

## 혈관평활근세포 증식과 신생혈관내막증식 생성 촉진작용에 대한 내피세포 미세입자의 작용기전

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### Indoxyl Sulfate-induced Endothelial Microparticles Stimulate Vascular SMC Proliferation and Neointimal Hyperplasia Formation through TGF- $\beta$ Induction

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**Background:** Vascular access stenosis occurs frequently and predominantly as a result of neointimal hyperplasia (NH) formation due to vascular smooth muscle cell (SMC) proliferation. Endothelial microparticle (EMP) is closely associated with vascular dysfunction and atherosclerosis. We are to investigate the effects of EMP on TGF- $\beta$  signaling associated with proliferation of vascular SMC and NH formation.

**Methods:** HUVECs were cultured and stimulated by indoxyl sulfate. EMPs were collected by ultracentrifugation at 100,000 $\times$ g with cell culture media and determined by flow cytometry analysis. Human aortic SMCs were treated by collected EMPs ( $2 \times 10^6$ ) or TGF- $\beta$  (10 ng/mL) (with or without anti-TGF- $\beta$  neutralizing antibody). Western blot analysis for phospho-specific TGF- $\beta$  signalings (Akt, ERK1/2, p38 MAPK, and Smad3) was done with EMP or TGF- $\beta$ -stimulated aortic SMCs. SMC proliferation was measured with BrdU cell proliferation assay. TGF- $\beta$  production was measured with ELISA in cell culture media. Ex vivo experiments were also performed on porcine internal jugular veins with or without EMPs for 12 days, and immunohistochemistry for phospho-specific TGF- $\beta$  signalings was done with porcine venous tissue.

**Results:** Indoxyl sulfate-induced EMPs increased phosphorylation of Akt, ERK1/2, p38 MAPK, and Smad3, and stimulated the proliferation of aortic SMCs. These effects were reduced when anti-TGF- $\beta$  neutralizing antibody was administered. EMPs induced the TGF- $\beta$  secretion from aortic SMCs. In ex vivo culture, NH was significantly developed in EMP-treated venous tissues. The activation of TGF- $\beta$  signalings (Akt, ERK1/2, p38 MAPK and Smad3) was observed in the neointimal hyperplasia.

**Conclusion:** EMPs generated by indoxyl sulfate induce TGF- $\beta$  in vascular SMC. TGF- $\beta$  stimulates vascular SMC proliferation and neointimal hyperplasia formation through Akt, ERK1/2, p38 MAPK, and Smad3 signaling pathway. Further investigation is needed to demonstrate the role of EMPs on vascular access stenosis.

**Key Words:** 내피세포 미세입자, 투석접근로 폐쇄, TGF- $\beta$

Endothelial microparticle, Vascular access stenosis, TGF- $\beta$