学 位 論 文 の 要 旨

氏名　石川 雅子

学 位 論 文 名　A *BTB/POZ* Gene, *NAC-1*, a Tumor Recurrence-Associated Gene, as a Potential Target for Taxol Resistance in Ovarian Cancer

発 表 雑 誌 名　Clinical Cancer Research
（巻，初頁～終頁，年）　14(10):3149-3155, 2008

著 者 名　Masako Ishibashi, Kentaro Nakayama, Shamima Yeasmin, Atsuko Katagiri, Kouji Iida, Naomi Nakayama, Manabu Fukumoto and Kohji Miyazaki

論 文 内 容 の 要 旨

Introduction

Ovarian cancer is the most lethal gynecological malignancy in the world. In more than 70% of cases, the tumor has disseminated beyond the ovaries at the time of diagnosis. Patients who relapse, and those who do not initially respond to chemotherapy, are thought to carry hidden drug-resistant cells, which are the cause of tumor relapse and lethality. A number of mechanisms have been proposed that might be targets of novel chemotherapeutic agents. Therefore, pharmacologic research could be directed at enhancing this process, either by directly activating apoptosis or by lowering the threshold for its initiation by cytotoxic drugs.

The genes of the *BTB/POZ* family participate in several cellular functions including proliferation, apoptosis, transcription control, and cell morphology maintenance. BTB/POZ proteins including *BCL-6*, *PLZF* (promyelocytic leukemia zinc finger), *leukemia/lymphoma related factor (LRF)/Pokemon*, *HIC-1 (hypermethylated in cancer-1)* and *Kaiso* are involved in cancer development. By serial analysis of gene expression (SAGE) levels in all 130 deduced human *BTB/POZ* genes, we identified *NAC-1* as a carcinoma-associated *BTB/POZ* gene. NAC-1 is a transcription repressor and is involved in self renewal and maintenance of pluripotency in embryonic stem cells. NAC-1 is significantly overexpressed in several types of human carcinomas including ovarian serous carcinoma, the most common and malignant type of ovarian cancer. The levels of NAC-1 expression correlate with tumor recurrence in ovarian serous carcinomas; and, intense NAC-1 immunoreactivity in primary ovarian tumors predicts early recurrence. Taken together, our previous studies suggest that NAC-1 is a tumor recurrence-associated gene with oncogenic potential. The molecular mechanisms underlying these observations are unknown and how NAC-1 expression contributes to chemotherapy resistance in ovarian cancer is yet to be studied.

The current study examined the role of NAC-1 in ovarian cancer recurrence, investigated the relationship between NAC-1 expression and tumor recurrence and finally assessed whether NAC-1 is a useful prognostic factor in patients with ovarian cancer.

Purpose

The current study examined the role of NAC-1 in ovarian cancer recurrence, investigated the relationship between NAC-1 expression and tumor recurrence.
We finally assessed whether NAC-1 is a useful prognostic factor in patients with ovarian cancer.

Materials and Methods

Tissue samples and immunohistochemistry

The paraffin-embedded tumor tissues were obtained from the Department of Obstetrics and Gynecology at the Shimane University Hospital. These included 43 advanced stage (stage III, IV) ovarian carcinomas. All patients were primarily treated with cytoreductive surgery and adjuvant conventional platinum and taxane chemotherapy at least 6 cycles. Acquisition of tissue specimens and clinical information was approved by an institutional review board (Shimane University). Written informed consent was obtained from each patient.
That were organized into tissue microarrays(TMA), and we examined overexpression of NAC-1 in the immunohistchemistry studies.

Quantitative PCR analysis

We performed quantitative real-time PCR to assess the correlation between NAC-1 gene expression level and NAC-1 immunointensity.

Cell culture and Western blot analysis

Western blot analysis was done on 4 ovarian carcinoma cell lines KF28, KF28TX, KFr13, and KFr13TX which included paclitaxel - and cisplatin-resistant.

siRNA knockdown of *NAC-1* gene expression

NAC-1 siRNA was transfected into KF28TX and KFr13TX cells, which included paclitaxel resistant. We examined whether *NAC-1* gene knockdown in both cell lines rescued paclitaxel and CDDP sensitivity.

Result

Overexpression NAC-1 in advanced stage ovarian carcinoma

Overexpression of NAC-1 correlated with shorter relapse free survival in patients with advanced stage (stage III, IV) ovarian carcinoma treated with platinum and taxane chemotherapy. ($P<0.001$、 $P=0.026$)

Relationship between clinicopathological findings and NAC-1 expression

Both residual tumor (≧1cm) and overexpression of NAC-1 were correlated with shorter relapse free survival, and overexpression of NAC-1 were correlated with shorter relapse free survival than overall survival.

Correlation of NAC-1 protein expression and NAC-1 gene expression

NAC-1 gene expression levels were significantly correlated with higher immunointensity in ovarian carcinomas.

NAC-1 expression is higher in paclitaxel resistant KF28TX and KFr13TX cells than in parental KF28 and cisplatin resistant KFr13 cells

NAC-1 expression levels were measured and compared among the human ovarian cancer cell line (KF28), cisplatin-resistant cell line (KFr13) induced from KF28, and paclitaxel-resistant cell lines (KF28TX and KFr13TX) induced by exposing KF28 and KFr13 to dose-escalating paclitaxel. Overexpression of NAC-1 was observed in only the taxol-resistant KF28TX and KFr13 TX cells, but not in KF28 or cisplatin-resistant KFr13 cells.

Suppression of NAC-1 in paclitaxel resistant cell lines KF28TX and KFr13TX and drug resistance to paclitaxel

KF28TX and KFr13TX cells were then transfected with NAC-1 siRNA or luciferase siRNA .We found that there were significantly fewer KF28TX and KFr13TX cells following transfection with NAC-1 siRNA than after transfection with luciferase siRNA ($p<0.05$).

Functional analysis of NAC-1 expression

The normal epithelial cell line RK3E were transfected with a vector expressing NAC-1 and three independent clones were randomly selected for functional analysis. All of the NAC-1 expressing clones were resistant to paclitaxel compared to vector transfected cells. There was no difference in sensitivity to carboplatin between vector transfected cells and the NAC-1 expressing clones.

Conclusion

We demonstrated that NAC-1, a transcriptional repressor, was expressed in a subset of advanced stage ovarian cancers, and was associated with tumor recurrence. Of special interest is the finding that overexpression NAC-1 correlates with a shorter relapse free survival in advanced stage ovarian cancer. NAC-1 may be a useful marker for predicting recurrence within 6 months of cytoreductive surgery and first line platinum and taxane based chemotherapy. Using complementary gene knockdown and gene overexpressing systems, we also demonstrated that overexpression of NAC-1 was essential for paclitaxel resistance. Its role in paclitaxel resistance makes NAC-1 an attractive target for designing chemotherapeutic agents for patients resistant to conventional taxane based regimens.