学 位 論 文 の 要 旨

氏名　　片桐　浩

学 位 論 文 名　Loss of Autophagy-related Protein Beclin-1 may Define Poor Prognosis in Ovarian Clear Cell Carcinoma

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著 者 名　Hiroshi Katagiri, Kentaro Nakayama, Sultana Razia, Kohei Nakamura, Emi Sato, Tomoka Ishibashi, Masako Ishikawa, Kouji Iida, Noriyosi Ishikawa, Yoshiro Otsuki, Satoru Nakayama, Satoru Kyo

論 文 内 容 の 要 旨

INTRODUCTION

OVARIAN CLEAR CELL CARCINOMA (OCCC), which constitutes around 25% of ovarian carcinoma in Japan, is highly resistant to conventional platinum based chemotherapy.

Autophagy is a process of degradation and recycling of cytoplasmic components for energy utilization. Autophagy also occurs in response to certain forms of therapeutic stress, including cytotoxic chemotherapy. BECLIN-1 plays key roles in mammalian autophagy.

The aim of this study was to clarify the role of autophagy in cisplatin (CDDP) sensitivity in OCCCs and the role of BECLIN-1 in OCCC progression.

MATERIALS AND METHODS

Autophagy was measured using (1) Western blot analysis of LC3 and p62 and (2) microscopic observation of GFP-LC3 puncta by OCCC cell lines, ES-2 and TOV-21G. Autophagy was suppressed using chloroquine and BECLIN-1 siRNA.

Formalin-fixed, paraffin-embedded tissue samples of 60 ovarian clear cell carcinomas were used in this study. Surgical specimens were examined for

BECLIN- 1 protein expression by immunohistochemistry. The correlations between the loss of BECLIN-1 expression and clinicopathological characteristics, prognosis, and chemosensitivity were investigated.

This study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects.

RESULTS AND DISCUSSION

Inhibition of autophagy by chloroquine or BECLIN-1 siRNA did not enhance the sensitivity of the ES2 and TOV-21G OCCC cell lines to CDDP. These results suggest that CDDP induces canonical autophagy. However, induction of CDDP-induced autophagy does not have a protective role in OCCC cell lines subjected to CDDP cytotoxicity.

Loss of BECLIN-1 expression was observed in 38.3% (23/60) of the analyzed tumors. We examined the effect of loss of BECLIN-1 protein expression on the prognosis for progression-free survival. Kaplan-Meier estimates of progression-free/overall survival. Of the 60 patients diagnosed at stages I-IV, 23 patients with loss of BECLIN-1 expression had a shorter progression-free survival than those with positive BECLIN-1 expression (P = 0.0273; Log rank test). Univariate analysis demonstrated that FIGO stage III, IV (P < 0.01; Log rank test), CA125 levels (P = 0.01; Log rank test), residual tumor (≧ 2 cm) (P < 0.01; Log rank test), and loss of BECLIN-1 expression (P = 0.0273; Log rank test) correlated with shorter progression-free survival. When the data were stratified for multivariate analysis, only residual tumor (≧ 2 cm) remained a significant (P = 0.03) factor for shorter disease-free survival. Loss of BECLIN-1 expression also tended to correlate with shorter overall survival in OCCC patients treated with platinum–based chemotherapy (P = 0.1616; Log rank test). Univariate analysis demonstrated that FIGO stage III, IV (P < 0.01; Log rank test), CA125 levels (P = 0.01; Log rank test), and residual tumor (≧ 2 cm) (P < 0.01; Log rank test) significantly correlated with shorter overall survival. When these data were stratified for multivariate analysis, only residual tumor (≧ 2 cm) remained a significant (P = 0.04) predictor for shorter overall survival. There were no significant correlations between loss of BECLIN-1 expression and FIGO stage, CA125 levels, patient age, status of endometriosis, Ki-67 labeling index, chemotherapy regimen, or status of residual tumor. There was a marginally significant correlation between loss of BECLIN-1 expression and lymph node metastasis (P = 0.0579).

Statistical analysis showed no correlations between loss of BECLIN-1 expression and *Kras* and *PIK3CA* mutation status in OCCCs. In contrast, loss of BECLIN-1 expression

was significantly correlated with *ZNF 217* amplification (P = 0.024), and tended to correlate with loss of ARID1A expression in OCCCs (P = 0.057).

The findings of this study suggest that BECLIN-1 is a potential tumor suppressor in OCCCs. To assess the contribution of BECLIN-1 expression to OCCC cell growth and survival, OCCC cell lines were treated with BECLIN-1 siRNA and BECLIN-1 levels and cell growth were assessed. Following BECLIN-1 knockdown, cell growth increased in ES-2 and TOV-21G OCCC cell lines with positive BECLIN-1 expression. However, there was no profound inhibition or activation of cell migration and invasion observed in BECLIN-1 siRNA-treated OCCC cells

CONCLUSION

Autophagy defects caused by loss of BECLIN-1 are not related to chemoresistance and metastasis, but may be associated with malignant phenotype and poor prognosis of OCCC.