HYPOXIA-INDUCIBLE FACTOR-1α REGULATES MIGRATION, PROLIFERATION AND ANGIOGENESIS IN REPLICATIVE ENDOTHELIAL SENESCENCE INDEPENDENTLY OF MICRORNA-126 EXPRESSION

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INTRODUCTION AND OBJECTIVES

Endothelial damage contributes to the development of some renal and cardiovascular diseases and is associated with premature ageing in chronic kidney disease. Endothelial senescence is linked to endothelial dysfunction and therefore to age-related renal and cardiovascular diseases.

The aim of this study is the role of the hypoxia-inducible factor 1-alpha (HIF-1α) and microRNA-126 (miR126) in the replicative senescence associated with endothelial damage.

To test the hypothesis that HIF-1α plays a role in aging: a) we have analyzed its expression in endothelial cells using a well-validated and robust replicative senescence model. b) we studied whether miR-126 expression is dependent on HIF-1α expression. c) we have analyzed both HIF-1 and miRNA-126 content in young and senescent endothelial cells-released microvesicles (MVs). d) we investigated whether Hsp90 is a relevant regulator of HIF-1α expression in our model.

METHODS

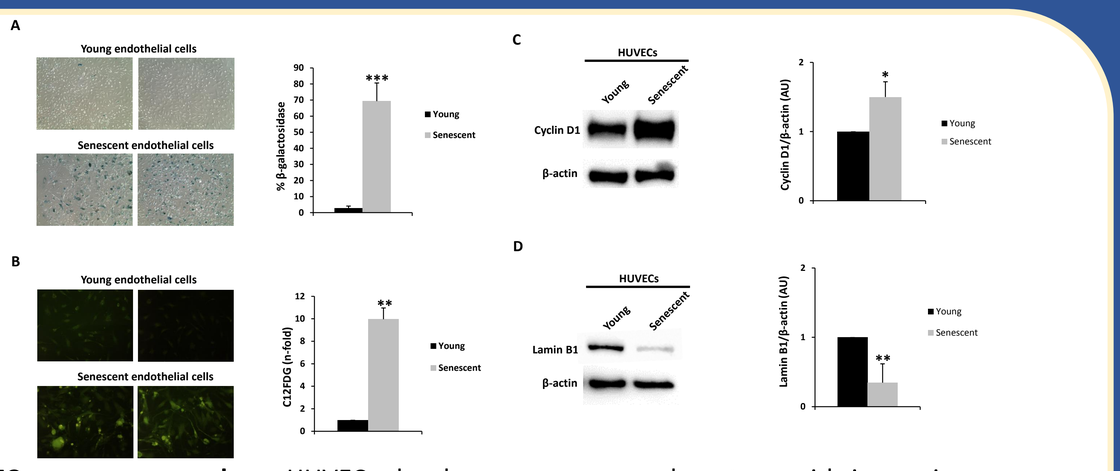
Human umbilical vein endothelial cells (HUVEC) were grown and serially passaged until they reached senescence (replicative senescence model). Senescence-associated β-galactosidase activity analyses were performed to check the replicative senescence model. Wound healing assays and endothelial tube formation assays were performed to test the endothelial cell functionality. HIF-1α expression was evaluated by real time PCR and western blot, and miR126 levels were measured by real time PCR of mature microRNAs. Data are represented as mean ± SD., and the significance was calculated by the Student's t-test. *p<0.05, **p<0.01 and ***p<0.001.

CONCLUSIONS

The results demonstrate that the expression of HIF-1α and miRNA126 play an essential key role in the endothelial cell homeostasis and their protective and repairing functions are independent, suggesting as a potential therapeutic targets for age-related disorders associated with chronic kidney diseases.

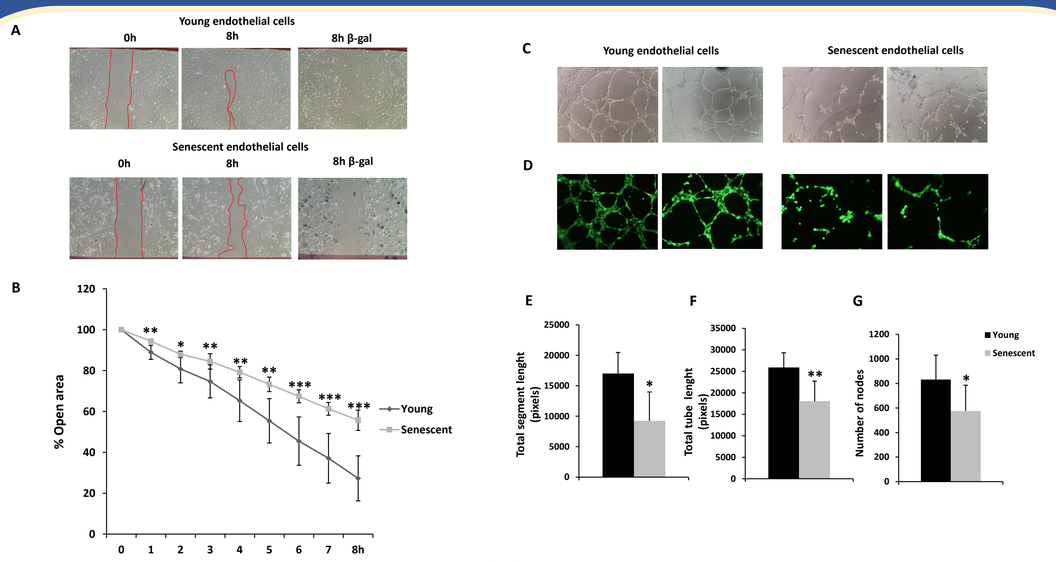
RESULTS

1) PD in primary HUVEC > 96 display characteristics of senescence



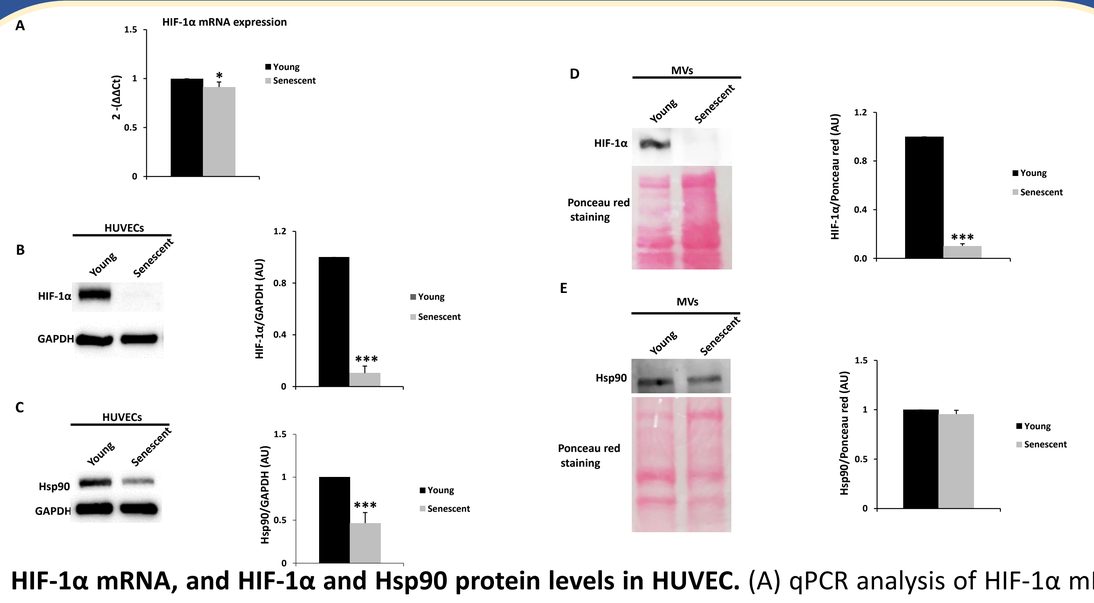
HUVEC senescence markers. HUVECs develop a senescence phenotype with increasing passage number *in vitro*. The percentage of senescent HUVECs at different passages was determined by (A) senescence-associated β-galactosidase staining and (B) C12FDG fluorescence staining. n=6; Magnification, x10. (C) Cyclin D1 and (D) Lamin B1 representative Western blots in young and senescent HUVEC pools. n=3 pools.

2) Senescent HUVEC show alteration in endothelial function



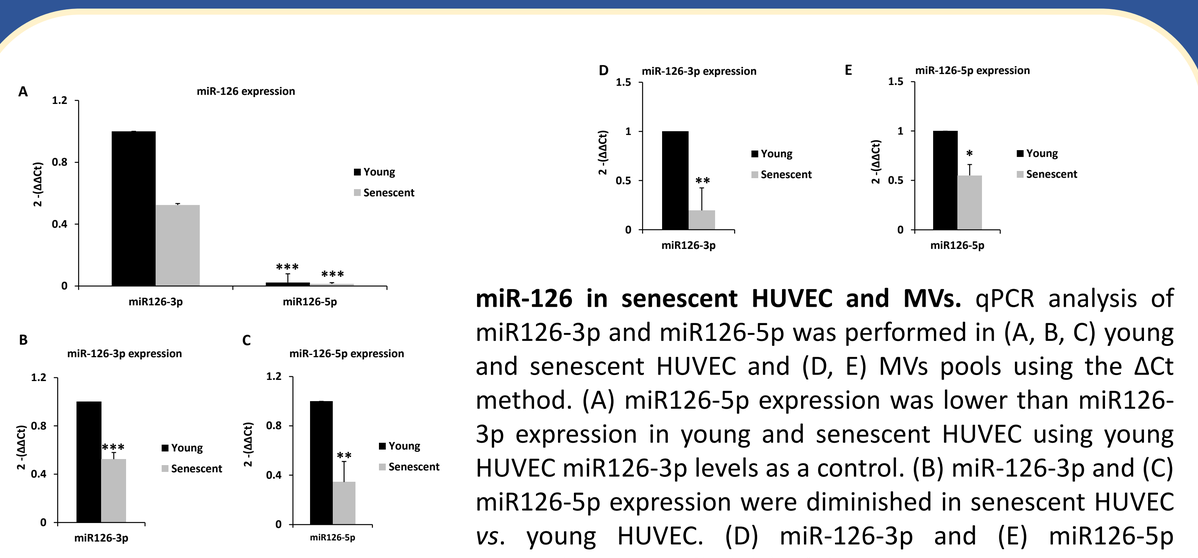
Wound healing in HUVEC monolayers. (A) Representative photomicrographs of young and senescent HUVEC monolayers 8 hours after wounding. (B) Time course of changes in the size of the remaining wound. The data points represent the % open area. **Endothelial tube formation in HUVEC.** The spontaneous formation of capillary-like structures by HUVEC on Matrigel was used to assess angiogenic potential. (C) Light and (D) fluorescent microscope pictures (calcein AM) of young and senescent HUVEC after 6 h. (E, F, G) Total segment length, total tube length and the number of nodes were quantitated from photographs. Magnification: 10x. n=9 in triplicate.

3) HIF-1α and Hsp90 expression are reduced in senescent endothelial cells and MVs



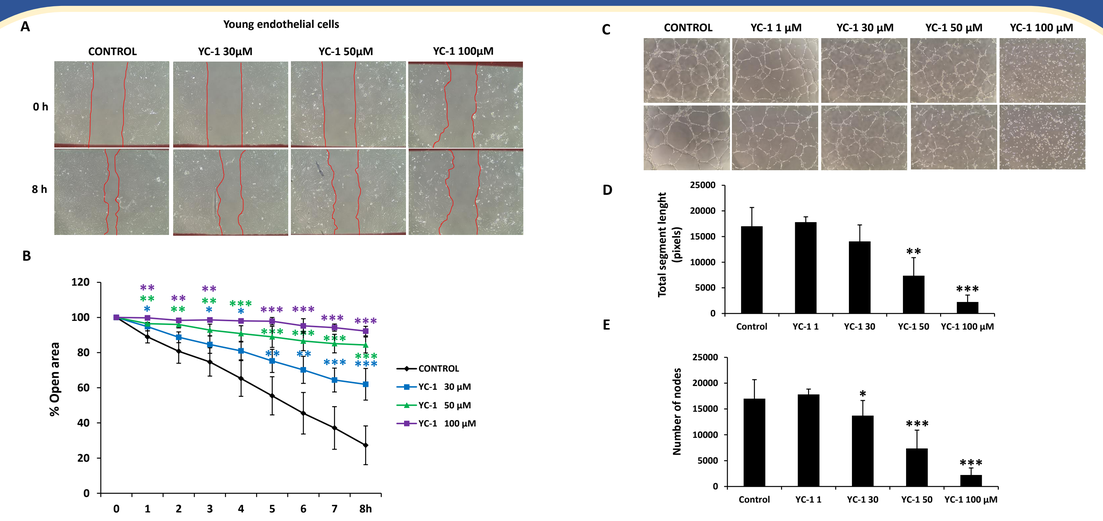
HIF-1α mRNA, and HIF-1α and Hsp90 protein levels in HUVEC. (A) qPCR analysis of HIF-1α mRNA levels in young and senescent HUVEC pools using the ΔCt method; HPRT1 mRNA was used for normalization. (B, C) Representative HIF-1α and Hsp90 western blot of young and senescent HUVEC pools. **HIF-1α and Hsp90 protein levels of MVs released by HUVEC**. (D, E) Representative HIF-1α and Hsp90 western blot of young and senescent MVs pools. n=3 pools.

4) MicroRNA-126 is decreased in senescent endothelial cells



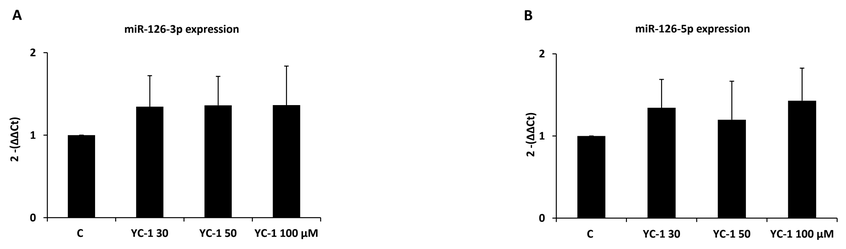
miR-126 in senescent HUVEC and MVs. qPCR analysis of miR126-3p and miR126-5p was performed in (A, B, C) young and senescent HUVEC and (D, E) MVs pools using the ΔCt method. (A) miR126-5p expression was lower than miR126-3p expression in young and senescent HUVEC using young HUVEC miR126-3p levels as a control. (B) miR-126-3p and (C) miR126-5p expression were diminished in senescent HUVEC *vs*. young HUVEC. (D) miR-126-3p and (E) miR126-5p expression were diminished in senescent MVs compared with young MVs. n=3 pools.

5) Effect of HIF-1α inhibition in young endothelial cells



Effect of YC-1 (inhibitor of HIF-1α) in wound healing assay. (A) Representative cell pictures 8 h after wounding. Magnification 10x. (B) Time course of changes in the size of the remaining wound. The data points represent the % open. n=4 in duplicate; YC-1 treated *vs.* Control at the same time. **Effect of YC-1 on tube formation in HUVEC.** (C) Light microscope pictures of HUVEC seeded on Matrigel-coated wells and treated with different YC-1 concentrations (6 h). Control HUVEC migrated to form connected tubular networks. YC-1-treated HUVEC significantly attenuated network formation. (D, E) Quantitative analysis of the total segment length and the number of nodes were performed. Magnification: 10x. n=4 in triplicate. YC-1 treated *vs.* Control.

6) HIF-1α does not regulate microRNA-126 levels in endothelial cells



miR126-3p and miR126-5p in YC-1-treated HUVEC. qPCR analysis of miR126-3p (A) and miR126-5p (B) was performed in YC-1-treated HUVEC using the ΔCt method; U6 snRNA was used for normalization. HUVEC were YC-1-treated with different doses (30, 50, 100 μM) for 16h. The data are expressed as fold induction with respect to control values (control HUVEC). n=4.

ACKNOWLEDGEMENTS

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