the specific primary antibodies overnight for 4°C, and were also washed by TBST. This was followed by incubating with appropriate secondary antibodies for combining primary antibodies. After washing 3 times, membranes were treated with ECL Kit (Thermo Fisher Scientific) to amplify and appear complex, and signals were analyzed by Quantity One software (Bio-Rad).

**Dual-luciferase reporter assay**

Common fragments between miR-197-3p and IGF1R or BCL2 were predicted by starBase. In order to verify the interaction of them, based on the common sequences, the binding sites of miR-197-3p and wildtype (named as IGF1R-WT) or mutant (named as IGF1R-MUT) of IGF1R were amplified by PCR and then inserted into pGL3 firefly luciferase reporter vector (Promega, Madison, WI, USA). The vectors of wildtype (named as BCL2-WT) or mutant (named as BCL2-MUT) of BCL2 were constructed in line with the above description. After that, vectors (IGF1R-WT, IGF1R-MUT, BCL2-WT or BCL2-MUT) were co-transfected with miR-197-3p mimics or control into HCAECs using lipofectamine 2000 transfection reagent (Invitrogen). Furthermore, pRL-CMV vector (Promega) was also transfected with the above sequences as an endogenous control for standardizing transfection efficiency. Subsequently, luciferase intensities were then identified using the dual-luciferase reporter assay system (Promega) after transfection for 48 h, and renilla luciferase activity was used to normalize firefly luciferase activity in the assay.

**Statistical analysis**

In the study, every experiment was repeated three times independently. The data from each single assay was expressed as the mean ± standard deviation (mean ± SD). Data analysis was carried out using SPSS 22.0 software. Differences between paired groups and among multiple groups were analyzed using Tukey’s test and analysis of variance, respectively. It was considered to indicate a statistically significant difference when $P$ less than 0.05.

**Results**

**Level of miR-197-3p is highly expressed in acute KD**

To verify whether miR-197-3p plays an important role in KD, the level of miR-197-3p was measured by qRT-PCR. The serum samples donated by children were classified according to different clinical characteristics, including healthy control (n=18), acute KD (n=32) and convalescent KD (n=20). QRT-PCR results exhibited that miR-197-3p level was higher than that in of healthy control group and convalescent KD group (Figure 1). The data meant that miR-197-3p may play a critical role in the progression of children KD.
Knockdown of miR-197-3p suppresses cell apoptosis, induces proliferation and migration in KD serum-stimulated HCAECs

Given the high expression of miR-197-3p in acute KD sera, we further investigated whether miR-197-3p participating in cell behaviors of HCAECs, such as cell proliferation, apoptosis, and migration. Firstly, 20% KD serum was used to stimulate HCAECs for establishing cell model, and miR-197-3p inhibitor or control were transfected into treated cells. The level of miR-197-3p was significantly induced by KD serum, while the promotor effect was obviously decreased by miR-197-3p inhibitor in HCAECs (Figure 2A). Cell behavior of proliferation was determined by MTT assay, the results showed that miR-197-3p notably increased in 20% KD serum induced HCAECs (Figure 2B). Moreover, flow cytometry results proved that the induced apoptotic rate by KD serum was remarkably hindered after transfection of miR-197-3p in HCAECs (Figure 2C). At the same time, transwell assay demonstrated that KD serum could dramatically hamper cell migration capacity, while the inhibitory effect was accelerated by miR-197-3p inhibitor (Figure 2D). Subsequent assay also determined the apoptosis-related proteins, C-caspase 3 and C-PARP using western blot assay, and the results also supported the above cell apoptosis conclusion (Figure 2E and 2F). Finally, E-cadherin, N-cadherin, and Vimentin, which functioned as transition-related proteins and were measured using western blot assay, the high expression of E-cadherin and low expression of N-cadherin and Vimentin revealed that KD serum exactly restrained cell transition, whereas the inhibiting effect on cell transition was relieved by miR-197-3p inhibitor. The conclusion was the same as the above description (Figure 2E and 2G). In brief, HCAECs were induced by KD serum and this stimulation indicated repression effect on cell proliferation and migration as well as promotor effect on cell apoptosis, while these effects of KD serum were reversed by miR-197-3p inhibitor.

IGF1R is a target gene of miR-197-3p

Subsequently, the study aimed to clarify how miR-197-3p exerted its role in KD. StarBase was employed to predict the common fragments between miR-197-3p and target gene, and show that there were binding sites between miR-197-3p and IGF1R (Figure 3A). In addition, the results of decreased luciferase activity in transfecting IGF1R-WT group and no evident change in mutant group revealed that IGF1R was a target gene of miR-197-3p (Figure 3B). To further investigate the regulatory mechanism between miR-197-3p and IGF1R, miR-197-3p mimics or inhibitor were transfected into HCAECs, qRT-PCR and western blot assays were performed and the results displayed that both mRNA level and protein expression of IGF1R were markedly blocked by miR-197-3p mimics, while greatly enhanced by miR-197-3p inhibitor (Figure 3C and 3D). Simultaneously, the study also researched the expression of IGF1R in KD serum induced HCAECs; qRT-PCR proved that IGF1R was prominently decreased by KD serum. Interestingly, the repression effect was rescued by miR-197-3p, while aggravated after transfection with miR-197-3p mimics (Figure 3E and 3F). Moreover, the results of western blot also agreed with the above conclusion in protein level of IGF1R (Figure 3G and 3H). This data discovered that miR-197-3p directly targeted IGF1R and the level of IGF1R was opposite with miR-197-3p.

MiR-197-3p directly targets BCL2

To further find the target gene of miR-197-3p, starBase also showed that there were binding sites between miR-197-3p and BCL2 (Figure 4A). Then the dual-luciferase reporter assay was performed to verify the interaction between miR-197-3p and BCL2, the result exhibited that luciferase activity was distinctly curbed by wild-type of BCL2, whereas there was no significant difference in mutant group (Figure 4B). Subsequent assays uncovered the molecular mechanism between miR-197-3p and BCL2. Firstly, miR-197-3p mimics or inhibitor were transfected into HCAECs, severally, the levels in both mRNA and protein were conspicuously impeded in miR-197-3p mimics group, but was obviously induced by miR-197-3p inhibitor (Figure 4C and 4D). Then the level of BCL2 was assessed after transfection with miR-197-3p inhibitor or control in KD serum induced HCAECs. QRT-PCR and western blot assays indicated that mRNA level and protein expression of BCL2 were drastically repressed in KD serum stimulated HCAECs. Excitedly, the inhibiting effect was regained through transfecting with miR-197-3p inhibitor, while further blocked
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by miR-197-3p mimics in KD serum stimulated HCAECs (Figure 4E-H). These results displayed that BCL2 was the other target gene of miR-197-3p and the expression of BCL2 was regulated by miR-197-3p in KD serum induced HCAECs.

Effects of miR-197-3p inhibitor on cell behaviors were abolished by either IGF1R or BCL2 knockdown in KD serum induced HCAECs

In the assay, the study systematically verified the modulation mechanism between miR-197-3p and the cell behaviors in KD serum induced HCAECs.
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3p and IGF1R or BCL2. HCAECs treated with KD serum were transfected with anti-miR-NC, anti-miR-197-3p, anti-miR-197-3p+si-NC or anti-miR-197-3p+si-IGF1R, severally. The results discovered that the promotor effect of miR-197-3p inhibitor on IGF1R expression was recovered by knockdown of IGF1R in KD serum stimulated HCAECs (Figure 5A and 5B). Utilizing the similar method, after co-transfected with miR-197-3p inhibitor and BCL2 siRNA or scramble, the acceleration effect of miR-197-3p inhibitor on BCL2 expression in both mRNA and protein levels were abrogated by BCL2 siRNA (Figure 5C and 5D). Additionally, cell viability was evaluated by MTT assay. The results revealed that the facilitation effect of miR-197-3p inhibitor on cell proliferation was restored by either si-IGF1R or si-BCL2 in KD serum induced HCAECs (Figure 5E). Meanwhile, any one siRNA of IGF1R and BCL2 abolished the curb effect of miR-197-3p inhibitor on cell apoptosis in HCAECs treated with KD serum (Figure 5F). Cell migration was detected by transwell assay, the results displayed that cell migration, which was boosted by miR-197-39 inhibitor, was abrogated by transfecting either si-IGF1R or si-BCL2 into KD serum stimulated HCAECs (Figure 5G). Simultaneously, apoptosis proteins including C-caspase 3 and C-PARP were examined by western blot for further investigating of the regulatory mech-

Figure 3. IGF1R is a target gene of miR-197-3p. A. The binding sites between miR-197-3p and IGF1R were predicted by starBase. B. Dual-luciferase reporter assay was carried out to confirm the relation between miR-197-3p and IGF1R. C and D. QRT-PCR and western blot assays were employed to examine IGF1R mRNA level and protein expression after transfection miR-197-3p mimics or inhibitor. E and F. The mRNA level of IGF1R was evaluated by qRT-PCR after transfected with miR-197-3p mimics or inhibitor in 20% KD serum-stimulated HCAECs. G and H. Western blot assay was conducted to measure IGF1R protein level after induced accordingly. *P<0.05.
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Figure 4. MiR-197-3p directly targets BCL2. A. Wildtype and mutant of putative miR-197-3p targeted sequences in BCL2 mRNA were shown. B. Analysis of luciferase activity in HCAECs, vectors co-transfected with miR-NC or miR-197-3p into HCAECs was performed, respectively. C and D. MRNA level and protein expression of BCL2 were detected by qRT-PCR and western blot assays, respectively, post-transfection with miR-197-3p mimics or inhibitors. E and F. After HCAECs were treated with 20% KD serum, miR-197-3p or anti-miR-197-3p were transfected into cells; analysis of mRNA level BCL2 via qRT-PCR was implemented. G and H. The protein of BCL2 was analyzed using western blot assay post treatment accordingly. *P<0.05.

Figure 5. A. Wildtype and mutant of putative miR-197-3p targeted sequences in IGF1R mRNA were shown. The result agreed with the conclusion of either si-IGF1R or si-BCL2 reversed the inhibitory role of miR-197-3p inhibitor in cell apoptosis (Figure 5H and 5I). Furthermore, the proteins, E-cadherin, N-cadherin, and Vimentin which were viewed as transition-related proteins were identified by western blot and the results were in line with the results of transwell (Figure 5H and 5I). All data indicated that knockdown of either IGF1 or BCL2 overturned the effect of miR-197-3p on cell proliferation, apoptosis, and migration in KD serum stimulated HCAECs.

Discussion

KD is characterized by acute, febrile rash, principally affecting among children less than 5 years old and caused CCAs or ectasia. Besides, KD has become the reason of the cardiac complications in children [24] and is closely related to inflammatory response [25]. Owing to the aberrant expression of miRNAs in KD patients, researchers have discovered the association between partial miRNAs and the progression of KD or vascular injury. The mechanism of damaged coronary artery endothelium which was caused by paradoxical response to intracoro-
nary artery acetylcholine has been displayed. Other researchers reported that ECs damage was associated with KD [26]. Here, the study used KD serum and HCAECs to establish cell model of KD.

Over the past decades, miRNAs, which were small non-coding RNAs, has been proved to participate in human diseases development, such as cardiovascular diseases, and KD [9, 27]. Prior to this study, many miRNAs functioned in KD have been proved. Specially, miR-125a-5p in plasma acted as a novel biomarker for KD with coronary artery aneurysm [29]. The target gene of miR-197-3p was focused on its role in the progression of cancers. Such as, miR-197-3p regulated chemoresistance of non-small cell lung cancer through being targeted by long non-coding RNA Metastasis Associated Lung Adenocarcinoma Transcript 1 [30]. However, the role of miR-197-3p in KD diseases progression and ECs injury was not investigated previously. In this study, the main finding was aberrant expression of miR-197-3p in KD serum and KD serum induced cell model. Subsequent assay proved that cell apoptosis was promoted, whereas proliferation and migration were induced and treated with KD serum in HCAECs. Interestingly, miR-197-3p

Figure 5. Effects of miR-197-3p inhibitor on cell behaviors were abolished by either IGF1R or BCL2 knockdown in KD serum induced HCAECs. (A and B) MiR-197-3p inhibitor was co-transfected with si-NC or si-IGF1R into 20% serum-treated HCAECs, qRT-PCR and western blot assays results exhibited IGF1R mRNA and protein levels, respectively. (C and D) MRNA and protein levels of BCL2 were evaluated by qRT-PCR and western blot assays, respectively, after co-transfection with miR-197-3p inhibitor and si-NC or si-BCL2 into 20% KD serum explored HCAECs. (E-J) HCAECs induced by 20% KD serum were transfected with anti-miR-NC, anti-miR-197-3p, anti-miR-197-3p+si-IGF1R or anti-miR-197-3p+si-BCL2, severally. (E) Cell viability was determined by MTT assay. (F) Flow cytometry was performed to detect apoptotic cells. (G) Cell migration capacity of HCAECs was examined using transwell assay. (H) Proteins of C-caspase 3, C-PARP, E-cadherin, N-cadherin and Vimentin were estimated by western blot assay. (I and J) Apoptosis-related proteins, C-caspase 3 and C-PARP (I), as well as transition-related proteins, E-cadherin, N-cadherin and Vimentin (J) were quantified via Image J software. *P<0.05.
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inhibitor reversed the above effect of KD serum on cell proliferation, apoptosis and migration in vitro.

For IGF1R, except the reporter of that IGF1R, was involved in gestational age [31], previous studies have proven that the expression of IGF1R was highly expressed in pediatric gastrointestinal stromal tumors [32]. Besides, the signaling pathway of IGF1R could act as the target for cancer therapy [33]. Nevertheless, why IGF1R was selected in the study? Firstly, the study found that miR-197-3p was a pro-apoptotic factor on HCAECs, thereby, an anti-apoptotic gene may be a directly targeted by miR-197-3p. Secondly, starBase online software predicted that there were binding sites between miR-197-3p and IGF1R. Thirdly, IGF1R expression was lowered in KD serum stimulated HCAECs. Above all, subsequent assays confirmed that IGF1R was directly targeted by miR-197-3p.

For BCL2, increasing researches discovered that BCL2 as an apoptosis-relative gene, played a critical role in human diseases. Firstly, BCL2 family was switch in regulating of the cellular life-or-death switch [34]. What’s more, BCL2 was also a target in relapsed chronic lymphocytic leukemia [35] and BCL2 antisense therapy was applied to chemo-sensitization of malignant melanoma [36, 37]. Apart from this, the expression of BCL2 was limited in KD serum induced HCAECs and subsequent assay also uncovered that there were common fragments between miR-197-3p and BCL2. Importantly, the role of miR-197-3p inhibitor in cell proliferation, apoptosis, and migration was relieved by BCL2 siRNA. That was to say, miR-197-3p exerted its roles in cell behaviors by targeting BCL2.

In summary, the level of miR-197-3p was significantly enhanced in acute KD serum and KD cell model. While expression of IGH1R and BCL2 was both curbed in KD cell model, compared with matched control. The functions of miR-197-3p on cell behaviors of proliferation, apoptosis, and migration were totally overturned by IGF1R or BCL2 in KD.

Conclusion

The level of miR-197-3p was significantly augmented in acute KD serum compared with healthy control and convalescent KD samples.

On the function, miR-197-3p inhibitor repressed cell apoptosis, enhanced proliferation and migration in KD serum stimulated HCAECs. Interestingly, silencing either IGF1R or BCL2 abolished the effect of miR-197-3p inhibitor on cell proliferation, apoptosis, and migration in KD serum induced HCAECs. Mechanically, miR-197-3p directly targeted IGF1R and BCL2. MiR-197-3p functioned its roles in cell behaviors via targeting IGF1R and BCL2 in KD.

Disclosure of conflict of interest

None.

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References

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