Sex Hormone–Dependent Regulation of the Renin–Angiotensin System by miR-125b-5p in Brain Cells

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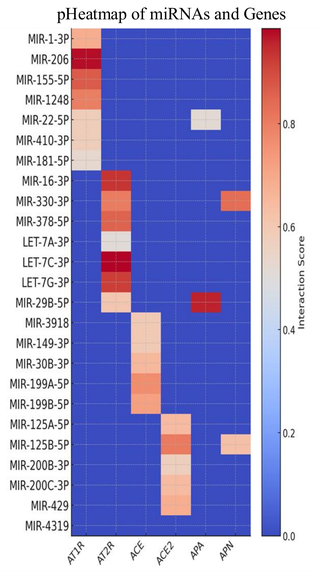
Introduction

Dysregulation of the **renin-angiotensin system (RAS)** is a key driver of chronic hypertension and cardiovascular disease.

MicroRNAs have emerged as novel epigenetic regulators in chronic diseases, including hypertension

Our lab identified MircoRNA-125b-5p (miR-125b-5p) as a potential regulator of RAS genes such as ACE2 and ATRAP

Computer-based analysis suggest conserved binding sites for miR-125b-5p in these critical genes.



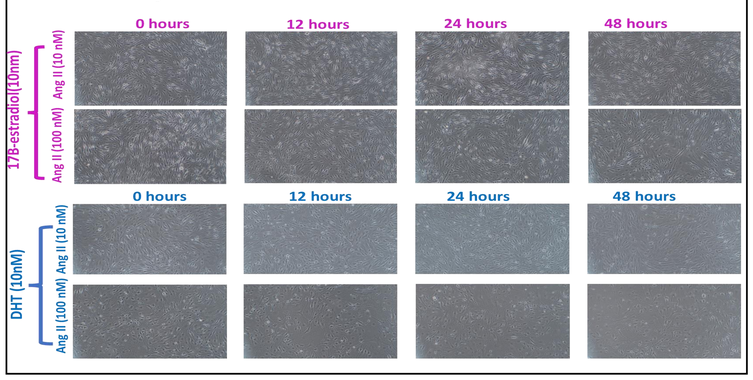
Objective

- This study investigates the role of miR-125b-5p in regulating RAS components under **Angiotensin II** (**Ang II**) stress and explores the influence of sex hormones on its activity

Methods & Materials

Cells and Culture Conditions

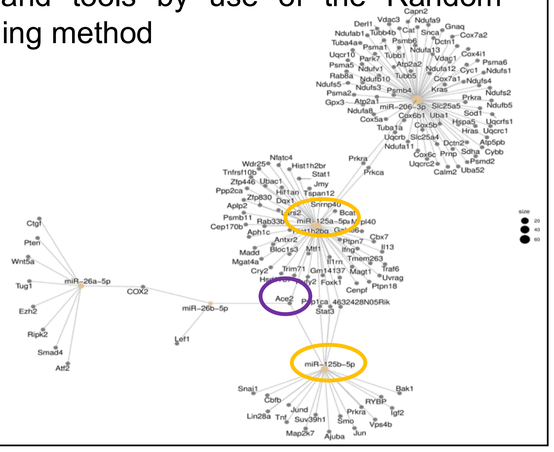
- Two brain-derived endothelial cell lines, bEND.3 (mouse) and LUHMES (human) were treated with 100 nM of Ang II to mimic a hypertensive environment.
- β-estradiol (E2) was used to treat cell lines that would mimic the female hormonal environment and dihydrotestosterone (DHT) for those cells mimicking the male hormonal environment.
- Agomirs and antagomirs were designed specifically for our microRNA, miR-125b-5p, and tested in the two cell lines.
- Cells were treated with 20.5 nmol of either the agomir or antagomir in the presence of Ang II
- Following a 48-hour treatment period, cells were harvested, and total proteins were extracted for Western blot analysis



Genetic Mapping

Our team gathered all strong targets of miR-125 using multiple databases and tools by use of the Random Forest machine learning method

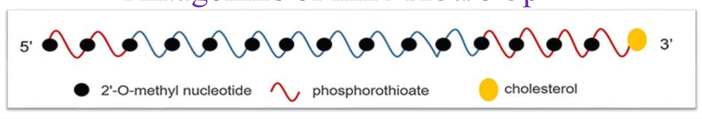
·We then mapped these targets to create a cNETmap. Our results indicate that ACE2 can be considered a target for both miR-125a-5p and miR-125b-5p



Designing Antagomir

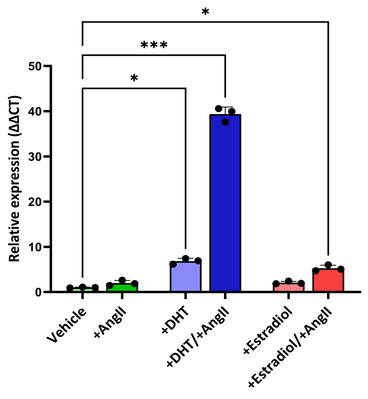
In designing an AntagomiR that targets miR-125, the first three 5' nucleotides and the last five 3' nucleotides of the AntagomiR were phosphorylated to prevent degradation in the physiological influents. Additionally, we introduced a methyl group to the 2' hydroxyl of the ribose to enhance the stability and half-life of the drug, allowing the AntagomiR to remain stable for over four weeks. Furthermore, we conjugated cholesterol to the 3' end of the AntagomiR to facilitate membrane penetration, particularly across exosome membranes, ensuring efficient delivery.

Antagomirs of miR-125a/b-5p



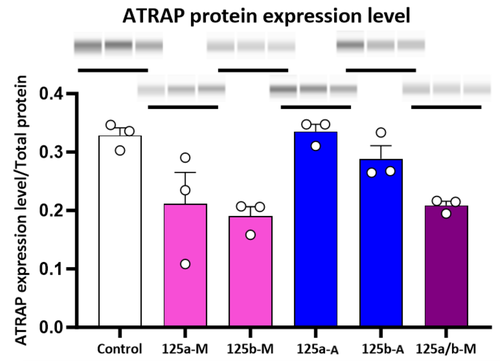
miR-125-5p expression is regulated by sex hormones

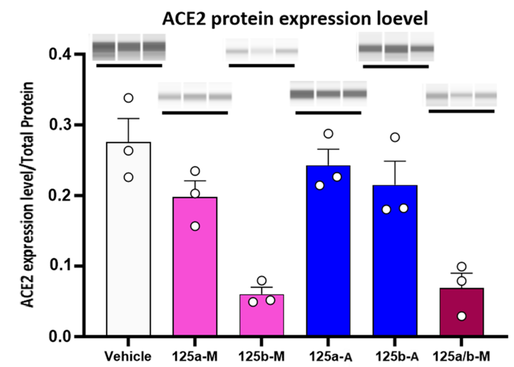
Expression levels of miR-125a/b-5p in cells treated in presence of angiotensin II (Ang II, 100 nM) to mimic hypertensive stress, β-estradiol (E2) to simulate the female hormonal environment, and dihydrotestosterone (DHT) to simulate the male hormonal showed an upregulation by Ang II or DHT but not with E2.



Results

We observed a decrease in the expression of both ATRAP and ACE2 in cells treated with the miR-125 mimic, suggesting that this microRNA targets both key molecules in the RAS.





Furthermore, cells treated with the miR-125b-5p antagomir exhibited a significant increase in ATRAP and ACE2 protein levels ($P < 0.05$). This effect was reversed in cells treated with the miR-125b-5p agomir, indicating a direct regulatory role of this miRNA. Collectively, these results suggest that miR-125b-5p negatively regulates the expression of both ATRAP and ACE2

Conclusion

1. *Upregulation of miR-125a/b in the hypertensive mice models is linked to decreased levels of ACE2/ATRAP in the plasma.*

2. *ACE2/ATRAP was validated as a target of miR-125a/b-5p.*

3. *Our findings suggest that members of the miR-125 family may contribute to the development of cardiometabolic diseases by targeting RAS components such as ACE2 and ATRAP, whereas miR-125 antagomirs may offer therapeutic benefits.*