論文の内容の要旨

論 文 題 目　**Fibroblast Growth Factor 23 accelerates phosphate-induced calcification of vascular smooth muscle cells**

（**FGF23** はリンによる血管平滑筋石灰化を促進する）

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Background: The prevalence of chronic kidney disease (CKD) is increasing worldwide. Patients with CKD and particularly end-stage renal disease (ESRD) are at increased risk of mortality, mainly from cardiovascular disease (CVD).

Vascular calcification (VC) is the depositions of calcium (Ca) phosphate (P) salt in the vasculature. VC in the medial layer, also known as Mönckeberg's calcification, is common in CKD patients and is associated with increased risk of CVD. The mechanism of VC is thought to be a highly complex process that shares many similarities with the mineralization of bone. *In vivo* and *in vitro* studies have shown that P induces osteoblastic differentiation of vascular smooth muscle cells (VSMCs).

Fibroblast growth factor 23 (FGF23) is the most recently discovered FGF

which belongs to the FGF19 subfamily. It is synthesized in bone and acts mainly on the kidney and parathyroid as a P-regulating hormone. The presence of the co-receptor Klotho, which binds FGF receptors (FGFRs), is necessary for induction of the FGF23-specific signaling.

Circulating levels of FGF23 increase as kidney function declines. Clinical studies suggest that increased level of FGF23 is associated with mortality and VC among CKD patients but its direct effect on VSMC is unknown.

Methods: VSMCs were isolated from thoracic aorta of 8-wk-old male Sprague-Dawley rats. The mRNA expressions of FGFRs and Klotho in VSMCs were examined by reverse transcription-polymerase chain reaction (RT-PCR). The protein expressions of Klotho in VSMCs and the aorta were analyzed by western blot and immunohistochemistry. Extracellular signal-regulated kinase (ERK) 1/2 phosphorylation in normal VSMCs or Klotho-overexpressed VSMCs (Klotho-VSMCs) by recombinant FGF23 (2-10 ng/ml) was analyzed by western blot.

Calcification of Klotho-VSMCs was induced by 5 mM P medium (high-P medium) in the presence or absence of FGF23 (2-10 ng/ml). The Ca depositions were visualized by Alizarin Red S calcification staining solution and quantified by methylxylenol blue method. The mRNA expressions of osteoblastic transcription factors

(Msx2 and osterix) and sodium dependent P transporters (Pit 1 and Pit2) were evaluated by real-time RT-PCR.

Results: FGFR 1, 2, 3 and Klotho mRNA were expressed in cultured VSMCs. Klotho protein was detected in the medial layer of the aorta but not in the isolated VSMCs.

ERK 1/2 phosphorylation by FGF23 was increased in Klotho-VSMCs in a dose dependent manner but not in the normal VSMCs. ERK 1/2 phosphorylation by FGF23 in Klotho-VSMCs was inhibited by FGFR1 inhibitor (SU5402) (2-10 μM) or mitogen-activated protein kinase kinase (MEK) inhibitor U0126 (2-10 μM).

FGF23 accelerated P-induced calcification of Klotho-VSMCs in a dose dependent manner. P induced Msx2 and osterix expressions were enhanced by FGF23 and U0126 inhibited the additive effect of FGF23 on the expression of these osteoblastic markers. The expressions of Pit1 and Pit 2 were not affected by either increased P level or FGF23.

Conclusion: We have shown that FGF23 enhances P-induced calcification in Klotho-VSMCs by promoting osteoblastic differentiation and its effect might be mediated by the ERK 1/2 pathway. Our study indicates that we have to take into consideration the effect of FGF23 on VC during CKD patient’s management.