STRESS FIBERS IN AORTIC SMOOTH MUSCLE CELLS ALTER TEHIR DIRECTION TO ELEVATED STRAIN DIRECTION UNDER HYPERTENSION

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Introduction

Aortic walls become hypertrophy under hypertension [1]. The hypertrophy is believed to be caused by the homeostasis to keep circumferential (θ) stress constant [1], though its formation mechanism remains unknown. One possible process includes an increase in tension in stress fibers (SFs) in the vascular smooth muscle cells (VSMCs) as a result of elevated θ stress due to elevated intraluminal pressure. Since the SFs connect to the nucleus [2], the elevated tension in SFs transmits the force to the nucleus, which might change the gene expressions.

In our previous study [3], we found that SFs of VSMCs in aortas oriented at ~30° from the θ direction in radial (r)-θ plane, and normal strain in this direction due to the pulse pressure equals almost 0. This result indicates that SFs in VSMCs do not stretch due to the changes in blood pressure under a normal physiological state. We hypothesized that the strain increases at the initial phase of hypertension and returns to 0 after hypertrophy is completed since the cell responses are also completed. To evaluate the hypothesis, we measured the strain in the SF direction at the initial phase of hypertension and after completion of hypertrophy.

Methods

All animal experiments were approved by the Review Board of the Animal Committee of Nagoya Institute of Technology. Spontaneously Hypertensive Rats (SHR/Izm, 10-14 weeks, Japan SLC) and their control (Wistar Kyoto Rats, WKY/Izm, Japan SLC) were used as test models. The SHR was regarded as a model in which hypertrophy was completed.

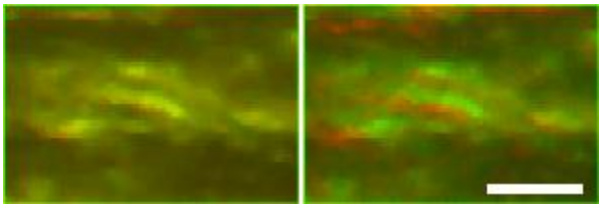
After systolic and diastolic pressures were measured (BP-98A-L, Softron), a rat was sacrificed, and its thoracic aorta was excised. The pressure-diameter test was then performed to obtain the normal strains in the θ direction of the aorta, such as strain at the diastolic ε_{dia} and at the systolic pressures ε_{sys} from strain at the 0 mmHg ε_0. For WKY rats, the strain at 179 mmHg intraluminal pressure ε_{hyp} was also measured as an initial phase of hypertension. The aortic specimens were sectioned into the 200-μm-thick ring specimen with a micro-slicer (DTK-1000, Dosaka-EM). The SFs in the sectioned specimen were stained in ×200 diluted Alexa Fluor 647 Phalloidin (A22287, Thermo Fisher Scientific) for 2 h.

The specimen was stretched under a confocal laser scanning microscope (FV3000, Olympus) with a custom-made tensile tester. At ε_0, ε_{dia}, ε_{sys}, and ε_{hyp} of the tissue stretch, SFs and elastin autofluorescence images were captured. Local and normal strains in the θ $\varepsilon_{\theta\theta}$ and r direction ε_{rr} and shear strain in the r-θ axes $\varepsilon_{r\theta}$ were measured from the strain markers created at the elastic laminas, as stated previously [4]. SF direction α_{SF} from the θ direction in the r-θ plane was measured from the fluorescence images of SF, and its strain ε_{SF} was calculated from the $\varepsilon_{\theta\theta}$, ε_{rr}, $\varepsilon_{r\theta}$, and α_{SF}.

Results and Discussion

Typical images of the SFs were shown in Fig. 1. SFs oriented in the α_{SF} = 19 ± 2° at the ε_{sys} of WKY, α_{SF} = 13 ± 1° at the ε_{hyp} of WKY, and α_{SF} = 20 ± 2° at the ε_{sys} of SHR. These results indicate that SFs changed their alignment closer to the θ direction under hypertension and returned to their original direction after the completion of hypertrophy.

Strain in the SF direction was ε_{SF} = 0.01 ± 0.03 from ε_{dia} to ε_{sys} of WKY, ε_{SF} = 0.18 ± 0.04 from ε_{dia} to ε_{hyp} of WKY, and ε_{SF} = 0.03 ± 0.01 from ε_{dia} to ε_{sys} of SHR. In a physiological state of WKY, the strain in the SF direction was almost 0, as confirmed in a previous study [3]. At the initial hypertension condition, SF strain drastically increased, while the strain ε_{SF} again reached almost 0 levels after the completion of hypertrophy. These results indicate that the SF transmits the force applied to the aorta by changing its direction under hypertension and does not after the completion of hypertrophy.



(a) *(b)*

Figure 1: Superimposed images of SFs at different tissue strain levels. (a) Diastolic (green) and Systolic (red) strain levels. (b) Diastolic (green) and 179 mmHg intraluminal pressure strain levels. Bar = 20 μm.

References

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Acknowledgements

This work in part was supported by KAKENHI 21H04955 from the JSPS, Tatematsu foundation, JP20gm0810005 from AMED.