International Core Journal of Engineering Volume 8 Issue 3, 2022
ISSN: 2414-1895 DOI: 10.6919/ICJE.202203_8(3).0081

# piRNA-62898 Inhibits the Migration of Cardiac Microvascular Endothelial Cells

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## Abstract

**Recent studies have shown that piRNAs not only play an important role in regulation of reproductive cells, but also in other somatic cells. In present study, we show as first that piRNA-62898 treatment inhibits migration of CMECs but fails to conduct effects in proliferation and apoptosis of CMECs. The finding of this study clearly reveals that piRNA, such as piRNA-62898, plays an important regulative effect in migration of CMECs.**

## Keywords

**piRNA-62898; Cardiac Microvascular Endothelial Cells; Cell Migration.**

## 1. Introduction

piRNA (Piwi interacting RNA) is a kind of small RNA with a length of 24-31nt originally isolated from mammalian germ cells (most of the piRNAs length is concentrated in 29-30nt) [1]. At the beginning, piRNAs were found in the germ cells[2]. piRNAs and piwi containing Argonaute protein, which constitutes a key part of piRNA pathway, were believed to be mainly involved in silencing germ cell transposon elements[3]. The functions of piRNAs are found to regulate the growth and development of germ line cells. Its main role is related to genome rearrangement, epigenetic regulation, and protein regulation of the reproductive system [4-5].

Recent studies reveal that except reproductive system, piRNA is expressed in somatic cells. piRNA is involved in the regulation of cardiovascular dysfunction[6]. The recent study of our laboratory found that piRNAs were involved in the cardiac telocytes (CTs) derived exosomes. Therefore, we hypothesized that piRNAs might play a role in cardiac microvascular endothelial cells (CMECs). In this study, one of the CT-derived exosomal piRNAs, piRNA-62898 was investigated. Our results demonstrated that piRNA-62898 is able to inhibit the migration of CMECs.

## 2. Materials and Methods

### 2.1 Animals

Three-month-old female Sprague-Dawley (SD) rats (250-300 g) were utilized in the present study. The rats were housed for 2 weeks to allow them to adapt before experimentation. They were provided with food and water ad libitum. Animal care, surgery and handling procedures were performed according to regulations established by The Ministry of Science and Technology of the People's Republic of China ([2006] 398) and approved by Jinan University Animal Care Committee.

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### 2.2 Synthesis of piRNA-62898 Agmior

The piRNA-62898 agmior and the agmior negative control (NC) were obtained from GenePharma. The piRNA-62898 agmior sequence is 5′-GACUCUUAGCGGUGGAUCACUCGGC-3′. In the mimic, 2′-O-methylation modification was carried out in all the bases, and cholesterol was added to the 3′ end. The sequence of the mimic-NC is 5′-UUCUCCGAACGUGUCACGUTT-3′.

### 2.3 Isolation of CMECs from Adult Rat Hearts

The rats were sacrificed by cervical dislocation, followed by immerse in 75% ethanol for 3-5 min to disinfection. The heart was cut out in clean bench and washed with precooled ADS buffer. The hearts were minced into small pieces of approximately 1–2 mm3 in size, and then and then incubated with 10 mg/mL type collagenase II at 37 °C on a shaker for 20 min. The suspension was filtered by 100-μm filter and centrifuged at 200 × g for 10min. The supernatant was then removed and the pellet was resuspended in 1 mL of precooled PBS buffer (pH=7.4). The cells suspension was layered onto gradient buffer formed by 40% percoll and 20% percoll, and then centrifuged at 400 × g for 30 min. After the percoll gradient centrifugation, the middle cells layer was collected and diluted with PBS buffer, followed by centrifuged at 1000 × rpm for 3 min. The cells pellet were resuspended in the CMEC medium (medium 199 basic supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin and endothelial cell growth supplement) and cultured at 37 °C and 5% CO2 in a 95% air incubator.

### 2.4 Transfection of piRNA-62898 Agmior in CMECs

The isolated CMECs were cultured to 80% confluence with complete culture medium as described above at 37 °C in a 5% CO2 and 95% air incubator and then transfected with piRNA-62898 agmior (5'-GACUCUUAGCGGUGGAUCACUCGGC-3'; 100 nM; GenePharma) or the scrambled control (5'-UUCUCCGAACGUGUCACG-3'; 100 nM; GenePharma) using Lipofectamine RNAiMAX (cat. no. 13778150; Invitrogen). The details are followed the manual. The transfected cells were used for cell viability assays, migration assays or apoptosis assays. In the above assays, 3 identical wells were observed in each analysis, and three repeat experiments were conducted.

### 2.5 Migration Assay

For the wound healing migration assay, CMECs transfected with piRNA-62898 agmior or scrambled control were cultured on 12-well plates at a density of 2×105 cells per well. The monolayer CMECs (approximately90% confluence) were scratched with 100 uL pipette tips and washed with PBS to remove the cell debris. Images were captured using an inverted phase microscope (Olympus IX71, Japan) at 0 h, 36 h after the cell layer was wounded. The migration distance = (average scratch width after dosing - initial average scratch width) / 2Average width = scratch area in field of view / scratch length in field of view [7].

### 2.6 Apoptosis Assay Using Flow Cytometry

CMECs transfected with piRNA-62898 agmior or scrambled control were cultured in 10% serum medium 199 basic at 37 °C in a 5% CO2 and 95% air incubator for 24 h. An Annexin V/PI kit (FXP023-100, 4A Biotech) and subsequent flow cytometry analysis were applied to investigate the apoptosis of treated CMECs according to the manufacturer's instructions. Briefly, the treated CMECs (1×106) were washed with cold PBS, resuspended in 100 μL of binding buffer and stained with 5 μL of Alexa Fluor 647-conjugated Annexin V. Following a 5-min incubation in a dark room, 10 μL of PI and 400 μL of binding buffer were added. Finally, the cells were analyzed using a flow cytometer (Cytoflex; Beckman Coulter). Early and late apoptotic cells were examined using plots of fluorescence 2 (FL2 for PI) versus fluorescence 1 (FL1 for Annexin V). A total of 10,000 events were collected and analyzed for each sample.

### 2.7 Cell Viability Assay

Cell viability was measured using the CCK-8 assay kit (cat. no. CK04; Dojindo) according to the manufacturer's instructions. Briefly, 10 μL of CCK-8 solution was added to each well (100 μL of

ISSN: 2414-1895 DOI: 10.6919/ICJE.202203_8(3).0081

medium) and incubated for 1 h at 37 °C in a 5% CO2 and 95% air incubator, and the absorbance was then measured at 450 nm in a microplate reader (Biotek).

### 2.8 Statistical Analysis

All data were analyzed and charted by graphpad prism 8 statistical analysis software. T-test was used for inter group analysis, $P < 0.05$, indicating that the difference between groups was statistically significant.

## 3. Results

### 3.1 piRNA-62898 Inhibited the Migration of CMECs in Vitro

To evaluate the biological functions of piRNA-62898 in migration of CMECs, wound-healing assay was applied. CMECs were cultured and transfected with piRNA-62898 agomir or agmior negative control. It was found that the migration distance of piRNA-62898 treated CMECs in 36 h after treatment was significantly less than that of the negative control ($P < 0.01$, Fig.1).

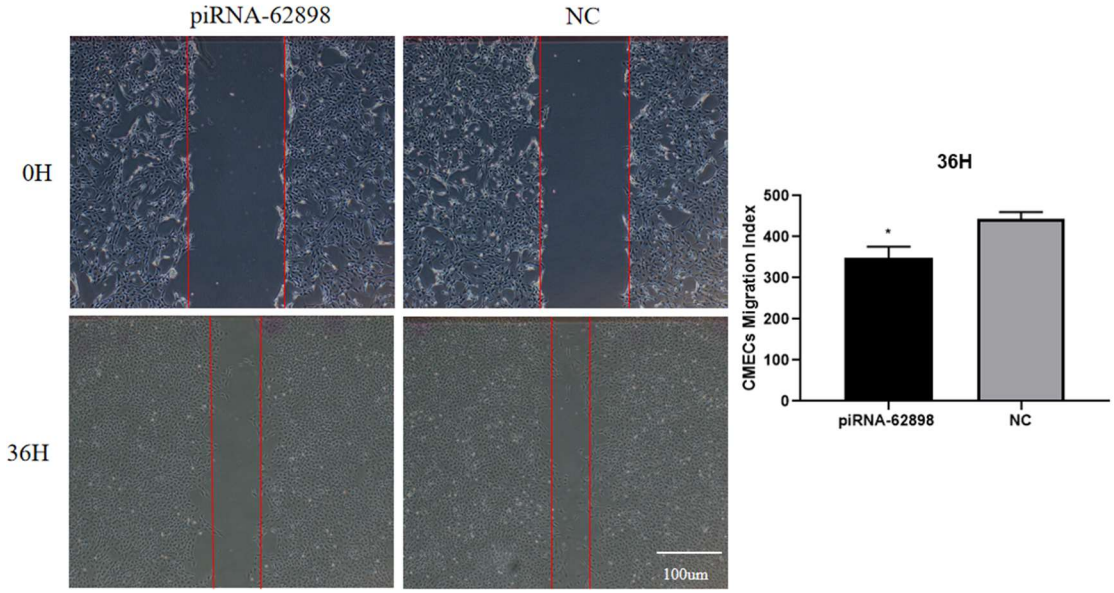


**Fig. 1** piRNA-62898 inhibited the migration of CMECs in vitro. *: $P < 0.05$.

### 3.2 piRNA-62898 did Not Promote the Proliferation of CMECs in Vitro

CCK-8 assay was conducted to determine the biological functions of piRNA-62898 in cell proliferation and viability of CMECs. The results of CCK-8 assay revealed that the OD value of the piRNA-62898 treated groups in 24 h, 48 h, 72 h and 96 h after transfection was similar to the negative control groups respectively ($P > 0.05$, Fig.2).

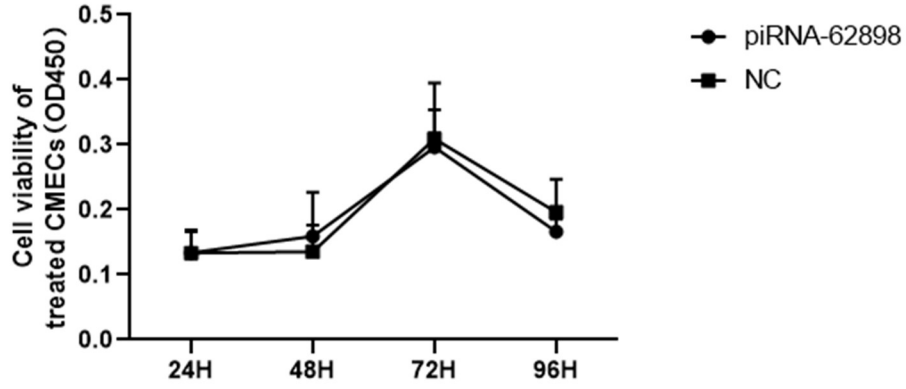


**Fig. 2** piRNA-62898 did not promote the proliferation and viability of CMECs in vitro.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202203_8(3).0081

### 3.3 piRNA-62898 had No Effect on the Apoptosis of CMECs

To assess the role of piRNA-62898 in apoptosis of CMECs, the annexin V/PI kit and subsequent flow cytometry analysis were applied. It was found that the difference of apoptosis of CMECs between piRNA-62898 agmior treated group and negative control group was not statistic significance ($P > 0.05$, Fig.3).

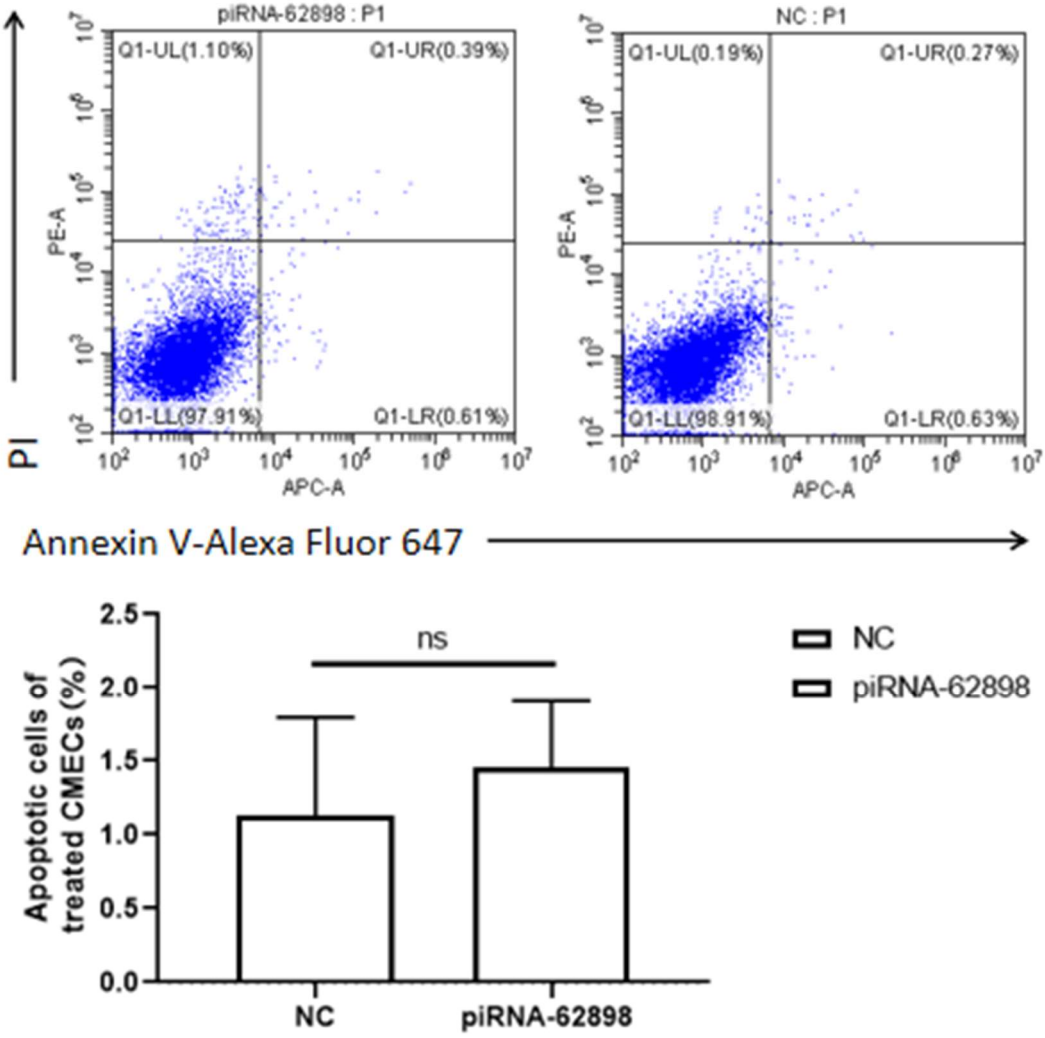


**Fig. 3** piRNA-62898 did not play an effect in apoptosis of CMECs. ns: The difference is not statistic significance.

## 4. Discussion

The results of present study document as first that piRNA-62898 treatment inhibits migration of CMECs but fails to conduct effects in proliferation and apoptosis of CMECs. The finding proposes that piRNA-62898 derived inhibition of CMECs migration is not due to inhibition of CMECs proliferation and increasing apoptosis of CMECs. It is attributed to the direct effect of inhibition in cell migration. The finding also reveals that piRNA, such as piRNA-62898, plays an important regulative effect in migration of CMECs. The exact molecular mechanism of piRNA-6289 derived inhibition of CMECs migration need to be further studied in future.

## Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (82001466).

ISSN: 2414-1895 DOI: 10.6919/ICJE.202203_8(3).0081

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