学 位 論 文 の 要 旨

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学 位 論 文 名　Tumor Suppressor Function of PGP9.5 Is Associated with Epigenetic Regulation in Prostate Cancer - Novel Predictor of Biochemical Recurrence After Radical Surgery

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論 文 内 容 の 要 旨

INTRODUCTION

Protein Gene Product 9.5 (PGP9.5) is presumed to be a common antigen in both neuroendocrine (NE) and secretory cells in the prostate. In human solid tumors, the expression level of *PGP9.5* is frequently down-regulated because of promoter CpG hypermethylation, and in fact, *PGP9.5* has been considered as a tumor suppressor gene. Unfortunately, only a limited amount of information is available on *PGP9.5*, in regards to the human prostate glands, making it difficult to evaluate its functional role as a tumor-suppressant and its active involvement in NE differentiation.

The aim of the present study was to assess the correlation between expression of the *PGP9.5* gene and epigenetic mechanisms. In addition, we evaluated the associations between the expression of *PGP9.5* or CpG methylation of the PGP9.5 status and clinical parameters or biochemical recurrence (BCR) in cases of localized prostate cancer (PC) following a radical prostatectomy (RP).

MATERIALS AND METHODS

To screen for epigenetic alterations in the *PGP9.5* gene, prostate cancer cell lines of LNCaP (androgen-dependent) and PC-3 (androgen-independent) were treated with a de-methylating agent of 5-aza-2′-deoxycytidine (5-aza-dC) in duplicate. The difference in

expression level of the *PGP9.5* mRNA transcripts, before and after 5-aza-dC treatment, was analyzed by a quantitative RT-PCR using cDNA from these cell lines. To determine the functional role of the *PGP9.5* gene, methyl thiazolyl tetrazorium (MTT) and apoptosis assay were performed using LNCaP or PC-3 cells transfected with the *PGP9.5* gene's small interfering RNA (siRNA).

The promoter CpG hypermethylation and mRNA transcript level of *PGP9.5* gene was analyzed in prostate cancer cell lines—226 localized PC samples from radical prostatectomy cases, and 80 pathologically proven benign prostate hyperplasia (BPH) tissues. From these samples, genomic DNA, bisulfite-modified DNA, and cDNA were prepared. Both methylation specific and un-methylation specific primers were designed by a MethPrimer (http://www.urogene.org/ methprimer/), and PCR was done using bisulfite-modified DNA as a template. For bisulfite DNA sequencing, a pair of universal primers, in which no CpG islands were present, was used to confirm the methylation status of each CpG island. The mRNA transcript level of the *PGP9.5* gene was measured by a quantitative RT-PCR using G3PDH as a reference gene.

Parametric data were statistically analyzed through an ANOVA test followed by a *post hoc* test. Non-parametric data were analyzed by a X-square test. The probability factor of BCR-free probability was estimated using the Kaplan-Meier method, and survival curves among groups were compared statistically using the log-rank test. An age-adjusted logistic regression model was applied to determine the independent factors contributing to the BCR. A P-value of less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In this present study, we hypothesized that expression of *PGP9.5* in PC tissue is regulated by epigenetic alteration of CpG methylation and that the harboring of *PGP9.5* in PC functions as a tumor-suppressant. Irrespective of the androgen-dependent potential of PC cell lines used, a 5-aza-dC treatment significantly increased the mRNA transcript of *PGP9.5* when compared to the non-treatment of 5-aza-dC, suggesting the probability of CpG hypermethylation as a regulator of the *PGP9.5* gene expression in human PC cells. Bisulfite DNA sequencing of the *PGP9.5* promoter showed that complete methylation was found in PC3 cells, and partial, but not complete, methylation was dominant in LNCaP cells. This probably explains why the detectable level of *PGP9.5* mRNA transcript was found even before the 5-aza-dC treatment in LNCaP cells.

We found that the siRNA transfection of *PGP9.5* in LNCaP cells, in which partial methylation was found in the CpG promoter and wherein weak levels of mRNA transcript of *PGP9.5* was detected, significantly enhanced cell viability through the acceleration of the

inhibitory effect on cultured cells in spite of a significant lower amount of *PGP9.5* mRNA transcript in LNCaP. Furthermore, we found a significant difference in apoptotic cells between the *PGP9.5* siRNA transfection and the control siRNA transfection in LNCaP cells, which suggests the enhancement of the apoptosis after the *PGP9.5* siRNA transfection. On the contrary, in PC3 cells in which the *PGP9.5* mRNA transcript was not absolutely detectable due to complete methylation of the CpG promoter, the inhibitory effect of siRNA transfection of *PGP9.5* on cell viability of PC3 cells was not found. These findings strongly indicate the potential of *PGP9.5* as a tumor-suppressant in human PC tissues.

Subsequently, we investigated the prognostic relevance of *PGP9.5* methylation or *PGP9.5* expression, focusing on the probability of early detection of BCR after radical surgery. Univariate analysis showed that the lower BCR-free probability was significantly associated with higher *PGP9.5* expression or negative *PGP9.5* methylation. Age-adjusted multivariate analysis clearly demonstrated the prognostic relevance of *PGP9.5* methylation to predict an early BCR after surgery following the pT category. We also identified *PGP9.5* methylation as an independent factor superior to the Gleason score. In PC tissues, perineural invasion is supposed to be a substantial gateway for PC cells to spread out from the prostate. The perineural invasion of PC cells was found to be relevant to BCR after surgery showing significant correlation with *PGP9.5* methylation.

In general, a high Gleason score, high pT category, increased tumor volume, and positive surgical margins are all major concerns for BCR and might constitute independent factors for predicting BCR following RP; however, they do not always exactly predict BCR due to the biological variability of PC. In this study, we showed that further stratification with the pT category, in addition to methylation status, identified stepwise reduction of the BCR-free probability, in which the highest BCR-free probability was found in the PCs with both methylation-negative and a pT category of less than 2; and the lowest was in those PCs with methylation-positive and a pT category of more than 3a. Based on our data, we derived that CpG methylation of the *PGP9.5* status, in combination with a pathological T stage, and a more accurately predicted BCR, may help identify and select patients who should be submitted to additional treatment following RP.

CONCLUSION

The epigenetics of promoter CpG hypermethylation of *PGP9.5* may be one of the mechanisms associated with the down-regulation and functional loss of PGP9.5 in human PC. We have concluded that CpG methylation of the *PGP9.5* status, in combination with a pathological T stage, can more accurately predict BCR, and may help to identify and narrow-down the selection of patients in need of additional treatment following RP.