Hypoxia-Induced MMP-13 Expression and Destruction of Tight Junction Protein ZO-1 in Astrocytes are an Important Factor Leading to Hyperpermeablility of Blood-Brain Barrier

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Aims: Blood-brain barrier (BBB) integrity protects the neuronal microenvironment. If this integrity is lost, inflammatory cells and fluid penetrate the brain, causing edema and cell death. Matrix metalloproteinase(MMPs) are involved in the remodeling of the extracellular matrix (ECM) in a variety of pathological conditions encountered with ischemia/hypoxia (e.g. stroke). This study is to examine the MMP-13 expression in astrocytes in response to hypoxia.

Methods: The mRNA level of MMP-13 was analyzed by RT-PCR. c-Fos and c-Jun are assessing as signaling pathways involved in the regulation of hypoxia-stimulated MMP-13 expression in rat astrocyte. The paracellular permeability was evaluated by measuring FITC-dextran leaked through endothelial monolayer. To determine whether hypoxic-conditioned medium (Hx-CM)-induced increase of endothelium permeability is due to the effect of MMP-13 on tight junction, zonular occludens-1(ZO-1) protein was examined by immunofluorescent staining and Western blot analysis. $P<0.05$ are considered significant(Student's t-test).

Results: Exposure to hypoxia up-regulated the expression of MMP-13, c-Fos and c-Jun time-dependently. Hypoxia-induced MMP-13 overexpression was antagonized by transfection with antisense oligodeoxynucleotides (AS-ODN) of c-Fos or c-Jun. Furthermore, Hx-CM collected from astrocytes exposed to hypoxia increased paracellular permeability of adult rat brain endothelial cells (ARBECs). Administration of MMP-13 neutralizing antibody antagonized Hx-CM-induced paracellular permeability of ARBECs. Furthermore, pre-transfection of astrocytes with AS-ODN of c-Fos, c-Jun or MMP-13-shRNA significantly decreased hyperpermeability of ARBECs induced by Hx-CM. The arrangement of tight junction protein (TJP). ZO-1 of ARBECs disorganized in response to Hx-CM. Administration of Hx-CM to ARBECs also resulted in the production of proteolytic fragments of ZO-1, which was antagonized by transfection of MMP-13-shRNA in primary astrocytes. These results suggest that hypoxia-induced MMP-13 expression in astrocytes is regulated by c-Fos and c-Jun. MMP-13 is an important factor leading to the disorganization of ZO-1 and hyperpermeablility of blood-brain barrier in response to hypoxia.

Conclusions: It was found that hypoxia enhanced the expression of MMP-13 in astrocytes, which was regulated by c-Fos and c-Jun. In addition, MMP-13 was able to cause hyperpermeability of BBB, suggesting that MMP-13 and ZO-1 protein may be an important factor in the pathogenesis of stroke. **(*This study was accepted by "Journal of Cellular Physiology "** *for publication at July, 2009; Funded by:National Science Council of Taiwan no. 97-2314-B-039-039)*