論　文　内　容　要　旨

XRCC3 polymorphism is associated with hypertension-induced left ventricular hypertrophy

(XRCC3 多型は、高血圧誘発左心室肥大に関連する)

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XRCC3 is a DNA repair protein that participates in a DNA double-strand break repair pathway, particularly homologous recombination. The polymorphism at exon 7 of XRCC3 gene leads to Thr to Met change at codon 241. This polymorphism may affect DNA repair efficiency. Previously we have reported that XRCC3 inactivation in a human colon cancer cell line showed increased endoreduplication and polyploidy. Endoreduplication is a cell cycle variation in which nuclear genome is replicated without cell division. So this results in increased DNA content and increased cell size.

The purpose of this study was to investigate whether XRCC3 polymorphism is associated with hypertension-induced left ventricular hypertrophy and to determine the mechanism.

77 hypertensive patients who were under hemodialysis were studied. Their genotypes were determined by PCR-RFLP. LVH was diagnosed using 2D echocardiography. We defined LVH as the wall thickness of both ventricular septum and left ventricular posterior wall more than 12mm. The association between XRCC3 polymorphism and LVH was statistically analyzed by Chi-square test.

In table shows the baseline characteristics of the patients. 59 out of 77 patients were Thr/Thr and 18 patients were Thr/Met. There were no patients with Met/Met in these 77 patients. Between Thr/Thr and Thr/Met groups, there were no significant differences in gender, age, BMI, body weight gain between the treatment, duration from the treatment initiation, systolic and diastolic BP before and after the treatment.

In echocardiographic measurements, the LV posterior wall was significantly thicker in Thr/Met than Thr/Thr. The statistical analysis showed that there were significant differences in both genotypic an allelic frequencies between LVH- and LVH+ groups. The relative risk of LVH in Thr/Met compared with that of Thr/Thr was 4.61 and that of Met allele compared Thr allele was 3.64.

Summarizing these findings, the prevalence of LVH was higher in the hypertensive HD patients with Thr/Met than Thr/Thr. Also, XRCC3 Thr241Met polymorphism was a risk factor of LVH among the HD patients.

We investigated the possible mechanisms underlying these observations. First we examined whether XRCC3 241Met variant induced cell hypertrophy. CHO cells transfected either with empty vector, human XRCC3 241Thr or 241Met together with LacZ were stained with X-gal and the surface area was analyzed. We found that cells with XRCC3 241Met variant were significantly larger than 241Thr.

We next examined the phenotypic change of the cells with XRCC3 241Met variant by introducing human XRCC3 into NIH3T3 cells. Spontaneous DNA double-strand breaks accumulated to a greater degree in NIH3T3 cells with XRCC3 241Met than in those

expressing 241Thr. DNA damage caused by radiation induced cell senescence more frequently in NIH3T3 cells with XRCC3 241Met. We evaluated the mRNA expression of inflammatory cytokines. Both basal and TNF-α stimulated mRNA expression and protein secretion of MCP1 was significantly increased in NIH3T3 cells with XRCC3 241Met compared with the cells with 241Thr. Furthermore, we evaluated the cell cycle distribution and DNA content of 3T3 cells with XRCC3 241Met variant by using a flow-cytometer. We found that the cell percentage of G2/M phase and polyploidy was significantly increased and that of G1 was decreased in 3T3 cells with 241Met variant compared with those with 241Thr. In addition, we found that there was a positive correlation between MCP1 expression and the cell percentage of G2 phase and polyploidy.

Summarizing these findings, XRCC3 241Met induction increased cell surface area in CHO cells, and increased spontaneous DNA damage, SA beta-gal activity, MCP1 mRNA expression and MCP1 protein secretion in NIH3T3 cells. The cell percentage of G2/M phase and polyploidy was increased in cells with 241Met variant. Lastly, MCP1 mRNA expression was positively correlated with the cell percentage of G2/M phase and polyploidy.

In conclusion, these data suggest that the XRCC3 241Met increases the risk of LVH via accumulation of DNA damage, thereby altering cell cycle progression and inducing cell senescence and a proinflammatory phenotype.

Keywords: left ventricular hypertrophy, XRCC3, polyploidy, DNA damage, senescence.