学 位 論 文 の 要 旨

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学 位 論 文 名　ARID1B as a Potential Therapeutic Target for ARID1A-Mutant Ovarian Clear Cell Carcinoma

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論 文 内 容 の 要 旨

INTRODUCTION

AT-rich interactive domain 1A (ARID1A) and AT-rich interactive domain 1B (ARID1B) are subunits of the SWI/SNF chromatin complex. ARID1A is a tumor suppressor gene that is frequently mutated (46%) in ovarian clear cell carcinomas (OCCC). Loss of ARID1B in an ARID1A-deficient background eliminates the intact SWI/SNF complex, indicating that ARID1B is essential for the formation or stabilization of an intact SWI/SNF complex and, thus, the survival of ARID1A-mutant cancer cell lines. In this study, we investigated the clinicopathologic and prognostic relevance of ARID1B in OCCC by immunohistochemical analysis of 53 OCCC patient samples and loss-of-function experiments in OCCC cell lines. We also examined whether ARID1B could be a therapeutic target or prognostic biomarker in OCCC.

MATERIALS AND METHODS

We conducted a SiRNA-mediated knockdown of ARID1B in ARID1A-mutant (OVISE, OVMANA, OVTOKO, OV207, and TOV-21G) and wild-type (ES2) cell lines and compared the proliferation in them. Then, we conducted a SiRNA-mediated knockdown of ARID1A and ARID1B in wild-type (ES2) cell line and assessed the proliferation.

We analyzed ARID1A and ARID1B expression in 53 OCCC samples. ARID1A and ARID1B immunoreactivity was detected in tumor cell nuclei. We examined the relationship between ARID1A and ARID1B protein expression in OCCC. First, we investigated the

relationship between ARID1B Expression and Clinicopathologic Factors. Second, we investigated whether ARID1B expression level is related to patient outcome by Kaplan-Meier analysis of progression-free survival and overall survival.

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

RESULTS AND DISCUSSION

ARID1A and ARID1B are typically co-expressed in cancer, but cancer driven by ARID1A mutation retains at least one functional ARID1B allele. Establishing the clinical relevance of ARID1A mutation and the role of ARID1B can lead to the development of more effective treatments for ovarian cancer. We addressed this in the present study using five OCCC cell lines (OVISE, OVMANA, OVTOKO, OV207, and TOV-21G) harboring ARID1A mutations. SiRNA-mediated knockdown of ARID1B suppressed cell growth in ARID1A-mutant but not the wild-type cell line. In the latter, proliferation was inhibited only by silencing both ARID1A and ARID1B. These results provide evidence for the functional redundancy of ARID1A and ARID1B.

We also examined the relationship between ARID1A and ARID1B protein expression in 53 OCCC patient specimens by immunohistochemistry. ARID1A nuclear expression was observed in 13.4% of samples whereas ARID1B was expressed in all samples at high (85%) or low (15%) levels. Samples lacking ARID1A expression in the nucleus were more likely to exhibit low ARID1B immunoreactivity, which was also associated with shorter progression-free survival. On the other hand, overall survival was unaffected by ARID1B expression level. The multivariate analysis showed that only CA125 level ($p = 0.0446$) and low ARID1B expression were significantly associated with progression-free survival.

Loss of ARID1A might be correlated with lower expression of ARID1B, which may create a specific vulnerability in ARID1B compared to cells without ARID1A mutations. These results suggest that low ARID1B expression can potentially serve as a prognostic biomarker in patients with recurrent OCCC. However, additional studies with a larger study population are required to establish the percentage of OCCC cases with low ARID1B expression and to determine the association between loss of ARID1A immunoreactivity and downregulation of ARID1B. Further investigation is necessary to determine the molecular mechanism underlying ARID1A mutation and ARID1B downregulation. In addition, real time PCR and in vitro chemosensitivity testing are required for evaluation of the expression of ARID1A/ARID1B.

CONCLUSION

Our data demonstrate that ARID1B plays an important role in the growth of ARID1A-mutant OCCC. Our most important finding is that low ARID1B expression was

associated with shorter progression-free survival. This is the first report demonstrating that low levels of ARID1B protein can serve as a marker of poor outcome in OCCC patients. We also found a significant correlation between the loss of ARID1A immunoreactivity and reduced ARID1B levels, suggesting that ARID1B could be a target for anti-cancer therapy.