Methylation of the CD44 Metastasis Suppressor Gene in Human Prostate Cancer

Wei Lou, Diane Krill, Rajiv Dhir, Michael J. Beech, Jin-Tang Dong, Henry F. Frierson, Jr., William B. Isaacs, and Allen C. Gao

Department of Pathology and Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15213 [W. L., D. K., R. D., M. J. B., A. C. G.]; Department of Pathology, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908 [J-T. D., H. F. F.], and Department of Urology and Oncology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21231 [W. B. I., J. T. I.]

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., U-251, 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 mRNA (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-

nate is required for metastasis suppression by CD44 (8). The CD44 is normally expressed on the plasma membrane of human prostate glandular cells (9, 10). The standard CD44 expression is down-regulated both at the mRNA and protein level during human prostate cancer progression with down-regulation being correlated with higher tumor grade, aneuploidy, and distant metastasis (9, 10). Further studies by Noordzij et al. (11) demonstrated that loss of the standard CD44 expression by prostate adenocarcinomas predicts a poor prognosis, independent of stage and grade.

Although down-regulation of the expression of the metastasis suppressor gene CD44 is associated with prostate cancer progression, the mechanism of this down-regulation is currently unknown. Frequent cytosine methylation at CpG dinucleotides of the 5′ CpG island has been associated with transcriptional repression of tumor suppressor genes in a variety of human cancers (12, 13). Sequence analysis of the 1582-bp 5′ regulatory region upstream of the CD44 gene reveals a CpG island (14, 15). Here, the hypothesis that hypermethylation of the promoter of the CD44 gene contributes to the decreased CD44 expression in human prostate cancer was tested in human prostate tissues from normal, primary, and metastatic tumors.

Materials and Methods

Tissue Specimens and Cell Lines. Eighty matched normal and cancer tissues were obtained from radical prostatectomy specimens from the University of Pittsburgh Medical Center, the Prostate Tissue Resource of the Johns Hopkins SPORE, and the University of Virginia Health Sciences Center. Four distant organ site metastases were obtained at autopsy from men who died of prostate cancer at the University of Virginia Health Sciences Center. Human prostatic carcinoma cell lines (LNCaP, DU145, PC3, and TSU) were maintained in RPMI 1640 supplemented with 10% FCS. Genomic DNAs were isolated from tissue specimens and cell lines using standard procedures.

RT-PCR. Total RNAs were isolated by the Trizol kit (Life Technologies, Inc., Gaithersburg, MD), and cDNAs were reverse transcribed by using first-strand cDNA synthesis reagents (Pharmacia, Piscataway, NJ), according to the manufacturer’s instructions. A human CD44 cDNA was amplified by PCR using the following primers, based on the CD44 cDNA sequence: CD44 5′, 5′-CTCCGGACACCACCTGGACAAGT; and CD44 3′, 5′-CTCTTTCTTATGC-TATAACCTG (16). Briefly, 20 µg of total RNA were reverse transcribed, and 2 µl of the reverse transcription reaction mix were amplified. The reaction contained template, 50 µM dNTPs, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 15 mM MgCl2, and 12.5 µM CD44 primers. The mixture was denatured for 5 min at 95°C and cooled to 72°C, 2.5 units of AmpliTaq polymerase (Perkin-Elmer Corp., Norwalk, CT) were added, and the reaction was overlaid with mineral oil. It was then cycled for 30 s at 94°C, 1 min at 55°C, and 2 min at 72°C for 35 cycles. The PCR products were cloned into pcR II vector (Invitrogen, San Diego, CA), and inserts were confirmed by DNA sequencing using DNASeqII, according to the manufacturer’s instructions (Amerham, Arlington Heights, IL).

PCR Analysis for CD44 Methylation. The genomic DNAs (0.5 µg) were completely digested with a nC5-sensitive restriction endonuclease HpaII, followed by ethanol coprecipitation with glycogen. PCRs were optimized

Received 10/8/99; accepted 3/30/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Supported by the Developmental Fund of University of Pittsburgh Cancer Institute and NIH Grant P50CA47904. The Prostate Tissue Resource of the Johns Hopkins SPORE was supported by NIH Grant CA 58326.

To whom requests for reprints should be addressed, at BSTW1055, University of Pittsburgh Cancer Institute, 200 Lothrop Street, Pittsburgh, PA 15213. Fax: (412) 624-7737; E-mail: gaoac@msx.upmc.edu.

3 The abbreviation used is: RT-PCR, reverse transcriptase-PCR.
PC3  DU145  Tsu  LNCaP  Marker

GGCAATTGAGGCTG. Twenty µl of PCR product were loaded on non-denaturation 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-regu-
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
Methylation of the CD44 Metastasis Suppressor Gene in Human Prostate Cancer

Wei Lou, Diane Krill, Rajiv Dhir, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/10/2329

Cited articles
This article cites 16 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/10/2329.full#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/10/2329.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.