学 位 論 文 の 要 旨

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学 位 論 文 名　Expression of the Bric-a-Brac Tramtrack Broad Complex Protein NAC-1 in Cervical Carcinomas Seems to Correlate With Poorer Prognosis

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論 文 内 容 の 要 旨

INTRODUCTION

Uterine cervical cancer is the second most common malignancy among women worldwide. Although early diagnosis has decreased the death rate, cervical cancer is still a leading cause of cancer deaths in Japanese women. This is partly because some patients continue to be diagnosed with advanced-stage disease, for which conventional therapy is less effective. As a result, many cervical cancer patients in Japan develop and eventually succumb to recurrent disease. New, effective therapeutic agents are urgently needed to improve outcome in these patients.

The bric-a-brac tramtrack broad complex [BTB, also known as POZ] gene family is composed of several proteins that share a conserved BTB/POZ protein-protein interaction motif at the N-terminal that mediates homodimer or heterodimer formation. These proteins have been demonstrated to participate in a wide variety of cellular functions including regulation of transcription, cellular proliferation, apoptosis, cell morphology, ion channel assembly and protein degradation through ubiquitination. A subset of BTB/POZ proteins have been implicated in human cancer. Based on analyzing gene expression levels in all 130 deduced human BTB/POZ genes using serial analysis of gene expression [SAGE] data, we recently identified NAC-1 as a carcinoma-associated BTB/POZ gene. NAC-1 is a transcription repressor that is involved in self renewal and maintenance of pluripotency in embryonic stem cells. In human cancer, our previous study revealed that NAC-1 is significantly overexpressed in several types of carcinomas.

To extend these observations to cervical carcinomas, we measured NAC-1 expression levels in

cervical carcinoma tissue samples and cell lines and sought to determine the role of NAC-1 in cervical cancer.

MATERIALS AND METHODS

Tissue samples and immunohistochemistry

A total of 108 paraffin-embedded tumor tissue and normal cervical tissue samples were obtained consisting of 84 cervical squamous cell carcinomas, 7 adenocarcinomas, 5 adenosquamous cell carcinomas, 3 cervical intraepithelial neoplasm samples and 9 normal cervical tissue samples were used.

Quantitative PCR analysis

A total of 16 frozen cervical cancer samples and 6 samples of normal cervix were analyzed for NAC-1 transcript expression by quantitative real-time PCR.

Cell culture, apoptosis counting and Western blot analysis

Seven human cervical adenocarcinoma cell lines were used. To assess whether NAC-1 overexpression affected growth activity, a mammalian expression vector, pCMV/NAC-1 with V5 tag at the COOH terminus was constructed and was stably transfected into TCS, CaSki and Hela P3 cells using the Nucleofector II electroporator.

The apoptotic index was expressed as the percentage of apoptotic figures obtained by counting at least 300 4´-6´-diamidino-2-phenylindole (DAPI)-stained cells under fluorescence microscope.

siRNA knockdown of NAC-1 gene expression

Two small interfering RNA sequences [siRNAs] targeting NAC-1 were designed. Control siRNA was purchased from IDT. Cell number was determined indirectly by MTT assay and Annexin V , 72 hours after transfection with siRNA.

Cell proliferation assay

The cell number was determined indirectly by MTT assay.

Statistical methods for clinical correlation

Overall survival was calculated from the date of diagnosis to the date of death or last follow-up. Patients whose tumors showed NAC-1 expression and those whose tumors were without expression had similar age and performance status distributions. Data were plotted as Kaplan-Meier curves, and statistical significance was determined by the Log-rank test. Multivariate prognostic analysis was performed using a Cox proportional hazards model. Data were censored when patients were lost to follow-up. Student's t test was used to examine statistical significance in differences of growth assay data.

RESULTS AND DISCUSSION

NAC-1 expression is higher in adenocarcinomas/adenosquamous cell carcinomas than in squamous cell carcinomas

In contrast to normal cervical tissue and cervical intraepithelial neoplasms [CINs], both squamous cell carcinomas (SCC) and adenocarcinomas/adenosquamous cell carcinomas (AC) demonstrated higher NAC-1 immunoreactivity, with 10% and 50% of cases showing 1+ and 2+ positivity, respectively (chi-square test, $P<0.001$) (Fig. 1; Table1). Positive NAC-1 staining intensity (1+ and 2+) was more frequently found in cells of adenocarcinomas/adenosquamous cell carcinomas than in squamous cell carcinomas ($P< 0.01$, chi-square test) (Table 2). Increased NAC-1 gene expression level was significantly correlated with adenocarcinomas/adenosquamous cell carcinoma types from real time PCR analysis.

Expression of NAC-1 correlates with poor prognosis in patients with SCC who received radiation therapy

Among 69 patients, the 9 patients with NAC-1 expression had a shorter overall survival than the peers whose tumors did not express NAC-1 ($P=0.001$; Log rank test) (Fig. 3). Univariate analysis demonstrated that stage III or IV disease and positive NAC-1 expression correlated with shorter overall survival. When data were stratified for multivariate analysis, stage III or IV disease and positive NAC-1 expression remained significant ($P=0.022$ and $P=0.005$, respectively) for shorter overall survival

Functional analysis of NAC-1 expression

When compared with vector-transfected controls, all TCS, CaSki and Hela P3 clones with NAC-1 expression had higher cellular proliferation based on growth assay results. NAC-1. SiRNA treatment significantly reduced NAC-1 protein expression compared with control siRNA treatment which was likely a result of induced apoptosis. Therefore, NAC-1 is considered to be essential for cell growth and survival in cervical cancer cell lines that overexpress NAC-1.

CONCLUSION

In conclusion, we demonstrated that NAC-1, a member of the BTB/POZ family, is expressed in a subset of cervical carcinomas. Of special interest is the finding that expression is more common in adenocarcinomas/adenosquamous carcinomas that are more refractory to conventional radiotherapy. Furthermore, NAC-1 expression is a prognostic factor for patients whose squamous cell carcinomas were treated with radiotherapy. Therefore, NAC-1 expression may be considered as a predictive marker of resistance to radiotherapy in patients with squamous cell carcinomas. We further predict that a drug targeting NAC-1 might be useful in combination with radiotherapy for improvement of prognosis of patients with cervical carcinomas.