HIGH-FAT DIET INDUCED VASCULAR SMOOTH MUSCLE CELL STIFFENING IN APOE$^{-/-}$ MICE

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INTRODUCTION

Cardiovascular disease (CVD) causes about 30% of global death (estimated 17.9 million) and about 80% of CVD is atherosclerosis-related diseases [1]. Vascular smooth muscle cells (VSMCs) are the major cellular components of arterial wall and perform the major function for arterial contraction and extracellular matrix (ECM) production. During atherosclerosis, VSMCs undergo phenotypic switching to a synthetic phenotype capable of proliferation and migration. The surrounding environment of VSMCs in vivo undergoes alterations in ECM stiffness and composition in addition to an increase in cholesterol content. Our work is focused on the analyzing difference in the mechanics of VSMCs isolated from western diet fed apolipoprotein-E knockout (ApoE$^{-/-}$) and wild type (WT) mice during atherosclerosis.

METHODS

VSMCs were enzymatically isolated from the descending thoracic aorta of male ApoE$^{-/-}$ and male WT mice [2]. VSMCs were cultured on elastically tunable substrates. Atomic force microscope (AFM) was employed to study N-cadherin (N-Cad) mediated cell-cell adhesion, integrin mediated cell-ECM adhesion forces, and stiffness of VSMCs. AFM was also used to examine live VSMC submembranous cytoskeleton organization. Two-way ANOVA was used to test statistical significance for all experiments.

RESULTS AND DISCUSSION

No significant difference in cell stiffenss was observed between normal diet-fed ApoE$^{-/-}$ and WT VSMCs. After three-month high fat diet, ApoE$^{-/-}$ VSMCs were found to have a significantly higher stiffness compared to WT VSMCs (Figure 1) [2]. Increased stiffness of ApoE$^{-/-}$ VSMCs correlated with a greater degree of stress fiber alignment as evidenced by AFM-generated force maps and stress fiber topography images. ApoE$^{-/-}$ VSMCs had a significantly lower adhesion force to N-Cad compared to WT on the 28 and 103 kPa substrates.

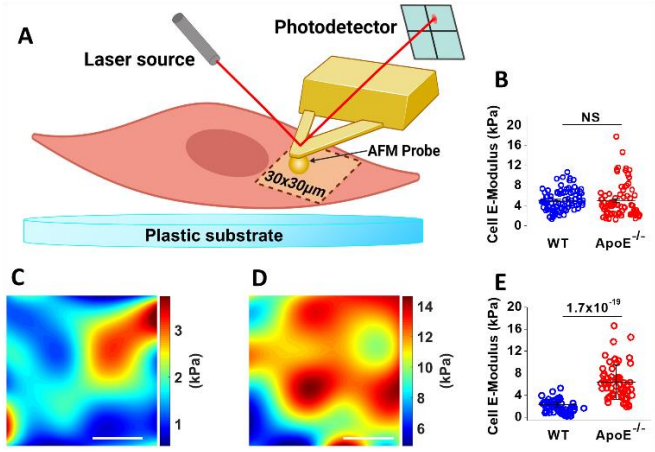

Figure 1 Live WT and ApoE$^{-/-}$ VSMC stiffness maps. **(A)** 30 × 30 μm cell surface areas were automatically scanned and indented at 6×6 positions with a glass bead. **(B)** Average stiffness for normal diet-fed ApoE$^{-/-}$ and WT VSMCs. **(C, D)** Stiffness force maps for WT and ApoE$^{-/-}$ VSMCs, respectively. **(E)** Average stiffness for Western diet-fed WT and ApoE$^{-/-}$ VSMCs. All data are presented as means ± SE (n > 60 cells across six different mice). Scale bar in lower right corner represents 10 μm. A was created with BioRender. com.

CONCLUSIONS

The results demonstrated a significant difference in cell mechanics, cytoskeletal organization, and migratory behavior of VSMCs isolated from WT and ApoE$^{-/-}$ mice. This supports our hypothesis that atherosclerosis alters the mechanical properties of VSMCs and provide insight into underlying mechanisms that may lead to future novel therapeutic approaches.

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