学 位 論 文 の 要 旨

氏名　　吉野　干城

学　位　論　文　名　　BCL-2 EXPRESSION AS A PREDICTIVE MARKER OF HORMONE-REFRACTORY PROSTATE CANCER TREATED WITH TAXANE-BASED CHEMOTHERAPY

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著　　者　　名　　TATEKI YOSHINO, HIROAKI SHIINA, SHINJI URAKAMI, NOBUYUKI KIKUNO, TATSUAKI YONEDA, KAZUSHI SHIGENO, MIKIO IGAWA

論 文 内 容 の 要 旨

INTRODUCTION

The Bcl-2 family plays a central role in the regulation of apoptosis, which is induced by a wide variety of stimuli. Members of this protein family can be divided into death antagonists, such as Bcl-2, and death agonists, such as Bak and Bax. The prevalence of Bcl-2 overexpression is higher in hormone-refractory prostate cancer (HRPC) compared with localized prostate cancer. Overexpression of Bcl-2 seems to enable the prostate cancer cells to survive in an androgen-deprived environment, and to confer resistance to antiandrogen therapy. The functional role of Bak and Bax is to inhibit the protection from apoptosis that is provided by Bcl-2.

The vast majority of metastatic prostate cancer patients will eventually develop hormone-refractory disease. Until these days, no definite treatment modalities have been available for HRPC. However, on the basis of recent phase Ⅲ randomized clinical trials, taxane-based chemotherapy is now considered standard first-line therapy for HRPC in the United States.

In vitro study, treatment of PC-3 cells with taxanes has been shown to induce Bcl-2 phosphorylation and apoptosis. On the other hand, treatment of Bcl-2-negative DU-145 prostate cancer cells with taxanes did not induce apoptosis. Considering that cancer cells that overexpress Bcl-2 are sensitive to taxane-induced apoptosis, therapeutic response and survival benefit of HRPC patients treated with taxane-based chemotherapy may depend on the

presence of Bcl-2 protein.

We hypothesized that (a) survival benefit in HRPC patients treated with taxanes is determined by the presence of Bcl-2 protein and (b) altered expression of Bak and Bax protein caused by genetic mutation is associated with biological aggressiveness of prostate cancer.

MATERIALS AND METHODS

Forty localized prostate cancer and thirty HRPC cases were enrolled in this study. Surgical specimens of localized prostate cancer and biopsy specimens of HRPC before treatment with taxane-based chemotherapy were used for immunostaining of Bcl-2, Bak and Bax as well as DNA extraction. In immunostaining method, the positive rate was expressed as the mean percentage of positively stained tumor cells against the total number of tumor cells counted. A specimen was regarded as positive when >10% of the tumor cells showed immunoreactivity. In addition, prostatic biopsy specimens of another consecutive 19 HRPC cases who were not exposed to taxanes-based chemotherapy as proper controls for HRPC treated with taxanes-based chemotherapy were also examined for Bcl-2, Bak and Bax expression at the time of HRPC diagnosis. Mutations in the Bak and Bax genes were screened by single-strand conformational polymorphism, and confirmed by direct DNA sequencing. All numerical data are expressed as mean $\pm$ standard deviation. Relationships between expression of Bcl-2, Bak or Bax protein and clinicopathological findings in localized PC were analyzed using the Kruskal-Wallis test. The difference in immunostaining between localized PC samples and HRPC samples was analyzed by the Mann-Whitney test. The relationship between immunostaining and therapeutic response to chemotherapy in HRPC was analyzed using the Mann-Whitney test. Multivariate survival analysis was carried out using a logistic regression model. A p-value of less than 0.05 was regarded as statistically significant.

RESULTS AND DISCUSSION

Regarding proapoptotic molecules, reduced expression of Bak and Bax predicts a poor clinical outcome for several malignant tumors. Our results showed that although Bak and Bax expression was not related to Gleason score or pT category, expression of both was significantly higher in localized prostate cancer than in HRPC. Additionally, decreased Bax expression was associated with an increased preoperative PSA level in localized prostate cancer and early disease progression in HRPC. These findings suggest that up-regulation of antiapoptotic Bcl-2 protein or down-regulation of proapoptotic proteins may interact with the processes involved in the development of HRPC as well as disease progression in prostate cancer.

In vitro, phosphorylation of Bcl-2 and subsequent activation of apoptotic processes has been

induced only in Bcl-2-expressing HRPC cells after treatment with taxanes, indicating that acceleration of apoptosis by taxanes can be recruited by the presence of Bcl-2 protein. Based on these findings, we hypothesized that therapeutic response to taxane-based chemotherapy in HRPC might be predicted by the level of Bcl-2 expression before treatment with taxane-based chemotherapy, and Bcl-2-positive HRPC cases might have a better therapeutic response to taxane-based chemotherapy than Bcl-2-negative cases. In this study, HRPC cases showing partial response to chemotherapy in lymph node metastasis and overall response had a significantly increased level of Bcl-2 expression compared with HRPC showing stable disease. Moreover, a longer cause-specific survival time was found in Bcl-2-positive HRPC cases than in Bcl-2-negative cases and multivariate analysis revealed that Bcl-2 expression was an independent predictor for cause-specific survival. Meanwhile, neither PSA levels at baseline nor PSA levels at nadir were related to therapeutic response to chemotherapy. Thus, Bcl-2 expression might be a reliable surrogate marker over the serum PSA level when predicting survival benefit in HRPC patients undergoing taxane-based chemotherapy. In other words, analysis of Bcl-2 expression before treatment in addition to PSA measurement could discriminate between HRPC patients who might benefit from taxane-based chemotherapy and those who might not. Furthermore, we have also evaluated an additional 19 HRPC cases who were not exposed to taxane-based chemotherapy as a control group. In this group, Bcl-2 expression was not an independent predictor for cause-specific survival. Consistent with our results, up-regulation of Bcl-2 has been reported as frequent events in HRPC. However, Bcl-2 mutation has been considered an extremely rare event in human malignancies. Taking into consideration, we focused on the mutational analysis of the Bak and Bax genes, and hypothesized that mutant forms of Bak or Bax protein would be unable to form a heterodimer complex with Bcl-2 and so could not inhibit the antiapoptotic function of Bcl-2. In the present study, neither Bak nor Bax gene mutation was found in localized prostate cancer, and only one missense mutation of the Bax gene was found in HRPC. Interestingly, because only DNA from HRPC tissue, but not that from either normal prostate or prostate cancer tissue before the development of HRPC, showed the Bax gene mutation, this genetic alteration may be a late event and might have been involved in the development of HRPC.

CONCLUSION

Our results support the hypothesis that the presence of Bcl-2 protein could indicate a survival benefit in HRPC patients scheduled for taxane-based chemotherapy. Further investigation based on this pilot study will hopefully lead to better clarification of the possible application of Bcl-2 expression as a predictive marker of HRPC treated with taxane-based chemotherapy.