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論文題目	Deletion of IKKβ in smooth muscle cells induces vascular calcification through β-catenin-Runx2 signaling (平滑筋における IKKβ欠損はβカテニン-Runx2 のシグナル伝達を介して血管石灰化を促進する)		

（論文内容の要旨）

[Background] Vascular calcification was previously considered as a degenerative process seen in advanced atherosclerosis lesions, but recent studies have indicated that such calcification can appear in a different manner. Calcification not only accompanies with atherosclerosis but also accompanies diabetes or renal failure in the absence of atherosclerosis. Moreover, recent clinical studies have revealed that statins, a medicine widely used for atherosclerosis, promote coronary calcification, which clearly indicates that calcification stands on a different basis from atherosclerosis. However, the difference has not been fully explained since the mechanism of vascular calcification is poorly understood. For example, the roles of NF-κB, a major regulator of inflammation, in vascular calcification are poorly explored, although its roles in atherosclerosis were well documented. In this study, the roles of NF-κB signaling in vascular calcification were investigated.

[Methods and Results] The mice with deletion of IKKβ, an essential kinase for NF-κB activation, in vascular smooth muscle cells (KO mice) were produced and subjected to the $CaCl_2$-induced aorta injury model. Unexpectedly, KO mice showed more calcification of the aorta than their wild-type (WT) littermates, despite the former's suppressed NF-κB activity. Cultured vascular smooth muscle cells (VSMCs) from the aorta of KO mice also showed significant calcification *in vitro*. In the molecular analysis, Runx2, a transcriptional factor accelerating bone formation, was upregulated in cultured VSMCs from KO mice, and its regulator β-catenin was more activated in KO VSMCs. Next, the mice with a knock-in of kinase-active IKKβ specifically in VSMCs (KA mice) were produced and subjected to a $CaCl_2$-induced aorta injury model, but no calcification was observed in aorta. Consistent with *in vivo* results, KA VSMCs showed an absence of calcium deposits. Both active and total β-catenin and Runx2 were significantly decreased in KA VSMCs when compared with that in WT VSMCs. These results in KA mice / VSMCs were opposite to the results seen in KO mice / VSMCs. Meanwhile, recent studies implicated that IKKβ has NF-κB independent, even kinase-independent function. To elucidate the kinase-independent roles of IKKβ in calcification, the mice expressing kinase-dead mutant (KD) of IKKβ in VSMCs on the background of KO mice were produced, and the cultured VSMCs from the mice were examined. Surprisingly, no increase of calcium deposit was observed in KD VSMCs compared with WT VSMCs, contrary to KO VSMCs. Osteoblast marker genes including osterix, alkaline phosphatase and osteocalcin were up-regulated in KO VSMCs, but not in KA and KD VSMCs. These results clearly indicated that the kinase-independent function of IKKβ has suppressive effect on calcification. In terms of molecular mechanism, active β-catenin and Runx2 in KD VSMCs were significantly downregulated compared with KO VSMCs, which were consistent with the results mentioned above. The ubiquitination of β-catenin in KO, KA and KD VSMCs was also examined as a further mechanism, and it was suppressed in KO VSMCs and augmented in KA and KD VSMCs. These results indicated that the kinase-independent function of IKKβ is involved in the regulation of β-catenin and Runx2.

[Conclusion] The deletion of IKKβ, which is known as essential kinase for NF-κB, in VSMCs promoted vascular calcification and it was mediated by the upregulation of β-catenin and Runx2. Further, kinase-independent function of IKKβ was involved in the regulation of such signaling. Considering that the accelerating roles of NF-κB signaling in atherosclerosis have been well analyzed, these findings would mechanistically explain the fundamental difference between atherosclerosis and calcification. Also, they can unveil the unknown relationship between vascular calcification and anti-inflammatory drugs including statins and aspirin.

（論文審査の結果の要旨）

炎症を制御する転写因子である NFκB の活性化に不可欠なキナーゼ IKKβの血管石灰化における役割を検討した。まず IKKβを平滑筋細胞特異的にノックアウトしたマウス（KO マウス）は、$CaCl_2$大動脈塗布モデルにおいて血管石灰化の亢進を呈した。逆に、キナーゼ活性が恒常的に活性化している kinase-active IKKβを平滑筋細胞に強発現させたマウス（KA マウス）においては石灰化の抑制が見られた。その分子メカニズムとして、KO マウス平滑筋細胞においてβカテニンと Runx2 が活性化しており、KA マウス平滑筋細胞においてはそれらが不活性化していることが見出された。さらに KO マウスを background としてキナーゼ活性を持たない kinase-dead IKKβ (KD)を平滑筋細胞に強発現させたマウスを作成し、その平滑筋細胞を検討したところ野生型マウス平滑筋細胞と同等までに石灰化が抑制されており、またβカテニンと Runx2 は KO マウス平滑筋細胞よりも著明に不活性化していた。すなわち、IKKβはキナーゼ活性非依存的にβカテニンや Runx2 を制御することによって血管石灰化を抑制していると考えられた。

以上の研究は血管石灰化における IKKβの役割の解明に貢献し、血管石灰化の病態解明に寄与するところが多い。

したがって、本論文は博士（医科学）の学位論文として価値あるものと認める。

なお、本学位授与申請者は、平成 30 年 2 月 27 日実施の論文内容とそれに関連した試問を受け、合格と認められたものである。

要旨公開可能日： 年 月 日 以降