P-88

글로보트라이아오실 세라마이드에 의한 파브리 병과 내피세포의 내피-중간엽 세포이행

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Globotriaosylceramide (Gb3)-induced Endothelial-to-Mesenchymal Transition (Endo-MT) in Fabry Disease and Cultured Endothelial Cells

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Introduction and Aims: The lysosomal storage disorder Fabry disease is characterized by excessive Gb3 accumulation in major organs including kidney. Endothelial dysfunction is known to be associated with Fabry disease, it is not known whether Gb3 per se induced endothelial dysfunction and progression of renal disease. We investigated whether Gb3 per se induced Endo-MT in cultured human umbilical venous endothelial cells (HUVEC) with an evaluation of in-vivo evidence of Endo-MT in the kidney of Fabry mouse.

Methods: Endo-MT was evaluated by the changes in cell morphology and a comparison of the expression of the endothelial marker, VE-cadherin and the mesenchymal marker, alpha smooth muscle actin (α-SMA) by real time PCR, Western blotting (WB) and immunocytochemistry in HUVEC exposed to Gb3. The activation of eNOS and MAPK was evaluated by WB, and production of reactive oxygen species (ROS) was evaluated by DCF-DA staining. Effects of recombinant of alpha-galactosidase A (Gla) (FabrazymeR), anti-oxidants and the inhibitors of MAPK on Gb3-induced Endo-MT were investigated. Endo-MT in the kidney of Fabry mouse was examined by double immunofluorescence staining of CD31 and α-SMA.

Results: Gb3 (0.1-20 μM) induced Endo-MT in HUVEC, as evidenced by an alteration of cell morphology and down-regulated the expressions of VE-cadherin with up-regulation of α-SMA. Gb3 (10 μM) decreased NO production with 1177Ser-eNOS de-phosphorylation and 475Thr-eNOS phosphorylation in HUVEC. Gb3 also activated MAPK from 15 minutes with an induction of ROS from 30 minutes of stimulation. Anti-oxidants or the inhibitors of MAPK as well as Fabrazyme reversed Gb3-induced Endo-MT. In Fabry mouse, Gb3 accumulation was observed in glomerular podocytes, tubular cell and peri-tubular capillaries (PTC). Immunostaining with CD31 and α-SMA revealed capillary rarefaction both in glomerular and PTC with de-novo expression of α-SMA in PTC, suggesting Endo-MT in the kidney of Fabry mouse.

Conclusions: Gb3 per se induced a phenotypic transition of endothelial cells via an activation of MAPK, an induction of oxidative stress and a differential phosphorylation of eNOS, which could be one of the mechanisms of endothelial dysfunction and nephropathy in Fabry disease.

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