学 位 論 文 の 要 旨

氏名　　西　　健

学　位　論　文　名　Low Expression of Nucleus Accumbens-Associated Protein 1 Predicts Poor Prognosis for Patients With Pancreatic Ductal Adenocarcinoma

発　表　雑　誌　名　Pathology International
（巻: 初頁～終頁等, 年）　(**68**: 802-810, 2012)

著　　者　　名　Takeshi Nishi, Riruke Maruyama, Takeshi Urano, Naomi Nakayama, Yasunari Kawabata, Seiji Yano, Manabu Yoshida, Kentaro Nakayama, Kohji Miyazaki, Keizo Takenaga, Tsuneo Tanaka, Yoshitsugu Tajima

論 文 内 容 の 要 旨

INTRODUCTION

Pancreatic carcinoma is a highly lethal cancer. It is important to elucidate the molecular etiology of pancreatic carcinoma and to provide new therapeutic targets for treating pancreatic carcinomas.

Nucleus accumbens-associated protein 1 (NAC1) is a nuclear protein that belongs to the broad complex, tramtrack, bric-a-brac /poxvirus and zinc finger (BTB/POZ) domain family. This family of proteins participates in several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance. NAC1 was identified as a cancer-associated BTB/POZ gene by serial analysis of gene expression in ovarian cancer. High expression of NAC1 is considered to have adverse effects on prognosis through negative regulation of growth arrest and DNA-damage–inducible 45-γ interacting protein 1 (GADD45GIP1) in ovarian and cervical carcinomas. Up-regulation of NAC1 in human cancers contributes to tumor growth and survival, and to resistance of tumor cells to chemotherapy. Moreover, NAC1 is significantly overexpressed in several types of carcinomas including ovarian carcinoma, colorectal carcinoma, breast carcinoma, renal cell carcinoma, cervical carcinoma, and pancreatic carcinoma.

In the present study, the expression of NAC1 in pancreatic ductal adenocarcinoma (PDA) was measured using immunohistochemistry and computer-assisted image analysis in order to

investigate its correlation with various clinicopathological parameters and prognosis.

MATERIALS AND METHODS

The present study population included 65 patients with PDA who underwent surgical resection between January 2000 and December 2010 at Shimane University Hospital. None of the patients had received preoperative chemotherapy or radiation therapy. The median follow-up duration after resection was 12.8 months (range: 0.3–72 months). PDAs were classified according to the staging system of the Japanese Classification of Pancreatic Cancer. All tumors were invasive PDAs and were divided according to UICC staging system. Immunohistochemistry was performed using the automatic system. Immunohistochemically stained slides with PDA tissue were scanned and converted to whole slide image, and they were analyzed using computer-assisted image analysis. Cases were categorized into two groups based on NAC1 positive rate (PR): a low PR (<30%) group and a high PR (≧30%) group. Small interfering RNA knockdown of NAC1 gene expression and Western blot analysis were performed using the human pancreatic carcinoma cell lines AsPC-1, MIA PaCa-2, PANC-1 and the cervical carcinoma cell line HeLa.

RESULTS AND DISCUSSION

In NAC1 high PR group, PDA cells exhibited same or higher NAC1 immunoreactivity, but in NAC1 low PR group, they showed lower immunoreactivity than normal pancreatic duct cells. Significant difference of staining intensity of NAC1 was observed neither between invasion front and center of the tumors, nor between intraductal component and invasive part of the tumors. Among PDA patients, twenty (30.8%) of 65 cases were classified into NAC1 low PR group, and 45 (69.2%) into NAC1 high PR group. Expression of NAC1 did not have any correlation with histological grading, but correlated with clinical outcome in patients with PDA. NAC1 low PR group had more frequent venous invasion and lymph node metastasis than NAC1 high PR group ($P = 0.047$ and $P = 0.0017$, respectively). In addition, when tumors were classified according to UICC staging system, the patients with low TNM-staging had relatively higher NAC1 expression than the patients with high TNM-staging ($P = 0.02$). In univariate analysis, NAC1 low PR group had a shorter disease-free survival and worse overall survival than NAC1 high PR group ($P = 0.0036$ and $P = 0.0010$, respectively). In multivariate analysis, however, NAC1 low expression wasn’t identified as an independent risk factor for disease-free survival and overall survival.

It was previously reported that GADD45GIP1 expression was negatively regulated by NAC1 in several ovarian cancer cell lines and cervical cancer cell line. The present report examined whether or not NAC1 regulates GADD45GIP1 expression in pancreatic carcinoma cell lines. siRNA knockdown of NAC1 induced GADD45GIP1 expression in HeLa cells, but not in pancreatic carcinoma cell lines.

In contrast to observations in ovarian cancer, a positive correlation was not observed between tumor aggressiveness and NAC1 expression in PDA. Moreover, the present results showed that a low NAC1 expression level was related to worse oncologic features such as incidence of lymphatic metastasis, venous invasion, and high TNM grading in PDA. The result of the univariate analysis supported this phenomenon as well. While increased NAC1 expression in either primary tumor or effusion was significantly correlated with shorter progression free survival in ovarian cancer, low NAC1 expression in primary tumor was correlated with shorter progression free survival and overall survival in PDA. This result was totally opposite from what was observed in ovarian cancer.

Some genes sometimes behave in a different manner in different tissues. Gene regulation of NAC1 may vary in carcinomas or the tissues from which they originate. NAC1 expression may also affect clinical outcome, depending on the carcinoma type.

The staining level of NAC1 expression in NAC1 high PR group is almost similar or higher than the normal pancreatic duct, on the other hand, it is lower in NAC1 low PR group. Another possibility was then considered; NAC1 expression is induced and takes important roles in the early steps of pancreatic carcinogenesis. Then, when tumor cells acquire the ability to invade other tissues and become progressive, NAC1 might become less important and its expression might be suppressed. At the present moment, the role of NAC1 in pancreatic carcinogenesis has not been very well elucidated. However, the present results provide a potentially useful method of prognosis: low NAC1 expression may be a predictive factor for poor prognosis in PDA patients.

The present results from the NAC1 knockdown in pancreatic carcinoma cell lines may provide a clue to the solution. NAC1 negatively regulates GADD45GIP1 expression to contribute to growth, survival and drug resistance in tumor cells that overexpressed NAC1 in ovarian cancer and cervical carcinoma. In the present report, endogenous NAC1 was knocked down in several carcinoma cell lines by siRNA and examined if expression of GADD45GIP1 was induced. As expected, expression of GADD45GIP1 was induced by NAC1 knockdown in the HeLa cervical cancer cell line. In contrast, NAC1 knockdown did not affect the expression level of GADD45GIP1 in any pancreatic carcinoma cell lines. At the present moment, it is still unknown what molecules can be regulated by NAC1 in PDA. However, it was shown clearly that the molecular mechanism involving NAC1 and GADD45GIP1 which provides a growth advantage in ovarian cancer does not apply to PDA.

CONCLUSION

Low NAC1 expression can be a predictive marker of prognosis of PDA patients. This finding may help physicians choose appropriate treatment options and give patients proper information about their prognosis.