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Role of microRNA-221 in mediating the protective action of Estradiol in vascular cells

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Introduction: Recent studies provide evidence that microRNAs (miRNAs) are involved in many clinically relevant biological and pathophysiological processes. They regulate cell differentiation, proliferation, migration and apoptosis in many cell types, including the cardiovascular system. Vascular remodeling associated with cardiovascular disease involves endothelial cell (EC) damage/dysfunction and abnormal growth of smooth muscle cells (SMCs). Several miRNAs, including microRNA-221 (miR-221), are known to influence both EC function and SMC growth. Estrogens are known to protect women against vasoocclusive disorders by promoting endothelial repair/recovery and inhibiting SMC growth. Thus, it is feasible that processes associated with vasoprotection and vascular repair are mediated by miRNA modulation. Hence, we hypothesize that the vasoprotective actions of estradiol (E2) may in part be mediated via modulation of miRNAs. In the present study we investigated the role of miR-221 in mediating the protective action of E2 in vascular cells.

Methods: To investigate the miR-221 expression, human umbilical vein ECs (HUVECs) and human coronary artery SMCs (HCASMCs) were treated with or without E2 (10-100nM) prior to small RNA extraction and RT-qPCR. Both cell types were then transfected with 25nM miR-221 mimics and antimirs or the respective controls, to assess the miR-221 role using different functional assays. Cell counts and a BrdU ELISA kit were employed to study the proliferation of HCASMCs. Matrigel microvessel formation and scratch assay were used to investigate the cell function of HUVECs. Western Blotting was performed to inspect protein expression. Experiments were repeated al least three times, *p<0.05, compared to respective control.

Results:

MiR-221 inhibits capillary formation and wound closure in HUVECs

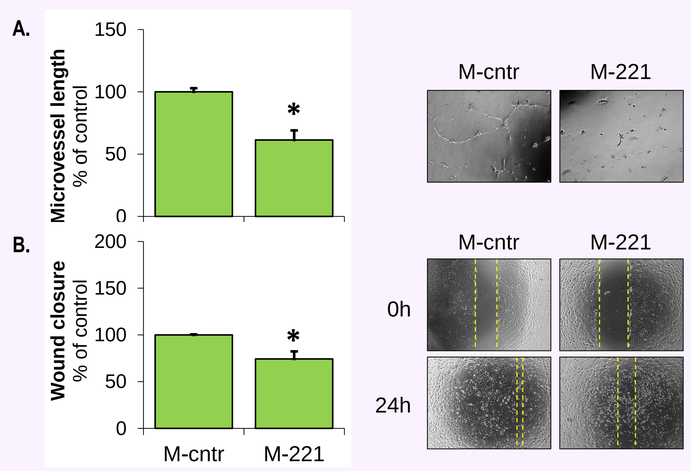


Fig. 1 HUVECs were transfected with control (M-cntr) or miR-221 mimic (M-221) for 24h prior to both assays. **A.** Capillary formation: images were taken 18h after cell seeding on 2D-Matrigel. **B.** Wound closure assay: cells were allowed to migrate 24h. Pictures were taken at both time points.

Antimir of miR-221 induces capillary formation and wound closure in HUVECs similar to E2

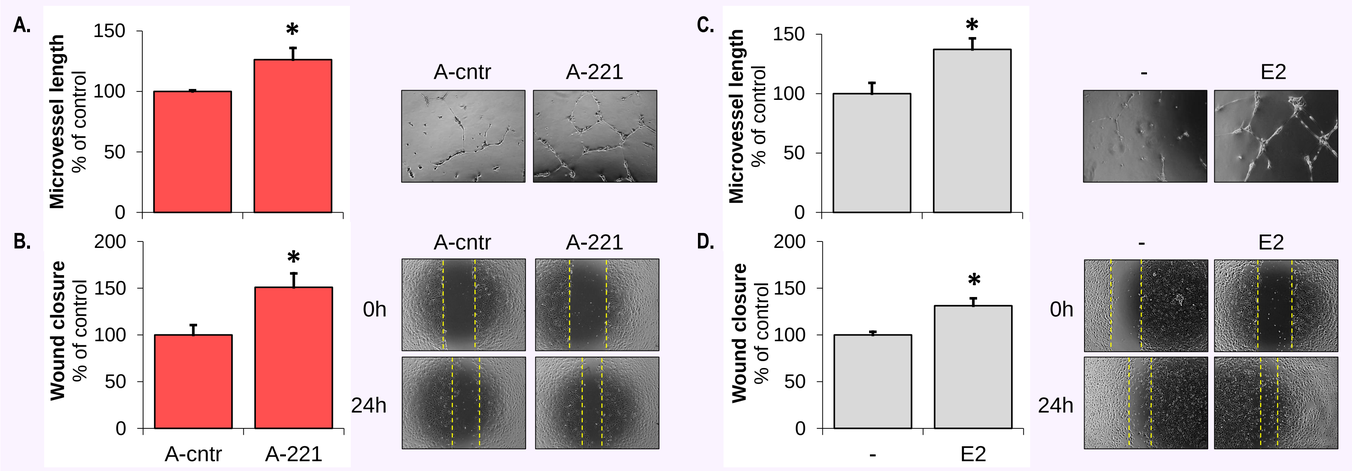
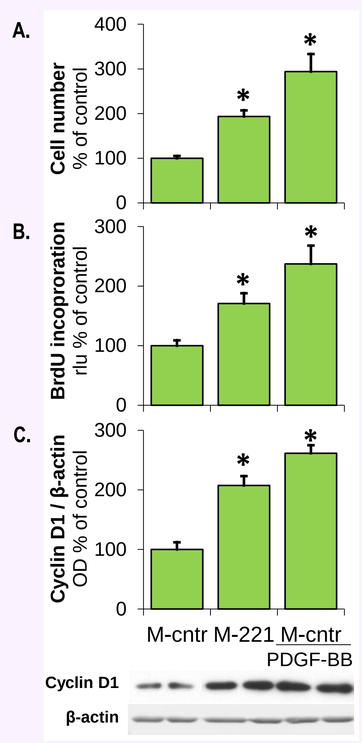


Fig. 2 Effect of miR-221 antimir and E2 on HUVEC function were compared using two different assays. HUVECs were either transfected with control (A-cntr) or miR-221 antimir (A-221) (**A**, **B**) or treated with or without E2 (10nM) (**C**, **D**). **A, C.** Capillary formation: images were taken 18h after cell seeding on 2D-Matrigel, either 30min after E2 treatment or 24h post-transfection. **B, D.** Wound closure: cells were allowed to migrate 24h, either in presence or absence of E2 or 48h after transfection with antimirs.

MiR-221 induces HCASMC proliferation



Antimir of miR-221 inhibits HCASMC proliferation similar to E2

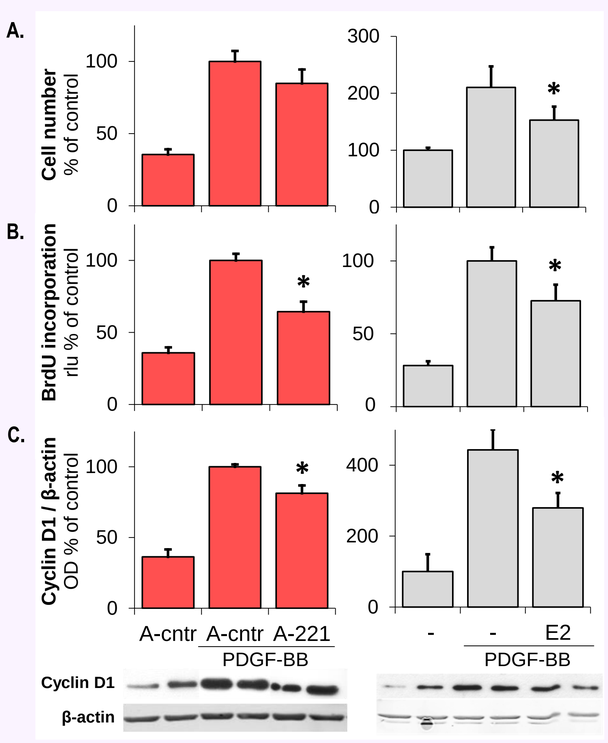


Fig. 3 HCASMCs were transfected with control or miR-221 mimics (M-cntr, M-221). PDGF-BB (20ng/ml) was used as a positive control. **A.** Cells count, 3 days after transfection. **B.** BrdU incorporation. **C.** Cyclin D1 protein expression. **Fig. 4** Effect of miR-221 antimir and E2 on proliferation were compared. Cells were transfected with control or miR-221 antimirs (A-cntr, A-221) and treated with or without 100nM E2 in presence of PDGF-BB (20ng/ul). Proliferation was measured using cell count (**A**), BrdU assay (**B**) and Cyclin D1 expression (**C**).

E2 inhibits miR-221 expression in both HUVECs and HCASMCs

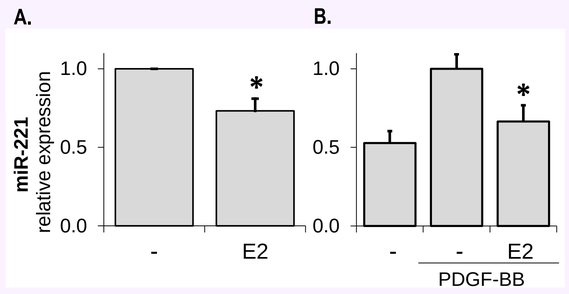


Fig.5 MiR-221 relative expression of HUVECs (**A**), treated 24h with or without E2 (10nM), and HCASMCs (**B**) treated with or without E2 (100nM) in presence of PDGF-BB (20ng/ul), was examined using RT-PCR after RNA extraction. U48 and U49 were used as endogenous controls.

Effect of E2 is reversed by miR-221 mimic

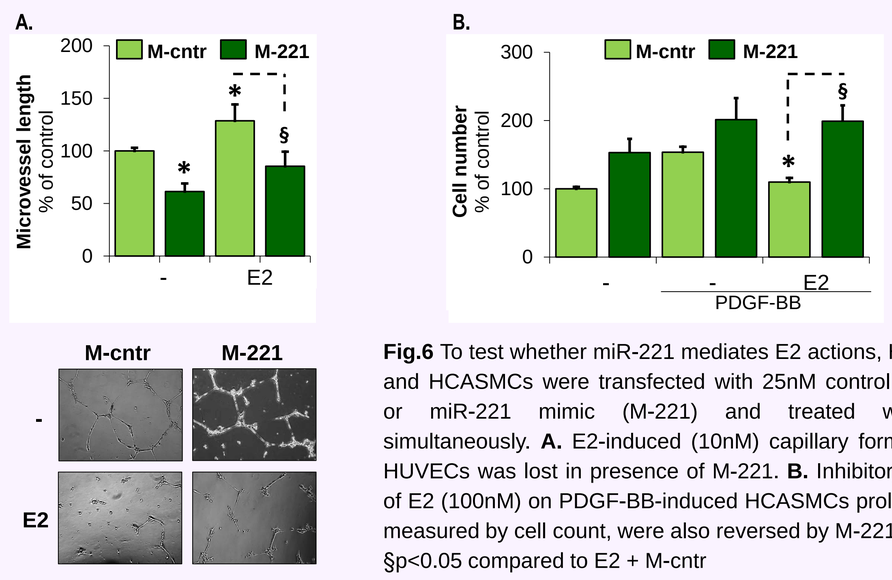
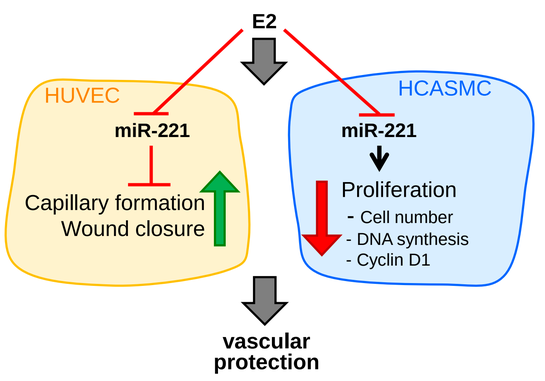


Fig.6 To test whether miR-221 mediates E2 actions, HUVECs and HCASMCs were transfected with 25nM control (M-cntr) or miR-221 mimic (M-221) and treated with E2 simultaneously. **A.** E2-induced (10nM) capillary formation in HUVECs was lost in presence of M-221. **B.** Inhibitory effects of E2 (100nM) on PDGF-BB-induced HCASMCs proliferation, measured by cell count, were also reversed by M-221. §p<0.05 compared to E2 + M-cntr

Discussion and Conclusion: Here, we demonstrate a differential role of miR-221 in regulating growth of endothelial and smooth muscle cells. Overexpression of miR-221 increased SMC proliferation and impaired EC-induced capillary formation and wound healing. We further show that E2 negatively modulates miR-221 expression in HCASMCs and HUVECs and that miR-221 downregulation by the antimir mimics the effects of E2. Interestingly, the miR-221 mimic was able to reverse the E2 effects in both cell types, suggesting that miR-221 modulation may represent a novel mechanism by which E2 mediates its protective actions on the cardiovascular system.



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