氏　　名	MD RASEL MOLLA
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学位論文題目	Vascular smooth muscle RhoA counteracts abdominal aortic aneurysm formation by modulating MAP4K4 activity. （血管平滑筋に発現する RhoA は MAP4K4 の活性調節によって腹部大動脈瘤の形成を抑制する）
審査委員	主査　教授　依馬　正次
	副査　教授　渡邉　嘉之
	副査　教授　大路　正人

別紙様式４（課程博士・論文博士共用）

論　文　内　容　要　旨

※整理番号	972	（ふりがな） 氏　　名	（むはんまど　らっせる　もーら） Md Rasel Molla
博士論文題目	Vascular smooth muscle RhoA counteracts abdominal aortic aneurysm formation by modulating MAP4K4 activity （血管平滑筋に発現するRhoAはMAP4K4の活性調節によって腹部大動脈瘤の形成を抑制する）		

＜目的＞ (Purpose)

Abdominal aortic aneurysm (AAA) is a life-threatening vascular disease that causes aortic rupture and sudden death. AAA is characterized by the alteration of vascular smooth muscle cell (VSMC) force generation, degradation and fragmentation of elastic fiber, loss of medial VSMCs, and vascular inflammation. Although the pathological mechanism underlying AAA is not completely understood, it is believed that reduced contractility and enhanced inflammation in VSMCs play key roles in the initiation and progression of AAA. RhoA, a small GTPase, abundantly exists in VSMCs. RhoA has multiple functions in regulating VSMCs and contributes to the morphological support and maintenance of the aortic structure. However, how RhoA in VSMCs regulates the homeostasis of the aorta remains unclear. The purpose of this study is to investigate the role of VSMC RhoA in the pathology of AAA formation.

＜方法＞ (Method)

Hematoxylin and eosin (H-E) and elastin-specific Verhoeff Van Gieson (VVG) staining were performed in AAA lesions and normal areas of the aortic wall of AAA patients. RhoA expression in AAA lesions and normal areas was also examined by immunostaining, qPCR and western blotting. VSMC-specific RhoA cKO mice using the Cre/loxP system were generated. To induce AAA in mice, angiotensin II (Ang II, 1000 ng/kg/min) and β-aminopropionitrile (BAPN, 37.5 mg/kg/d) were administered for 4 weeks using osmotic mini-pumps. Blood pressure (BP) in the mice was measured using the plethysmographic tail-cuff method. For measurements of aortic tension, the mouse aorta isolated from the RhoA cKO and control mice were applied to multi-wire myograph system. Collagen gel contractility assay was performed using VSMCs isolated from these mice. The histopathological staining, immunostaining, qPCR and western blotting were carried out to examine inflammation in the aorta and VSMCs, expression of contractility-associated genes, and endothelial layer disruption in RhoA cKO mice. Mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) expression and activity were determined in the aorta and VSMCs of RhoA cKO mice and control mice. A MAP4K4 inhibitor, DMX-5804, was administered to examine whether it could prevent AAA formation in RhoA cKO mice. To identify a novel molecular relationship between RhoA and MAP4K4, a proteomics analysis was carried out with liquid chromatography tandem mass spectrometry (LC-MS/MS). Further protein interaction analysis among RhoA, Set, PP2A and MAP4K4 was performed with co-immunoprecipitation assays.

（備考）１．論文内容要旨は，研究の目的・方法・結果・考察・結論の順に記載し ２千字程度でタイプ等で印字すること。

２．※印の欄には記入しないこと。

＜結果＞ (Results)

H-E and VVG staining revealed that the disrupted medial layer and loss of intact medial elastic fibers in the aortic wall of AAA patients. Immunostaining, qPCR and western blotting analyses demonstrated that RhoA expression was quite lower in the AAA lesions with enhanced inflammations and reduction of CD31, an endothelial marker, compared with the normal areas in patients with AAA. RhoA cKO mice exhibited that higher incidence of AAA formation compared to the control mice after treatment with AngII+BAPN that induced increase in BP and vulnerability of aortic wall, respectively. The contractility of the aortic rings and VSMCs from RhoA cKO mice was reduced and the expression of genes related to the VSM contractile phenotype was decreased by loss of RhoA. The enhanced inflammatory response and aortic wall degradation with impaired endothelial barrier function were observed in RhoA cKO mice. MAP kinase signaling, including MAP4K4, was highly activated in the VSMCs of RhoA cKO mice and human AAA lesions. This was related to the pathological pathway that induces the down-regulation of contractility and the up-regulation of inflammation in VSMCs. DMX-5804 administration in RhoA cKO mice decreased AAA formation, concomitant with the recovery of impaired contractile phenotypes and excessive inflammatory responses. The proteomics analysis with LC-MS/MS identified Set as a binding protein to active RhoA. When Set was bound to active RhoA, Set was dissociated from PP2A phosphatase, which then interacted with MAP4K4 to modulate the activation of MAP4K4. In the absence of RhoA, Set bound to PP2A to inhibit the PP2A–MAP4K4 interaction, resulting in the increased activation of MAP4K4.

＜考察＞ (Discussion)

In this study, the applicant identified the protective effects of RhoA in VSMCs on formation of AAA using human aortic tissues, a RhoA cKO mouse model, and in vitro approaches. The results indicate that loss of RhoA is involved in both dysfunction of VSMC contractility and enhancement of vascular inflammation. The novel discovery at the molecular level is that association between RhoA and MAP4K4 in VSMCs. This association is important for the prevention of AAA. A further novel finding of this study is the identification of a signaling molecule, Set, which links RhoA and MAP4K4 activity. Activated RhoA preferentially bound to Set, which dissociates Set from PP2A. The dissociation of Set from PP2A induces the interaction of PP2A with MAP4K4. Subsequently, PP2A inhibits the activity of this kinase. As for the translational and clinical implications, a MAP4K4 inhibitor like DMX-5804 may reverse the adverse phenomena for AAA formation induced by loss of RhoA in VSMCs. The applicant believes that providing the therapeutic potential to regulate RhoA expression and MAP4K4 activity must be crucial for AAA prevention.

＜結論＞ (Conclusion)

The inhibition of MAP4K4 by RhoA in VSMCs is important for the prevention of AAA formation. In conclusion, VSMC RhoA counteracts the AAA formation by suppressing MAP4K4 activity through Set and PP2A.

別紙様式９（課程博士・論文博士共用）

博士論文審査の結果の要旨

整理番号	９７２	氏　名	MD RASEL MOLLA
論文審査委員			

（博士論文審査の結果の要旨）

腹部大動脈瘤（AAA）は、破裂すると極めて死亡率が高い疾患である。AAA は血管平滑筋細胞の力学的特性、弾性繊維の断片化、血管炎症によって特徴づけられる。AAAの発症機構は、血管平滑筋細胞の収縮力低下と炎症亢進がAAAの形成と進展に中心的な役割を果たすと考えられている。RhoAは低分子量Gタンパク質の１つであり、血管平滑筋に高発現している。しかし、RhoAが血管の恒常性を調節しているメカニズムは分かっていなかった。そこでAAA形成におけるRhoAの役割を解析し、以下の点を明らかにした。

１）　RhoAは正常の血管平滑筋に高発現している一方、AAAの平滑筋層においては減弱していること。
２）　血管平滑筋特異的RhoAノックアウトマウス（RhoA CKO）は、昇圧剤添加により高頻度にAAAを呈すること。
３）　AAAでは炎症性サイトカインの亢進やマクロファージの浸潤が見られること。
４）　RhoA CKOではMAP4K4のリン酸化が亢進していること。
５）　MAP4K4阻害薬投与によってAAA形成が予防されること。
６）　MAP4K4活性はSetとP2Aによって制御されていること。

本論文は、血管平滑筋細胞のRhoAがMAP4K4活性を阻害することでAAA形成を予防する事を示したものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数５９７字）

（令和５年１月２４日）