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MicroRNA-19a-3p Regulates Abdominal Aneurysm Development and Progression via Direct Interaction with PMEPA1

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Abstract **Background:** Abdominal aneurysm (AA) is a fatal disease having high mortality rates. The therapeutic approaches and roles of microRNAs (miRNAs) and messenger RNA (mRNA) in treatment of AA has previously been explored. **Objective:** The aim of the study was to investigate microRNA-19a-3p influence on PMEPA1 in moderation of AA development and advancement. **Materials and Methods:** Quantitative Real Time-PCR was utilized to analyze the expression of microRNA-19a-3p and PMEPA1 in expanded vascular smooth muscle cells (VSMCs). Gene expression was modulated through cell transfection, either upregulating or downregulating the target genes, followed by assessment of cellular viability using the CCK-8 assay. RT-qPCR was employed to assess cellular proliferation and apoptosis, focusing on biomarkers Ki67 and PCNA for proliferation, and caspase-8 and caspase-3 for apoptosis. **Results:** The outcomes demonstrated upregulated microRNA-19a-3p levels in AA-VSM cells that promoted cellular viability. Silencing miR-19a-3p inhibited cell viability of AA VSM cells. Furthermore microRNA-19a-3p was wholly connected to the 3'-UnTranlated Region of PMEPA1 to negatively regulate PMEPA1expression and AA progression. While downregulated PMEPA1 strengthen the proliferation effect of microRNA-19a-3p in AA VSM cells. **Conclusion:** Our study presents further understanding in underlying molecular mechanisms of microRNA-19a-3p and PMEPA1suggesting a novel therapeutic approach to AA.

Key Words microRNA-19a-3p, PMEPA1, abdominal aneurysm, cell viability

1. Introduction

Abdominal aneurysm (AA) is an ailment accredited for high fatality rates. This diseases manifests itself as long-lasting abdominal aortic dilation having likely rupture and extensive hemorrhage and consequently fatal [1]. Aortic aneurysm is a disease that develops gradually or in stages by widening the aorta, specifically as a result of alteration of the aortic wall by atherosclerosis; deaths related to aortic aneurysm have been on the rise around the world due to lifestyle adjustments. Essentially, aortic aneurysm could result into terminal outcomes of aortic separation and fissure. The majority of aortic aneurysms show no symptoms until fissure, with the majority of aortic aneurysm patients getting detected during diagnostic imaging for differently assumed ailments. The abdominal aortic media has elastin filaments and collagen fibers organized in systematic denoting circle bands called lamellar units. Typically related with chronic aortic inflammation, usually sedentary proteases gets stimulated and a complex reconstruction process gets initiated, leading to disruption of systematic arrangement of elastin and collagen and extracellular matrix degradation [2]. However, research in AA has been marred by some challenges including but not limited to identification of safe medications for older patients with multiple comorbidities and development of precise diagnosis assessments [3], [4]. Regrettably, steadfast pharmacological agents are unavailable to limit AA expansion [5]. Finally, both human data and animal models indicate that a variety of leukocytes and inflammatory cytokines/mediators are also involved in the mechanisms of AAs [6]. The initial subsequent research has provided some evidences for the correlation between miRNAs and AA with some recent obtained miRNA microarray profiling advances. Research suggests that miRNAs exhibit various abnormalities in their overexpression or under expression with human diseases, such as cardiovascular disorders [7]. For example, some differently expresses lncRNA-miRNA-mRNA network in AA was established by performing data analysis from public databases [8]. Promotion of Invasion and Migration by Serum in Human Aortic

smooth Muscle Cells in relation to matrix metalloproteinase-2 and tissue inhibitor of MMP-1 [9]. p53-contingent vascular smooth muscle cell apoptosis and suppression of aneurysm by miRNA-504 [10]. Previous work has shown that miR-19a-3p is one of the miRNAs that can be of potential use as a biomarker for therapy due to aberrant expression of the given molecules. For example, one study shows that the released factors enhanced angiogenesis, clarified the nature of the involved cargo, and demonstrated their efficiency for regeneration and recovery of myocardial ischemia [11]. This study documents that miR-19a-3p contained exosomes are a vesicular content that leads to the observed effects and improved function of ischemic myocardium following treatment by shock wave therapy [12]. And inhibiting miR-19a-3p promoted cells chemo-sensitivity to osteosarcoma cells by enhancing PTE expression. MiR-19a-3p regulated cancer cells phenotype through down-regulation of IGFBP-3 expression in NF-κB-modulated human ovarian cancer cells. In addition, miR-19a targeting suppresses neurological functions of ischemic stroke by regulating glycogen metabolism and neuronal cell death. There were studies for the moderating function of microRNA-19a-3p in RA and it was determined that microRNA-19a-3p play a positive role in rheumatoid arthritis fibroblast-like synoviocytes.

A. Objectives

This study aimed at investigating the influence of microRNA-19a-3p on PMEPA1 in regulation of AA development and progression. It is envisaged that the microRNA-19a-3p/PMEPA1 axis may serve as a crucial biomarker for AA therapy.

2. Materials and Methods

A. Cell treatment and transfection

Primary human umbilical vein endothelial cells (HUVECs) were cultured in DMEM medium supplemented with 100 μg/ml streptomycin, 2 mM L-glutamine, and 20% fetal bovine serum (FBS), adhering to the manufacturer's instructions (Thermo Fisher Scientific, Inc.). The cells were maintained in a humidified atmosphere at 37 degrees Celsius with 5% CO2. After 48 hours of culture, the cells were harvested, and total RNA was extracted using Beyozol mixture following the manufacturer's protocol. Mimics of microRNA-19a-3p and PMEPA1, along with corresponding negative control mimics, were acquired. Cell transfection of the treated HUVECs was carried out using Lipofectamine 3000 according to the manufacturer's instructions, with microRNA-19a-3p and PMEPA1 mimics or inhibitors, including negative control mimics. Transfection efficiency was assessed after 48 hours for 24 hours. Subsequently, the cells (1x105 cells per well) were seeded in six-well dishes and stimulated with 120 nM angiotensin II.

B. Quantitative reverse transcription PCR assay

The RT-qPCR method was performed as describe below. Isolation of total RNA from the given specimen was done by using Beyozol mixture and for reverse transcription 1 μg RNA per specimen was done using BeyoRT Kit™ cDNA First Chain Synthesis.

microRNA-19a-3p (forward)	CCAATAATTCAAGCCAAGCA
microRNA-19a-3p (reverse)	CAGGCAGATTCTACATCGACA
PMEPA1 (forward)	GTGATTTAAAGGCTGTTCTGGG
PMEPA1 (reverse)	TCTGCAGAGAGGCCTTGG

Table 1: Primer sequences

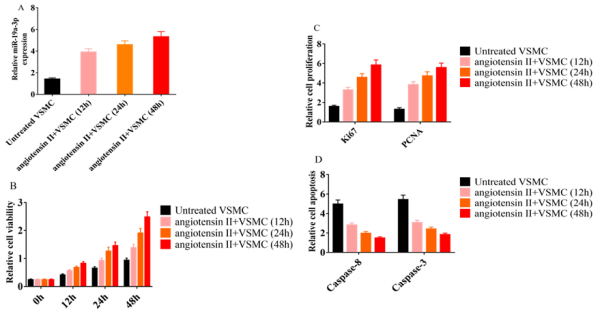

Figure 1: microRNA-19a-3p expression was upregulated in AA-mimic cells and promoted cellular viability

C. Bioinformatics analysis

The target putative binding sites for microRNA-19a-3p and PMEPA1were predicted using bioinformatics analyzing tool Targetscan.

D. Statistical analysis

Data were analyzed using GraphPad Prism 6 and SPSS 29.0. P-value < 0.05 to show a difference in statistical significance.

3. Results

A. microRNA-19a-3p was upregulated in AA-mimic cells and promoted cellular viability

According to the qRT-PCR assay, the expression of microRNA-19a-3p in angiotensin II-treated HUVECs increased significantly over time (0h-48h) compared to untreated cultured HUVECs (*P<0.05, Figure 1A). The highest level of expression was observed after 48 hours, with a progressive increase observed at each time point. Similarly, the CCK-8 assay demonstrated a notable increase in cell viability in angiotensin II-treated HUVECs over time (0h-48h) compared to untreated cultured HUVECs (*P<0.05, Figure 1B), with the highest expression observed after 48 hours.

The results for caspase-8 indicated a significant decrease in apoptosis rate in angiotensin II-treated HUVECs over time (0h-48h) compared to untreated HUVECs (Figure 1D, *P<0.05). The decrease improved with time, reaching its lowest expression after 48 hours. Similarly, the results for caspase-3 showed a significant decrease in cell apoptosis in angiotensin II-treated HUVECs over time (0h-48h) compared to untreated HUVECs (Figure 1D, *P<0.05), with the lowest expression observed after 48 hours. These findings

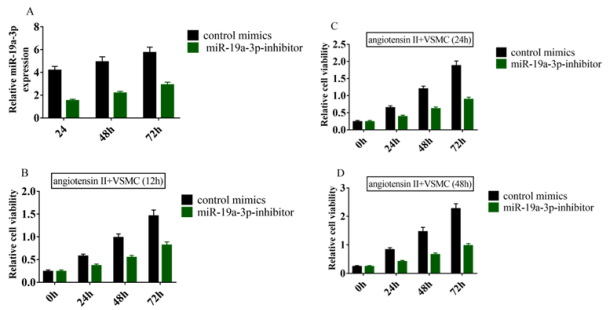

Figure 2: miR-19a-3p knockdown suppresses cell viability of AA cells. A

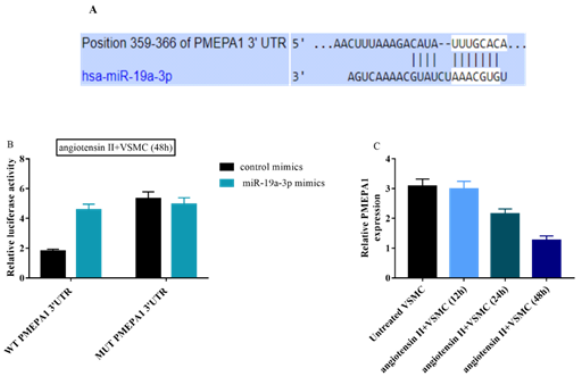

Figure 3: microRNA-19a-3p directly binds to 3'UTR of PMEPA1 to negatively regulate PMEPA1 expression and AA progression. A) ($p<0.05$)

suggest that elevated expression of microRNA-19a-3p in angiotensin II-treated HUVECs enhances cellular viability and reduces pathological apoptosis. The angiotensin II-treated HUVECs were utilized in subsequent experiments.

B. microRNA-19a-3p knockdown suppresses cell viability of AA cells

The impact of microRNA-19a-3p on the cellular viability of angiotensin II-treated HUVECs was assessed using the CCK8 assay. Initially, the HUVECs were treated with angiotensin II and then transfected with either control mimics or a microRNA-19a-3p inhibitor at different time points (12h-48h). Subsequently, RT-qPCR was utilized to evaluate the efficiency of microRNA-19a-3p knockdown. The results demonstrated a significant reduction in microRNA-19a-3p expression levels upon transfection with the miR-19a-3p inhibitor compared to control mimics at all time points examined (12h-48h).

microRNA-19a-3p might directly interact with PMEPA1 to regulate AA progression, bioinformatics analysis was performed using TargetScan. The matching pairing sequences among microRNA-19a-3p and 3'UTR of PMEPA1 were found.

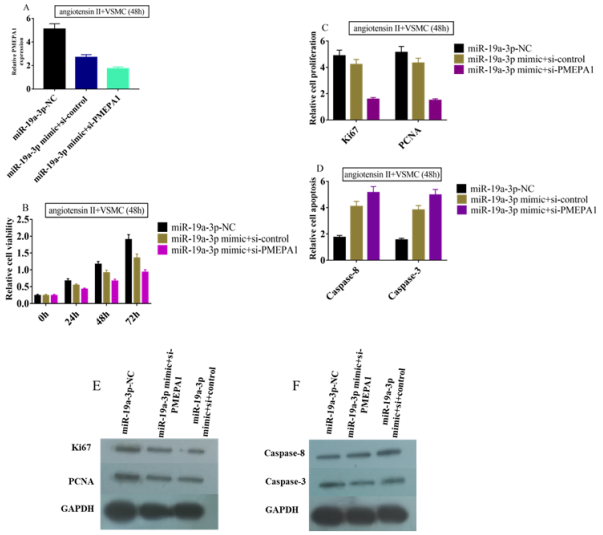

Figure 4: Down regulated PMEPA1 strengthen the proliferation effect of microRNA-19a-3p in AA cells

C. Downregulated PMEPA1

The findings indicated a reduction in PMEPA1 expression in the microRNA-19a-3p mimic+si-PMEPA1 group compared to both the microRNA-19a-3p-NC and microRNA-19a-3p mimic+si-control groups (Fig. 4A, $P<0.05$). Furthermore, the CCK-8 assay results showed a significant increase in cellular viability in the microRNA-19a-3p silenced group and control group (microRNA-19a-3p-NC), while decreased cell viability was observed in the overexpressed group (microRNA-19a-3p mimic+si-PMEPA1) (Fig. 4B, $P<0.05$).

4. Discussion

AA is a severe, deadly vascular ailment, which largely affects elderly men and its morbidity rate has increased gradually with threatening aspects including adulthood, commonly in men, smoking, lineage record, central overweight, low high-density lipoprotein cholesterolemia and hypertension [13]. AA comprises these main morphological features such as external cell matrix degeneration; external cellular medium in aorta contains collagen, elastin, fibronectin and laminin, as main constituent responsible to sustain physical reliability and flexibility of vascular wall [14].

MicroRNAs are a new kind of gene manifestation controlling factor, that suppress the transformation activity of messenger RNAs translating proteins via uniting with the targeted mRNA 3' untranslated region and stimulating controlling properties; they are also crucial in cell differentiation, proliferation, apoptosis and metabolism [15]. This study found that microRNA-19a-3p expression in angiotensin II treated HUVEcs was remarkably elevated at varied times. Cellular proliferation outcomes showed increased cell viability in angiotensin II treated HUVEcs at different times. The proliferation was verified by the Ki67 and PCNA biomarkers that showed an increased trend in proliferation. Thus,

the elevated levels of microRNA-19a-3p in angiotensin II treated HUVEcs promoted cell proliferation. In addition the cell apoptosis was dramatically reduced and confirmed by caspase-8 and caspase-3 suggesting that microRNA-19a-3p upregulated expression in angiotensin II treated HUVEcs affected apoptosis rate. However, microRNA-19a-3p silenced expression led to decreased cell proliferation implying that knocking down microRNA-19a-3p inhibited cellular viability in AA. The study has shown that PMEPA1 was significantly downregulated in angiotensin II treated HUVEcs for AA with time and enhanced cell proliferation. Thus the outcomes suggested that PMEPA1 was directly targeted by microRNA-19a-3p to negatively regulate AA progression. The strengthening and restoration assays revealed that silenced microRNA-19a-3p inhibited cellular proliferation and apoptosis in angiotensin II treated HUVEcs for AA [16]. While overexpressed miR-19a-3p expression coupled with silenced PMEPA1 expression resulted into increased cellular proliferation as verified by the proliferation biomarkers Ki67 and PCNA in angiotensin II treated HUVEcs for AA. In addition, the apoptosis rate was also affected with a dramatic decline in cell apoptosis as verified by the apoptosis biomarkers caspase-3 and caspase-8. Nevertheless, this study showed that protein expression levels for PMEPA1 was significantly reduced after microRNA-19a-3p was silenced as opposed to microRNA-19a-3p overexpression based on the proliferation biomarkers in angiotensin II treated HUVEcs for AA. Similarly, according to the apoptosis biomarkers the protein expression levels of PMEPA1 was significantly increased after microRNA-19a-3p was silenced as opposed to microRNA-19a-3p overexpression in angiotensin II treated HUVEcs for AA. Thus, downregulated PMEPA1 strengthens the proliferation effect by microRNA-19a-3p in AA mimic cell lines and connecting microRNA-19a-3p to PMEPA1 is crucial to the treatment of AA [17].

5. Conclusion

In summary, this paper puts forward a comprehension of molecular mechanism between microRNA-19a-3p and PMEPA1 on AA development and progression since there is no clear study to demonstrate their roles. Several assays have been performed to determine the expressions and interactions of these genes such as RT-qPCR, CCK-8, western blotting, bioinformatics and luciferase assay including statistical evaluation. The outcomes implied that microRNA-19a-3p expression level was elevated in AA-mimic cells and enhanced cellular viability and a notable pathological apoptosis. In addition, down regulated microRNA-19a-3p expression inhibited cell viability of AA cells. The interplay between the genes showed that, that PMEPA1 was directly targeted by microRNA-19a-3p to regulate AA progression and PMEPA1 strengthened the proliferation effect of microRNA-19a-3p in AA cells.

Conflict of interest

Author declares no conflict of interests. Author read and approved final version of the paper.

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