Methylation of the CD44 Metastasis Suppressor Gene in Human Prostate Cancer


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Abstract

Previous studies demonstrated that CD44 is a metastasis suppressor gene for prostate cancer and that the expression of CD44 both at mRNA and protein levels is down-regulated during prostate cancer progression, with down-regulation being correlated with higher tumor grade, aneuploidy, and distant metastasis. In this study, we evaluated DNA hypermethylation as a potential mechanism accompanying this decreased CD44 expression in human prostate cancer. Nucleotide sequence analysis revealed a CpG island in the CD44 transcriptional regulatory region. We found that cytosine methylation of CD44 promoter occurs in CD44-negative prostate cancer cell line (i.e., LNCap) but not in prostate cancer cell lines (i.e., TSU, PC3, and DU145) expressing this gene. In addition, we examined methylation status of CD44 in 84 matched normal and cancer prostate specimens. Hypermethylation of the 5′ CpG island of CD44 gene was observed in 31 of 40 primary prostate cancer specimens, 3 of 4 distant organ site metastases obtained at autopsy from men died of prostate cancer, and 4 of the 40 matched normal tissues. These results demonstrated that methylation of the 5′ CpG island of CD44 gene is closely associated with transcriptional inactivation, resulting in a decreased expression of CD44 in human prostate cancer.

Introduction

CD44, located on human chromosome 11 at p13 (1), is a transmembrane glycoprotein encoded by 20 exons over a length of ~60 kb, at least 10 of which are variably expressed due to alternative splicing of the nRNA (2). In addition to alternative splicing, variations in N-glycosylation, O-glycosylation, and glycosaminoglycanation can occur resulting in CD44 protein ranging from M, 85,000 to 230,000 (3). CD44 is an integral membrane phosphoprotein involved in cell-cell and cell-matrix interactions by serving as a receptor for the extracellular matrix component hyaluronic acid (4, 5) and osteopontin (6).

In previous studies, we demonstrated that down-regulation of the CD44 expression at the mRNA and protein level is associated with acquisition of high metastatic ability within the Dunning R-3327 system of rat prostate cancers (7). In addition, transfection-induced enhanced expression of the standard CD44 isoform (i.e., M, 85,000) suppresses the metastatic ability of the Dunning AT3,1 rat prostate cancer cell subline without suppression of tumorigenicity (7). The CD44 is a major cell surface receptor for the extracellular matrix glycosaminoglycan hyaluronate. We have demonstrated that the metastasis suppression by the standard CD44 is independent of its ability to bind to hyaluronate, suggesting that ligand(s) other than hyaluro-

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3 The abbreviation used is: RT-PCR, reverse transcriptase-PCR.

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PC3  DU145  Tsu  LNCaP Marker

GGGCAATGAGGCTG. Twenty μl of PCR product were loaded on non-denaturing polyacrylamide gels (5%) and visualized by ethidium bromide staining. For human prostate specimens, 1 μCi of [32P]dCTP was added to the PCR mixture. PCR products were electrophoresed on 5% polyacrylamide gels. The gels were dried and exposed to X-ray films.

Results and Discussion

Transcriptional Inactivation of the CD44 Gene by 5′ CpG Island Methylation in Human Prostate Cancer Cell Lines. CD44 expression was analyzed in prostate cancer cell lines derived from metastatic human prostate cancer by RT-PCR using CD44-specific primers. As shown in Fig. 1, the CD44 mRNA was detected in TSU, PC3, and DU145 human prostate cancer cell lines but not in LNCaP human prostate cancer cell line. This may represent a practical model system to study the mechanism of transcriptional inactivation of CD44 gene expression. Hypermethylation of CpG island is one of the mechanisms of transcriptional inactivation of gene expression. Sequence analysis of the 1582-bp 5′ flanking region of CD44 revealed a CpG-rich region fulfills criteria described previously for a CpG island (14, 15). Thus, we tested whether hypermethylation of this region is the mechanism responsible for the transcriptional inactivation of CD44 in prostate cancer cell lines.

To test whether hypermethylation is responsible for transcription inactivation of CD44 gene expression in LNCaP cells, we analyzed genomic DNA from the cell lines (i.e., LNCaP, TSU, DU145, and PC3), digested with the methylation-sensitive restriction enzyme HpaII and its methylation-insensitive isoschizomer, MspI. Genomic DNA (1 μg) of the prostate cancer cell lines was completely digested with either methylation-sensitive HpaII or methylation-insensitive MspI. PCR amplification using primers specific for the CD44 promoter sequences was performed. As shown in Fig. 2, in CD44 mRNA-positive cell lines (i.e., TSU, PC3, and DU145), CD44 promoter was found unmethylated resulting in no specific PCR product due to CD44 promoter digested by HpaII. In contrast, in a CD44 mRNA-negative cell line (i.e., LNCaP), CD44 promoter was methylated resulting a 791-bp PCR product. These results demonstrated that transcriptional inactivation of CD44 in human prostate cancer cell lines is due to hypermethylation of its promoter region.

Methylation of the 5′ CpG Island of the Promoter for the CD44 Gene Is Common in Prostate Cancer. The expression of CD44 is down-regulated during prostate cancer progression, with down-regulation

<table>
<thead>
<tr>
<th>Tissue types</th>
<th>No. of samples</th>
<th>No. of samples with methylation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>40</td>
<td>4 (10)</td>
</tr>
<tr>
<td>Cancer</td>
<td>40</td>
<td>31 (77.5)</td>
</tr>
<tr>
<td>Metastasis</td>
<td>4</td>
<td>3 (75)</td>
</tr>
</tbody>
</table>

using Advantage-GC Genomic PCR kit (Clontech, Palo Alto, CA) in a total volume of 20 μl with 1× Tth GC PCR buffer, 1 μl GC-mel, 25 pmol of each primer, 0.2 mm dNTPs, HpaII-digested DNAs (~ 250 ng), and 2.5 units of advantage Tth polymerase. Amplification was carried out on a thermocycler for 94°C for 45 s, 63°C for 2 min, and 72°C for 2 min for 40 cycles, followed by 72°C for 10 min. Primers, yielding a 791-bp product, were designed according to the published sequence of the 5′ region of CD44 (14). 5′ primer, 5′-GGATGGGCGGATGGAGAT; and 3′ primer, 5′-TCCGCT-
lation being correlated with higher tumor grade and distance metastasis (9–11). To examine whether this down-regulation of the expression of CD44 is also due to hypermethylation of its promoter, methylation analyses of CD44 in normal and tumor samples were performed. A total of 80 matched normal and primary prostate tumors and 4 metastases tissues were examined for CD44 methylation. Methylation of the CD44 promoter was observed in 31 of 40 primary prostate cancer specimens, 3 of 4 distant organ site metastases obtained at autopsy from men dying of prostate cancer, and 4 of the 40 matched normal tissues (Fig. 3 and Table 1).

In conclusion, CD44 was previously demonstrated to be a metastasis suppressor gene for prostate cancer (7). Down-regulation of the expression of the standard CD44 is correlated with increasing Gleason grade, aneuploidy, and distant metastasis (9–11). Here, we demonstrated that hypermethylation of CD44 promoter is associated with the down-regulation of the expression of CD44 during progression of prostate cancer to a metastatic state. Understanding the molecular mechanism for the methylation of the promoter of CD44 may provide insights into the progression of prostate cancer. In addition, these results warrant further investigation of CD44 promoter methylation as a prognostic marker for prostate cancer progression and as an intervention target for prostate cancer.

References
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