Anti-tumor effect of the cyclic peptides derived from flaxseed against giant cell tumor of the bone

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INTRODUCTION:

In recent years, the effectiveness of anti-receptor activator of NF-κB ligand (RANKL) therapy on giant cell tumor of the bone (GCTB) has been reported. Flax (botanical name: *Linum usitatissimum*) is a member of the family of *Linaceae* and raw material for linseed oil. Cyclolinopeptide (CL), derived from flax, suppresses the RANKL signal and osteoclast differentiation from myeloid progenitor cells. However, the anti-tumor effects of CL remain poorly understood. This study aimed to evaluate the anti-tumor effects of CL on GCTB *in vitro*.

METHODS:

Three cases of GCTB tissue were collected from a surgical specimen. The GCTB tissues were treated with collagenase and sub-cultured several times in D-MEM medium supplemented with 10% FBS. Three kinds of GCTB cell lines were established. Immunofluorescence of the GCTB cells were evaluated for RANKL expression and transmutation of histone H3F3A, a driver gene mutation in GCTB. GCTB cell growth inhibition by CL was evaluated using water-soluble tetrazolium salt (WST)-8 cell proliferation assay, cell-cycle assay and 5-ethynyl-2'-deoxyuridine (EdU) cell proliferation assay. The mRNA expression levels of RANKL and RUNX2 were evaluated using real-time polymerase chain reaction (PCR) before and after administration of CL.

RESULTS: During immunofluorescence, RANKL expression and transmutation of histone H3F3A were observed in all cell lines. In WST-8 cell proliferation assay, dose-dependent inhibition of GCTB cells was observed in the CL-administered group, compared to non CL-administered group ($P<0.05$, figure 1). During cell-cycle assay, the ratio of G0/G1 phase cells increased within the CL-administered group (figure 2). During EdU cell proliferation assay, the ratio of EdU positive cells decreased in the CL-administered group ($P<0.05$, figure 3). During real-time PCR testing, expressions of RANKL and RUNX2 mRNA were decreased within the CL-administered group ($P<0.05$, figure 4).

DISCUSSION AND CONCLUSION:

Our study suggests that CL has anti-tumor effects on GCTB *in vitro*.

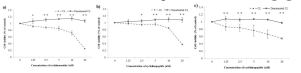

Figure 1 WST-8 assay
a) GCTB-1 b) GCTB-2 c) GCTB-3
Statistical analysis; Student t-test, * P<0.05, ** P<0.01

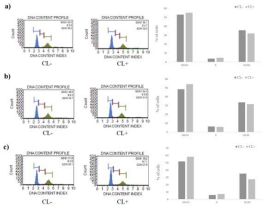

Figure 2 Cell-cycle assay
a) GCTB-1 b) GCTB-2 c) GCTB-3

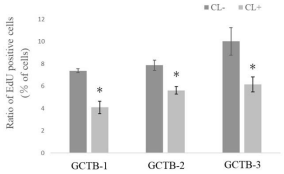

Figure 3 EdU assay
Statistical analysis; Mann–Whitney U test, * P<0.05

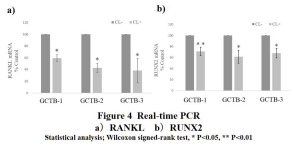

Figure 4 Real-time PCR
a) RANKL b) RUNX2
Statistical analysis; Wilcoxon signed-rank test, * P<0.05, ** P<0.01