Study of the phenotype changes and MMPs productions from SMCs under high Shear Stress using a co-culture model with EC

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論 文 内 容 の 要 旨

Chapter 1 Introduction

Vascular smooth muscle cells (SMCs) are lying in the tunica media layer outside the intimal endothelial cell (EC) monolayer, and could regulate vessel diameter to control blood pressure and flow. SMCs generally exhibit a contractile phenotype in normal blood vessels. And the SMC phenotype change from contractile to synthetic involving SMC migration and excessive proliferation in atherosclerotic lesions, is normally thought contributing to the growth of atherosclerotic plaques. Recent researches have suggested that SMCs could related to blood vessel remodeling during the initiation of cerebral aneurysms. Therefore, it is important to understand SMC behavior under different mechanical environments for better comprehension of blood vessel pathology.

However, SMCs are not in directly contact with blood flow, but surround the ECs, which expose to the blood. Therefore, EC-SMC co-culture models were developed for observing SMC behaviors in previous researches. The results revealed that ECs exposed to a physiological or a low shear stress (SS) affect unsheared SMC functions associated with the development of atherosclerosis through cellular interactions.

In most previous studies, SMCs were cultured in a synthetic state and/or as a monolayer, which was different from SMCs in the blood vessel. Considering that SMCs phenotype and function changes are important to the initiation of blood vessel diseases, it is important to control SMCs phenotype and function in the co-culture model. In the present study, the author constructed a co-culture model with SMCs 3D cultured in a collagen gel and SMCs phenotype controlled into a contractile phenotype, which are two aspects different from previously co-culture models. Since SMCs maintain in a contractile phenotype and extend spatially in the normal blood vessel, the

author think that the present co-culture model could be helpful for understanding SMCs behaviors in the blood vessel.

Earlier researches using co-culture models mainly focused on the SMC response to an SS of about 1.5–2 Pa, which is similar to the SS in a normal artery. However, cerebral aneurysms prefer to occur under high SS conditions. It is necessary to explore SMC response to high SS for better understanding of mechanism on blood vessel remodeling during cerebral aneurysm initiation. In the present study, the author explored how high SS affects SMCs and found that high SS could lead to a phenotype change from contractile to synthetic and higher MMP-2 and -9 production rates, which could induce blood vessel remodeling.

Chapter 2 Construction of an EC-SMC Co-Culture Model with 3D Cultured SMCs Controlled in a Contractile Phenotype

SMCs extend spatially with elastic tissue to consist of tunica media, and maintain in a contractile state in normal blood vessels. As to mimic spatially location of SMCs in blood vessels, the author constructed a new co-culture model with SMCs 3D cultured in a collagen matrix in this chapter. As to control SMC phenotype in a contractile state, a serum-free medium, quiescent medium was used in the present study.

The expression levels of three contractile proteins (i.e., α-SMA, calponin, and MHC) in SMCs were observed along with the culturing days to confirm the phenotype of SMCs, using a Western blotting method. The result showed that, SMCs changed to a contractile phenotype after 15 days cultured in QM (QM15). Then, QM15 SMCs were stained by antibodies, and observed by microscope to confirm SMCs location in the collagen gel. From the confocal microscopy observation, the author was able to confirm that SMCs located randomly at different height in the collagen gel.

After ECs cultured for 6 days until confluent on the membrane filter, ECs and QM15 SMCs were combined together to complete the co-culture model in the present study. As a result, QM15 SMCs were 3D cultured in a collagen gel and controlled to a contractile phenotype. With this two aspects, the author thinks that the current model could mimic SMCs spatially location and phenotype in the blood vessel. Therefore, it could be helpful for understanding SMC responses to mechanical stimulation and cellular interactions relevant to *in vivo* conditions.

Chapter 3 Influence of SS on Phenotype and MMP Production of SMCs in the Co-Culture Model

A high SS could have an influence on SMC and this relationship could be related to blood vessel remodeling during cerebral aneurysm initiation. However, SMCs responses to high SS are not studied yet. Therefore, in this chapter, the author applied a high SS to the co-culture model with 3D cultured SMCs, and evaluated the phenotype changes of SMCs. In addition, the author also observed the expressions of Matrix metalloproteinase-2 (MMP-2) and -9 from SMCs in the co-culture model under high SS. MMPs have the capacity to degrade all the components of extracellular matrix. Large amounts of MMP-2 and -9, which are the predominant MMPs, have been observed in the walls of well-developed aneurysms in human autopsy tissue and in animal models over weeks-months following the induction. Therefore, MMPs production could also be

important to the initiation of cerebral aneurysms.

SMCs phenotype changes after flow-exposure were determined by a WB method same as in chapter 2. Compared to QM15 SMCs, a physiological SS of 2 Pa could maintain the expression levels of contractile proteins (i.e., α-SMA, calponin, and MHC) in SMCs, whereas a high SS of 10 Pa could decrease the expression levels of α-SMA and calponin in SMCs in the co-culture model. In addition, high SS induced more MMP-2 and -9 production by SMCs in the co-culture model, compared to 2 Pa.

This result confirmed that high SS applied to ECs could affect SMCs phenotype change to a synthetic state in the co-culture model. High SS might influence gene expressions (i.e., TGF-β1) from EC, which could be one reason that results into a synthetic phenotype change of SMCs in the co-culture model. Besides SMCs phenotype changes, MMP-2 and -9 productions of SMCs under high SS were also higher than the productions under 2 Pa. SMC phenotype change from contractile to synthetic could be a factor that induces an inflammatory response on the blood vessel, and overexpression of MMPs could degrade extracellular matrix. Both are important to the blood vessel remolding during the initiation of cerebral aneurysms. Therefore, high SS flow-experiments using a co-culture model with 3D cultured SMCs in this chapter could be helpful for understanding the mechanisms of blood vessel remodeling during cerebral aneurysm initiation.

Chapter 4 The influence of TGF-β1 from ECs on SMC Phenotype and MMPs Production under SS in the Co-Culture Model

SMCs were not directly exposed to the flow, but under the ECs monolayer. It is possible that SMCs react to SS stimulations through EC induced signal transduction complexes. However, the communications between ECs and SMCs under a high SS were not explored yet. As to explain the results in chapter 3, the author observed the influence of one gene expression, transforming growth factor beta 1 (TGF-β1), from ECs on the phenotype change and MMPs productions of SMCs in the co-culture model under a high SS in this chapter.

TGF-β1 could promote SMCs phenotype change to a contractile state and suppress SMCs migration, and SS could influence TGF-β1 signaling in ECs. Therefore, it is reasonable to hypothesize that SS could influence TGF-β1 activation in ECs, and the TGF-β1 expression from ECs could modulate SMCs phenotype in the co-culture model. As to determine the effect of TGF-β1 expression from ECs on SMCs phenotype changes, the TGF-β1 expression from ECs was suppressed by a SiRNA transfection method in the present study, and the phenotype change of SMCs in the co-culture model was observed after flow-exposure experiments. MMP-2 and -9 productions of SMCs were also observed in the same conditions as to understand SMCs function changes.

As a result, the expression of α-SMA and calponin showed no obviously difference under each SS conditions in the co-culture model with SiRNA transfected ECs. Compared to QM15 SMCs, the expression levels of α-SMA and calponin here was at a lower level, suggesting that SMCs could change to a synthetic state in the co-culture model with transfected ECs after flow-exposure. As introduced in chapter 3, an SS of 2 Pa could maintain SMCs in a contractile state in the co-culture

model with normal ECs. While in this chapter, SMCs changed to a synthetic state under 2 Pa in the co-culture model with transfected ECs. This result suggests that TGF-β1 expression from EC could influence SMC phenotype change under an SS of 2 Pa in the co-culture model.

When TGF-β1 expression from ECs was suppressed by SiRNA, MMP-2 production of SMCs showed no obviously difference after flow-exposure under each SS conditions. In the co-culture model with normal ECs, MMP-2 production of SMCs decreased under 2 Pa compared to other SS conditions (i.e., 0.2, 6, and 10 Pa). And the MMP-2 productions of SMCs in the co-culture model with transfected ECs were generally lower than that in the co-culture model with normal ECs. Therefore, TGF-β1 expression from ECs could influence MMP-2 production of SMCs under different SS conditions in the co-culture model. Conversely, it seems that MMP-9 production of SMCs showed no obviously difference, whether TGF-β1 expression from ECs was suppressed or not.

In conclusion, the author confirmed that TGF-β1 expression from ECs could influence SMC phenotype change under SS conditions in the co-culture model. In addition, TGF-β1 expression from EC could also change MMP-2 production, but not MMP-9 production of SMC under SS conditions in the co-culture model.

Chapter 5 Concluding Remarks

In conclusion, an EC-SMC co-culture model with 3D cultured SMCs controlled in a contractile phenotype was constructed in the present study. Using this model, the author has confirmed the influence of high SS on SMC phenotype change and MMPs production, which is not explored before. A high SS could change SMC phenotype to a synthetic state, and induce higher MMPs productions from SMCs in the co-culture model. Finally, the author has confirmed that TGF-β1 expression from ECs could play a role in SMC responses to SS. TGF-β1 expression from EC could influence SMC phenotype change under an SS of 2 Pa, and could change MMP-2 production, but not MMP-9 production of SMC under SS conditions in the co-culture model. These results may help understand the mechanisms of blood vessel remodeling during the initiation of cerebral aneurysms, which predominately occur under high SS conditions.