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主　論　文　の　要　旨

論文題目　Dependency of deformation of cell nucleus on stretch direction of tissue: Relation to anisotropic response of aortic media to hypertension
（細胞核変形の組織引張方向依存性：高血圧に対する大動脈中膜の異方性反応との関係）

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論　文　内　容　の　要　旨

The deformation of the cell nucleus may cause dispersion of chromatin and eventually enhance transcription, translation, and protein expression. If this happens in the hypertensive artery, an excessive stretch of smooth muscle cell (SMC) nuclei caused by hypertension may provoke wall thickening. Particularly, the wall thickening was thought to be caused by the increased mechanical stimuli to the smooth muscle cells (SMCs), which induce the increase in the volume of SMCs in the media, as well as extracellular matrix (ECM), but detailed mechanism remains unclear.

Among many possible mechanisms explaining the wall thickening, I focused on the mechanical stimulation applied to the nucleus. I proposed that an increase in the deformation of the artery wall due to the hypertension causes the increase in the nuclear deformation that may elicit the dispersion of the chromatin which is normally densely packed in the nuclei of SMCs in the aortic media and eventually enhances the transcription, translation, and protein expression.

The wall thickening in response to the hypertension is thought to occur to maintain the circumferential stress developed under normal in vivo condition.　In contrast, the wall thickening does not seem to maintain the stress in the longitudinal direction.　It has been reported that no-load length of the aorta increases in response to hypertension, and thus the longitudinal stress in the hypertensive aorta decreases significantly.

I proposed that the directional difference in the deformation of the nucleus in response to stretch might explain the directional difference in the mechanical response of the aorta to the hypertension. By conducting the tensile test of thoracic aortic specimens obtained from Japanese White rabbits under a microscope in the circumferential and longitudinal directions, I found that the SM nuclei exhibited smaller deformations in the longitudinal.

Besides, the stress applied to the aortic wall is transmitted to the chromatin inside the nuclei through the cytoskeleton. Among them, the actin filaments (AFs) link extracellular matrix, such as elastin and collagen fibers through integrins and the nuclear envelope through LINC (linker of nucleoskeleton and cytoskeleton) complex, so that the AFs are thought to play a significant role in the deformation of the nucleus.

Based on these considerations, this thesis carried out 3D analysis in addition to 2D for the nucleus deformation and examined the deformations of the nuclei and their surrounding AFs simultaneously in the thoracic aorta tissue to reveal the reason to exert the directional difference in the nucleus deformation under the mechanical stretching.

Chapter 1 introduced the structure of the aortic wall, mechanical response of the artery wall to hypertension and the structure of the animal cell and mechanotransduction. Then, I proposed possible mechanotransduction mechanism of the wall thickening in response to hypertension and conceivable reason for the directional difference in mechanical response of the wall.

Chapter 2 introduced the details of the stretching experiment, including detailed procedures of specimen preparation, staining, and performing tensile test in the circumferential and axial directions. The thoracic aortas were cut into 0.2-mm-thick ring-like segments in the direction perpendicular to the aortic axial axis (for type 1 specimen), or 0.2-mm-thick strip-like segments in the direction perpendicular to the circumferential direction (for type 2 and 3 specimens). Then, the SMC and AF were stained and the tensile tests with the three types of specimens were performed under a laser scanning microscope. Then, the image stacks of the nucleus and AF taken at each step of the tensile tests were projected onto a single image. These projected images were used for measuring and calculating the following: 1) the macroscopic stretch ratio of the specimen in the horizontal and vertical directions of the image in the 2D analysis; 2) the macroscopic shear deformation between the laminas of the media and the microscopic heterogeneous deformation of the media; 3) nuclear stretch ratio in 2D and 3D view; 4) AF deformation in 2D view. Distinct methods were used to measure the

stretch ratios of the nucleus and AF in the stretched specimen because the nucleus and AF have different morphological shapes. An image analysis software ImageJ was employed to measure the nuclear deformation. Hand-made code written on the Matlab was used for measuring the stepwise stretch ratio of the AF network utilizing an image correlation method.

Chapter 3 showed the measurement and calculation results of the heterogeneous deformation of the media against the mechanical stretch. I calculated macroscopic shear deformation between the laminas of the media and microscopic heterogeneous deformation of the media. In conclusion, the SMLs in rabbit thoracic aorta exhibited heterogeneous deformation in response to circumferential tensile stretch at both the macroscopic and microscopic levels, and the heterogeneity was more prominent at the microscopic level.

Chapter 4 showed the deformation of the AF network against circumferential and axial stretches. The AF deformation was analyzed in the 2D view. The results showed that there was no significant difference in the deformation between AF and the entire cell for both stretch cases.

Chapter 5 showed results of the nuclear deformation against circumferential and axial stretches. The results showed different nuclear deformation behaviors depending on the stretch direction. To compare the deformation of the AF and the nucleus in the same framework, 2D analysis for the nucleus was performed first. Then, to consider the rotation of the nucleus in the direction perpendicular to the 2D plane, 3D analysis was performed. Both 2D and 3D analyses show qualitatively similar results that the nucleus deforms smaller in the axial stretch than the circumferential stretch. However, the elongation of the nucleus both in the nuclear longitudinal and transverse axes was smaller in the 3D analysis than in the 2D. The difference may be due to the rotation of the nucleus to out of the 2D plane. Different from the AF, the nucleus deformed significantly less than the cell body as well as the AF network for both circumferential and axial stretches.

Chapter 6 proposed two possible causes of the difference in the strain between the nucleus and cell body. They are 1) the nucleus is stiffer than the cell body and 2) the nucleus is connected to the cell body through weak and sparse link of AF. By mathematical derivation, I proved that difference in the mechanical properties of the

nucleus and cell body cannot explain the difference in their strain, thus the difference in the strain between the nucleus and cell body may be cause by the weak and sparse link between the nucleus and the cell body. Then I proposed a three-dimensional model, and performed qualitative and quantitative analysis to explain the anisotropic nuclear deformation against different stretches. This model supported my hypothesis on anisotropic deformation of the nucleus. Then I compared the present results with biaxial stretch case, and discussed the deformation of the nucleus and the cell body under physiological condition. I concluded that the circumferential strain used in this study was smaller than the value expected in a physiological state, that the future experiment may concentrate on the biaxial stretch case, which mimics the physiological state more closely.

Chapter 7 summarized the conclusions of this thesis. In this work, tensile tests of the aortic slices were performed in the circumferential and axial directions while observing the nuclear deformation both in 2D and 3D as well as the deformation of the AF network in 2D. The results showed that 1) the deformation of the nucleus is smaller than the macroscopic deformation of the tissue; 2) the deformation of the actin filaments was almost similar to that of the tissue; 3) the deformation of the nucleus is especially small when the cell is stretched in the direction perpendicular to its longitudinal (transversal) direction. Based on the deformations observed in the present study and the nanostructures of the cell reported in the literature, a novel cell model that explains the smaller nuclear deformation in the axial stretch was proposed. The deformation of this model corresponded well to the real deformation of the actin filament network. The minute deformation of the nucleus in response to the transversal stretch might indicate that the nucleus is insensitive to the deformation of the tissue in this direction. These findings might lead to a better understanding of that the artery walls do not maintain the axial stress unchanged while they maintain the circumferential stress in response to hypertension.