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Original Article

miR-155 regulates the expression of angiotensin II type 2 receptor in apocrine glands of human axillae from osmidrosis patients

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Abstract: Background: Axillary osmidrosis (AO) has become a benign disorder causing functional and emotional problems in patients. MicroRNAs (miRNAs) were known to play critical roles in numerous biological processes. However, the role of miRNAs in axillary osmidrosis is poorly understood and seldom reported. Methods: The expression of miR-155 and angiotensin II type 2 receptor (Ang II 2R) was detected in apocrine glands of human axillae from osmidrosis patients and the controls. The expression of Ang II 2R were examined in apocrine gland cells separated from healthy controls and transfected with miR-155 inhibitor, as well as in apocrine gland cells separated from osmidrosis patients and transfected with miR-155 mimic. Luciferase reporter assay was performed in 193A cells transfected with miR-155 inhibitor or miR-155 mimic. Results: We found the down-regulated expression of miR-155 and high expression of Ang II 2R in apocrine glands of human axillae from osmidrosis patients. When the apocrine glands cells were transfected with miR-155 mimic or miR-155 inhibitor, the expression of Ang II 2R was increased or decreased, respectively. In addition, the luciferase activity of 3'UTR of Ang II 2R was also reduced or enhanced by miR-155 mimic or miR-155 inhibitor in 193A cells. Conclusion: miR-155 regulates the expression of Ang II 2R in apocrine glands of human axillae from osmidrosis patients.

Keywords: miR-155, angiotensin II type 2 receptor, apocrine gland, axillary osmidrosis, apolipoprotein D

Introduction

Axillary osmidrosis is marked by special odor mainly stemming from the apocrine glands in human axillae [1]. Axillary osmidrosis influences the patients both physically and psychologically and leads to considerable social embarrassment to patients. To date, removing the apocrine glands in human axillae through surgery is an effective treatment for axillary osmidrosis [2]. Mounting evidence proved that the characteristic malodor in human axillary secretions were primarily generated by branched unsaturated C6-C11 acids, among which (E)-3-methyl-2-hexenoic acid (E3M2H) was the most important contributor [3]. Several factors are related to the pathology of axillary osmidrosis, such as genetic, hormones, neuroregulation and diet.

MicroRNAs (miRNAs) are a class of small non-coding RNAs molecules that regulate gene expression at a post-transcriptional level through imperfect binding to the 3' untranslated region (UTR) of target mRNAs [4]. It has been established that miRNAs play important roles in numerous biological processes, including signal transduction, cell fate determination, cell proliferation, apoptosis, differentiation and organ development [5, 6]. However, the role of miRNAs in axillary osmidrosis is poorly understood and seldom reported.

The renin-angiotensin system (RAS) is one of the significant hormonal systems regulating the body functions. Angiotensin II (Ang II) is a major active octapeptide of RAS that regulates body fluid homeostasis and blood pressure [7]. In addition to the best known roles of Ang II in cardiovascular homeostasis, Ang II also could be as a stimulator of tissue remodeling, pathologic scarring and cardiac hypertrophy [8]. The function of Ang II signals are mediated through two principal receptors, Ang II type 1 receptor (Ang

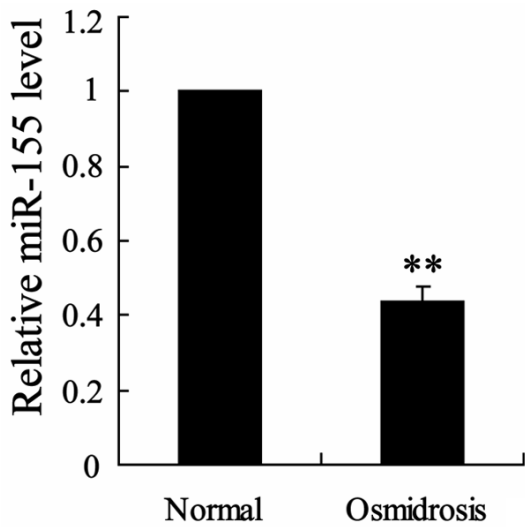

Figure 1. Expression of miR-155 in apocrine glands of human axillae from osmidrosis patients. All values are mean ± SD. **VS normal group, $P<0.01$.

II 1R) and Ang II type 2 receptor (Ang II 2R). High expression of Ang II 2R in the apocrine glands from osmidrosis patients was observed and the expression level of Ang II 2R was positively related to the expression of apolipoprotein D (ApoD) in apocrine glands from human axillae. In this study, we aimed to investigate the relationship of miRNA-155 and Ang II 2R in apocrine glands of human axillae from osmidrosis patients.

Materials and methods

Sample collection

A total of ten male patients with axillary osmidrosis were included from Zhengzhou Central Hospital Affiliated to Zhengzhou University from March 2014 to June 2014 and ten male subjects receiving surgery for scar repair or others served as controls. The fresh axillary skin containing adipose tissues (about 6×2×2 cm) was collected and stored at -80°C until experiments. The protocols were approved by the local ethics committee and all participants consented to molecular analyses.

Apocrine gland cell separation and culture

Adipose tissues were removed from the skin with D-Hanks solution. Then the skin was minced (1 mm^3) and digested in type II collagenase at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. One day later, the sweat gland was collected with an inverted phase contrast microscope and incubated in culture medium. After the sweat gland adhered to the flask wall, a 2-mL of medium were added and refreshed every 2-3 days. Generally, the sweat gland was mixed with fibroblasts which were less tolerant to the trypsin. To purify the sweat gland cells, digestion was performed with trypsin and fibroblasts were shedding. The sweat gland cells were maintained in DMEM containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

Western blot analysis

The proteins were extracted and quantified with Bradford assay. Fifty micrograms of protein were separated per lane by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose membranes (Invitrogen) using a Mini-Protein III system (Bio-Rad). Immunoblotting was performed using rabbit polyclonal primary anti-Ang II 2R (1:2000, Abcam, Cambridge, UK) and primary anti-β-actin. Specific peroxidase-conjugated anti-IgG secondary antibodies were used as the secondary antibody. Densitometric measurements were performed using the Quantity One image analysis software for Windows (Bio-Rad) for quantification. All Ang II 2R values were normalized to β-actin levels.

Quantitative real-time PCR

Total RNA was extracted by Trizol (Invitrogen) following the manufacturer's instruction. The cDNAs were generated by reverse transcription using M-MLV (Promega). The expression level of miR-155 was quantified with miRNA-specific TaqMan MiRNA Assay Kit. Real-time PCR was performed using fluorogenic SYBR Green with the Sequence Detection System 7700. qPCR was performed using an AB 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). Ang II 2R mRNA levels were normalized to that of GAPDH and miR-155 to U6.

Cell transfection and luciferase reporter assay

MiR-155 mimic and miR-155 inhibitor were used to overexpress miR-155 or inhibit the expression of miR-155, respectively. The region of Ang II 2R-3'UTR generated by PCR amplification were cloned into the pGL3-basic luciferase reporter plasmid and obtained pGL3-Ang II 2R-3'UTR. Transfection of miR-155 mimic, or miR-155 inhibitor, or the corresponding control were performed using the Lipofectamine 2000

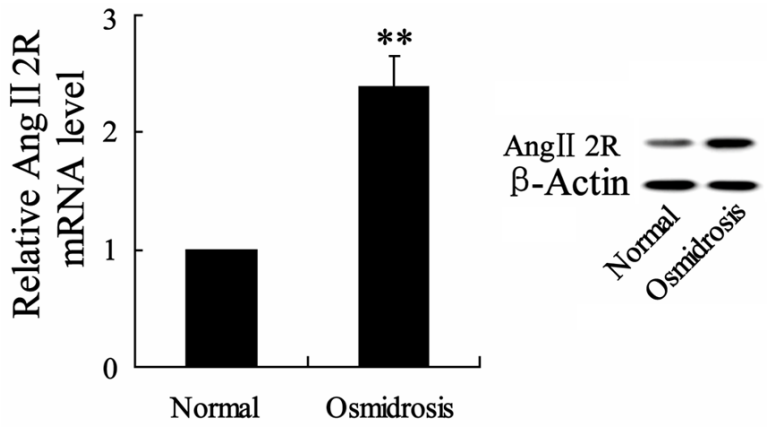

Figure 2. Expression of Ang II 2R in apocrine glands of human axillae from osmidrosis patients. All values are mean ± SD. **VS normal group, $P<0.01$.

reagent (Invitrogen) according to the manufacturer's instruction.

Statistical analysis

Quantitative data were expressed as mean ± standard deviation of separate experiments. One-way analysis of variance (ANOVA) followed by the Fisher's post-hoc test were applied for comparisons. Statistical analysis was performed with SPSS version 13.0. A value of $P<0.05$ was considered statistically significant.

Results

miR-155 was down-regulated in apocrine glands of human axillae from osmidrosis patients

To investigate the role of miR-155 in the development of osmidrosis, the mRNA level of miR-155 was detected in apocrine glands of human axillae from osmidrosis patients and normal control. As shown in **Figure 1**, the mRNA level of miR-155 was significantly decreased in apocrine glands of human axillae from osmidrosis patients than that in the control. This finding indicated that miR-155 might be involved in the pathology of osmidrosis.

Expression of Ang II 2R was increased in apocrine glands of human axillae from osmidrosis patients

Next, we examined the expression of Ang II 2R in apocrine glands of human axillae from osmidrosis patients and normal control. It has been shown that higher mRNA and protein level of Ang II 2R were observed in apocrine glands of human axillae from osmidrosis patients than that in the control (**Figure 2**).

Expression of Ang II 2R was increased in apocrine glands cells transfected with miR-155 inhibitor

To elucidate the regulation of miR-155 on the expression of Ang II 2R, apocrine glands cells separated from normal were transfected with miR-155 inhibitor to silence miR-155. MiR-155 inhibitor significantly reduced the expression of miR-155 in apocrine glands cells (**Figure 3A**). In addition, the mRNA and protein level of Ang II 2R were remarkably increased in apocrine glands cells transfected with miR-155 inhibitor (**Figure 3B**).

Expression of Ang II 2R was reduced in apocrine glands cells transfected with miR-155 mimic

Next, we detected the effect of miR-155 overexpression on the expression of Ang II 2R in apocrine glands cells. As shown in **Figure 4A**, the expression of miR-155 was up-regulated in apocrine glands cells transfected with miR-155 mimic. Moreover, miR-155 mimic reduced the mRNA and protein level of Ang II 2R in apocrine glands cells transfected with miR-155 mimic (**Figure 4B**).

Effect of miR-155 on the activity of 3'UTR of Ang II 2R in 193A cell

To further verify the relationship of miR-155 and Ang II 2R, 193A cells were transfected with miR-155 inhibitor or miR-155 mimic and the luciferase activity of 3'UTR of Ang II 2R was examined. As demonstrated in **Figure 5A**, the luciferase activity of 3'UTR of Ang II 2R was increased in 193A cells transfected with miR-155 inhibitor. On the other hand, the luciferase activity of 3'UTR of Ang II 2R were reduced in 193A cells transfected with miR-155 mimic compared with that in the control (**Figure 5B**).

Discussion

In this study, we found the down-regulated expression of miR-155 and high expression of

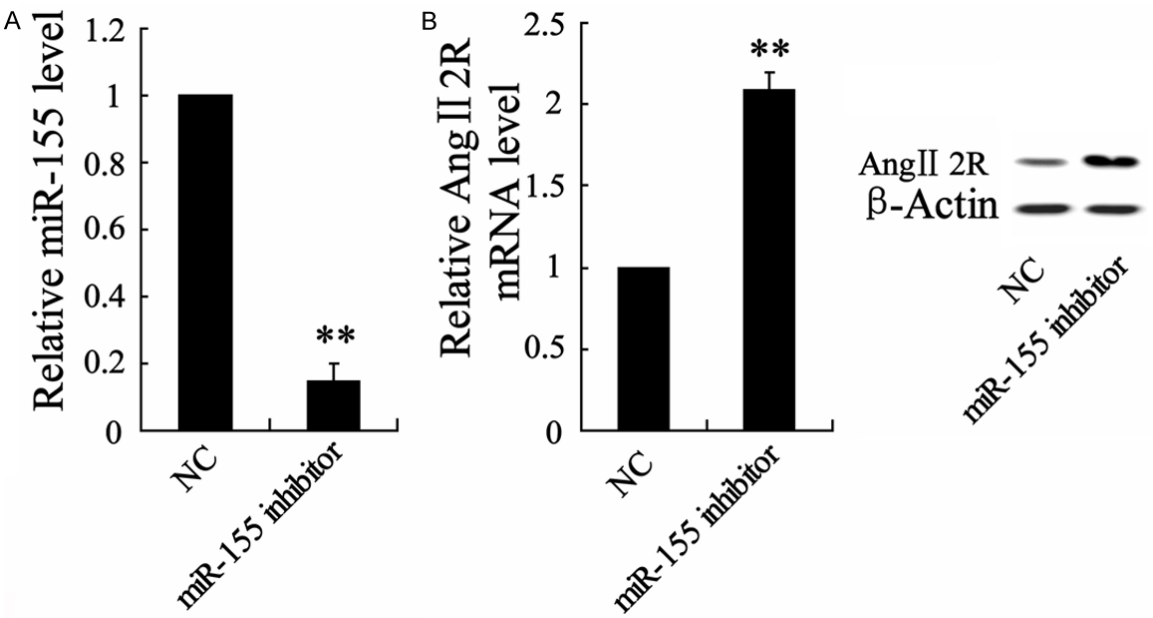

Figure 3. Effect of miR-155 inhibitor on the expression of Ang II 2R in apocrine glands cells from normal person. A. The mRNA level of miR-155 in apocrine glands cells transfected with miR-155 inhibitor. B. The expression of Ang II 2R in apocrine glands cells transfected with miR-155 inhibitor. All values are mean ± SD. **VS normal group, *P*<0.01.

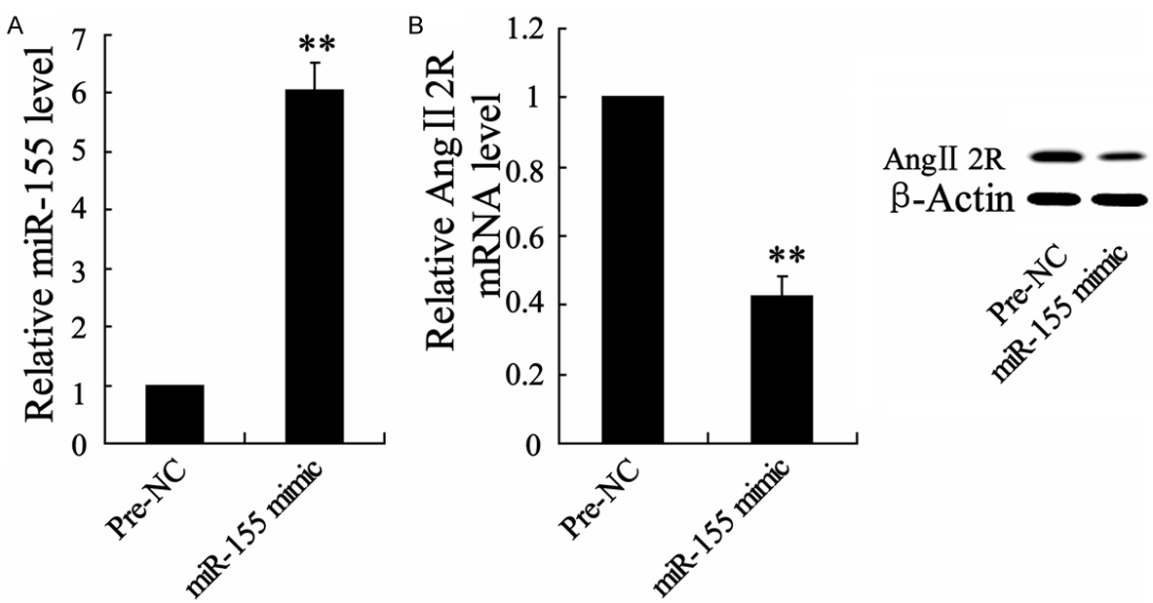

Figure 4. Effect of miR-155 mimic on the expression of Ang II 2R in apocrine glands cells from osmidrosis patients. A. The mRNA level of miR-155 in apocrine glands cells transfected with miR-155 mimic. B. The expression of Ang II 2R in apocrine glands cells transfected with miR-155 mimic. All values are mean ± SD. **VS normal group, *P*<0.01.

Ang II 2R in apocrine glands of human axillae from osmidrosis patients. When the apocrine glands cells were transfected with miR-155 mimic or miR-155 inhibitor, the expression of Ang II 2R was increased or decreased, respectively. In addition, the luciferase activity of 3'UTR of Ang II 2R was also reduced or enhanced by miR-155 mimic or miR-155 inhibitor in 193A cells. These data indicated that miR-155 regulated the expression of Ang II 2R

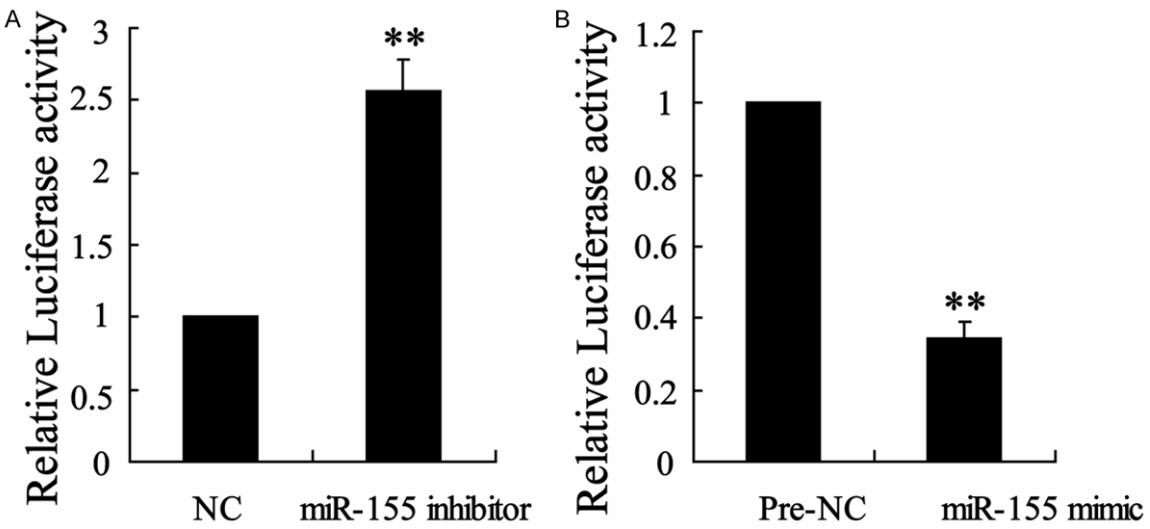

Figure 5. The effect of miR-155 on the activity of 3'UTR of Ang II 2R in 193A cell. A. The activity of 3'UTR of Ang II 2R in 193A cell transfected with miR-155 mimic. B. The activity of 3'UTR of Ang II 2R in 193A cell transfected with miR-155 inhibitor. All values are mean ± SD. **VS normal group, $P<0.01$.

in apocrine glands of human axillae from osmidrosis patients.

The majority of patients with axillary osmidrosis showed the marked hereditary feature. Genetic alteration is a major contributor to the initiation of axillary osmidrosis. Recently, a report indicated that a single nucleotide polymorphism (SNP) in gene *ABCC11* which caused a G180R substitution in the corresponding protein was closely correlated with the pathology of axillary osmidrosis [9]. ABCC11 is known to transport a variety of lipophilic anions. Martin et al. showed that ABCC11 was localized in the axillary apocrine sweat gland and was crucial for the secretion of odorants from the apocrine sweat gland [10]. SNPs regulated the gene expression by interfering with posttranscriptional activity, such as miRNA binding, polyadenylation, and protein binding [11].

To date, although the role of miRNA in the disease process has been elucidated much, few reports were related to the function of miRNAs in axillary osmidrosis. In the present study, we found the dysregulation of miR-155 in apocrine glands of human axillae from osmidrosis patients. miR-155 showed multifunction in several pathology, such as haematopoiesis, carcinogenesis, inflammation and the regulation of immune responses [12]. Sonkoly et al. showed that miR-155 was overexpressed in patients with atopic dermatitis and might contribute to chronic skin inflammation [13]. In addition, miR-155 played a significant role in the process of neoplastic diseases and predominantly acted as an oncomiR [14]. miR-155 appears to be participated in viral infections through targeting a number of transcriptional regulatory genes [15]. miR-155 was implicated in the regulation of Ang II 1R which was known to be associated with hypertension, cardiac hypertrophy and myocardial infarction [16]. In this study, we found miR-155 regulated the expression of Ang II 2R in apocrine glands of human axillae from osmidrosis patients.

The secretion of sweat gland is also regulated by nerve factor. Some adrenergic receptors were identified in apocrine gland and the dysregulation of adrenaline levels owing to emotion or physiological state might impact the function of apocrine gland. As an important factors in renin-angiotensin system, increased expression of Ang II 2R were found to cause cells apoptosis, such as endothelial cells, smooth muscle cells, fibroblasts, and pheochromocytoma [17-20]. Ang II 2R pathways is considered as a new therapeutic target for the relief of peripheral neuropathic and chronic inflammatory pain conditions [21]. The positively correlation between the expression of Ang II 2R and ApoD in apocrine glands from human axillae was observed. ApoD is a secreted glycoprotein and can regulate the E-3M2H secretion

[22]. These data indicated that Ang II 2R might involve in the development of axillary osmidrosis.

In conclusion, the dysregulation of miR-155 were observed in apocrine glands of human axillae from osmidrosis patients and it regulated the expression of Ang II 2R in apocrine gland cells. These findings shed light on the important roles of miR-155 and Ang II 2R on the pathology of axillary osmidrosis. Further study need to be performed to investigate the particular function of miR-155 and Ang II 2R in axillary osmidrosis.

Disclosure of conflict of interest

None.

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