学 位 論 文 の 要 旨

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学 位 論 文 名　Cystatin C Expression in Ischemic White Matter Lesions

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論 文 内 容 の 要 旨

Introduction

Cystatin C is an inhibitor of cysteine proteases, secreted from various types of cells and involved in regulation of local inflammation, tumor invasion and metastasis. Growing evidence shows that cerebrospinal fluid (CSF) cystatin C levels are one of the indicators for the evaluation of the condition of cysteine protease system in various neurologic diseases. However, the contribution of cystatin C to the pathology of various diseases, including cerebrovascular diseases and development of cerebral white matter lesions (WMLs), remains poorly understood. To clarify the role of cystatin C in the progression process of ischemic WMLs was the objective of our study.

Materials and Methods

Twenty-three patients of Fazekas' WMLs grade 3 were selected from the patients diagnosed as chronic ischemic cerebrovascular disease with lacunar infarctions, of whom 14 patients were with hypertension (W/HT(+); mean age ± SD, 75.1 ± 5.2 years) and 9 patients were without hypertension (W/HT(-); 75.9 ± 10.3 years). Thirty-eight patients with WMLs grade 0 or 1 (Control; 71.4 ± 11.6 years) were treated as a control group. By means of the established enzyme-linked immunosorbent assay (ELISA) method, cystatin C levels in CSF were compared among the groups of control, W/HT(+) and W/HT(-) groups. And also cystatin C levels in primary and established human neural cell cultures were investigated. For the immunohistochemical analysis, we examined the total of 10 brains, which included 3 brains of ischemic cerebrovascular disease (CVD) with severe WMLs (sWMLs), 3 brains of ischemic CVD with no or mild WMLs (mWMLs), and 4 controls with no significant WMLs. Cystatin C immunoreactivity was investigated in the white matter of patients with sWMLs, mWMLs or controls.

Results and Discussion

Cystatin C levels in the CSF of patients with Fazekas' WMLs grade 3 were lower than patients with grade 0-1 ($p = 0.0022$ and $p < 0.0001$, respectively). In human-derived culutured cells, cystatin C was detected in neurons, astrocytes, oligodendrocytes and microglia with each cell type-specific marker. Immuno-histochemical study showed that cystatin C immunoreactivity was found in astrocytes, and the number of astrocytes in the white matter in the sWML group was decreased as compared to those in the control ($p = 0.0027$) and mWML groups ($p = 0.0024$).

In human neural cell cultures, treatments with thrombin, matrix metalloproteinases and interleukin 1β increased the expression of cystatin C mRNA in human

astrocytes and hybrid neurons, but ELISA revealed that only thrombin significantly increased the production and secretion of cystatin C in astrocytes. Cystatin C expression in astrocytes for the sWML group appears stronger than the control group, and the number of cystatin C-positive cells are not statistically different among the three groups, although the number of astrocytes is decreased in the sWML group. These results suggest that the expression of cystatin C in the regressive astrocytes is up-regulated in the process of white matter degeneration. Upregulation of cystatin C is possibly one of the self-defense responses during neuroinflammation or neurodegeneration to inhibit protease release from lysosomes, where cystatin C localizes. In the pathology of WMLs, blood-brain barrier is disrupted as it progresses, which induces extravasation of serum proteins. Our immunohistochemical study did not reveal difference in thrombin immunoreactivity among the groups, but it is possible that continuous exposure to thrombin might exist in the areas around WMLs. The results of the present study suggest that astrocytes are the main source of secreted cystatin C in CNS and decreased number of astrocytes in WMLs leads to low concentration of cystatin C in CSF.

Conclusions

We reported for the first time that cystatin C level in CSF for the patients with sWML was decreased as compared to those for the patients with mWML and controls. Our study suggests that the low CSF level of cystatin C in ischemic WMLs is due to the decreased number of astrocytes that secrete cystatin C in response to the stimuli of proteases and inflammatory cytokines. As cystatin C secretion from astrocytes influences its CSF levels, further examination should be conducted to determine whether cystatin C modifies the pathological progression of WMLs.