Methylation-Mediated Repression of GADD45α in Prostate Cancer and Its Role as a Potential Therapeutic Target

Kavitha Ramachandran,1 Gopal Gopisetty,1 Edna Gordian,1 Loida Navarro,1 Christiane Hader,2 Isildinha M. Reis,1 Wolfgang A. Schulz,2 and Rakesh Singal2

1Sylvester Comprehensive Cancer Center, University of Miami, Miami, Florida and 2Department of Urology, Heinrich Heine University, Düsseldorf, Germany

Abstract

Defects in apoptotic pathway contribute to uncontrolled proliferation of cancer cells and confer resistance to chemotherapy. Growth arrest and DNA damage inducible, alpha (GADD45α) is up-regulated on docetaxel treatment and may contribute to docetaxel-mediated cytotoxicity. We examined the mechanism of regulation of GADD45α in prostate cancer cells and the effect of its up-regulation on sensitivity to docetaxel chemotherapy. Expression of GADD45α in PC3 cells was higher than that in Du145 and LNCaP cells (17- and 12-fold, respectively; P < 0.05). Although the proximal promoter region was unmethylated in all three cell lines, methylation of a 4 CpG region upstream of the proximal promoter correlated inversely with gene expression levels. Methylation was reversed by treatment of Du145 and LNCaP cells with DNA methyltransferase inhibitors, leading to reactivation of GADD45α expression in these cells. The 5’ 4 CpG region was also frequently methylated in prostate cancer tissues. Methylation of this region correlated inversely with gene expression in prostate cancer and benign prostate tissues. The methyl binding protein MeCP2 was associated with the methylated 4 CpGs in Du145 cells, and knockdown of MeCP2 in these cells (Du145 MeCP2−/−) led to a significantly increased expression of GADD45α (3-fold; P = 0.035) without affecting the methylation status of the gene. Enhanced sensitivity to docetaxel was observed by up-regulation of GADD45α in Du145 cells by recombinant expression of GADD45α or pretreatment with 5-azacytidine. Our results show that GADD45α is epigenetically repressed and is a potential target for treatment of prostate cancer. [Cancer Res 2009;69(4):1527–35]

Introduction

Apoptosis or programmed cell death is a crucial mechanism for maintenance of cell homeostasis. During carcinogenesis, there is increased cell proliferation and development of resistance to cytotoxic chemotherapeutic agents (1). The molecular mechanisms of this phenomenon are yet to be completely elucidated. It is believed that defects in apoptotic pathways play a key role in tumorigenesis. Genes involved in cell cycle control, apoptosis signaling, and DNA repair become methylated and epigenetically inactivated in most cancers (1–4).

Recently, there have been reports of methylation-mediated deregulation of proapoptotic genes, which could provide a mechanism through which tumor cells avoid apoptosis (5, 6). Growth arrest and DNA damage inducible, alpha (GADD45α) plays an important role in cellular response to DNA damage because it is involved in DNA repair, maintenance of genomic stability, cell cycle control, and apoptosis (7). GADD45α blocks G2-M transition thereby causing cell cycle arrest in response to DNA-damaging agents such as UV radiation, ionizing radiation, and methyl-methane sulfonate (8). The role of GADD45α in G2-M arrest is shown by its ability to interact with Cdc2 kinase resulting in inhibition of Cdc2/cyclin B1 complex formation, which is required for G2-M transition during cell cycle progression (9). Inhibition of growth by GADD45α occurs both in cell lines with functional p53 and those with a negative p53 status (7). GADD45α expression is critical for c-jun NH2-terminal kinase activation and apoptosis in tumor cells (10–12). Recombinant GADD45α expression in tumor cell lines by transfection reduces cell proliferation (13). Treatment of cells with genotoxic agents up-regulates GADD45α expression resulting in induction of apoptosis (14).

Previous studies showed that GADD45α was down-regulated in prostate cancer compared with benign prostate tissues. Down-regulation of gene expression was not associated with hypermethylation of the proximal promoter region (15). In this study, we show that transcriptional repression of GADD45α in Du145 prostate cancer cells occurs by an atypical methylation of 4 CpGs located upstream of the proximal promoter region. Methylation of the 5’ 4 CpG region is a frequent event in prostate cancer and correlates inversely with gene expression in prostate cancer and benign prostate tissues. Further, down-regulation of GADD45α in Du145 involves interaction of MeCP2 with GADD45α promoter. Induction of GADD45α by treatment of Du145 cells with DNA methyltransferase (DNMT) inhibitor increases sensitivity to docetaxel chemotherapy, thus identifying the role of GADD45α as a potential target gene in prostate cancer.

Materials and Methods

Cell culture. Du145, LNCaP, and PC3 prostate cancer cells were routinely cultured in RPMI 1640 (Mediatech) supplemented with 10% fetal bovine serum (Invitrogen), 2 mmol/L glutamine (Invitrogen), and 100 μg/mL penicillin-streptomycin (Invitrogen).

Tissue specimens. Tissue specimens used in this study were obtained from prostate cancer cases and benign prostate hyperplasia (BPH) controls as previously described (15, 16).

Drug treatment. 5-Azacytidine was provided by Pharmion Corporation and 5-azadeoxycytidine was purchased from Sigma-Aldrich. Du145 cells were treated with varying concentrations of 5-azacytidine or 5-azadeoxycytidine for 72 h, after which RNA/DNA/protein were extracted. For determining the sensitivity to docetaxel, Du145 cells were seeded for 24 h,
after which they were treated with 5-azacytidine for 72 h followed by 5 nmol/L docetaxel for 72 h.

**Plasmids and transfection.** pSuppressor/MeCP2 siRNA, SureSilencing MBD2 shRNA, and pCMV/GADD45α plasmids were obtained from Imgenex, SuperArray Bioscience Corporation, and Origene Technologies, Inc., respectively. Transfection was carried out using Amaxa Nucleofection system (Amaxa, Inc.). To generate stable clones of Du145 and LNCaP MeCP2/C0 cells, transfected cells were selected with 350 μg/mL G418. Stable clones of Du145 and LNCaP MBD2/C0 cells were generated by transfection followed by selection in medium containing 5 and 2.5 μg/mL puromycin.

**Quantitative reverse transcription-PCR.** RNA extracted from untreated and drug treated cells using RNA Stat 60 (TelTest, Inc.) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (USB Corp.). Real-time PCR amplification was done in triplicates with cDNA using primers for GADD45α. The average Ct was then used to quantitate relative mRNA levels by the comparative Ct method. RNA from untreated cells was used as a calibrator. Both samples and calibrator were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences for GADD45α are 5’-CGCCTGTGAGTGAGTGC-3’ (forward) and 5’-CTATCCATCTTTCGGTCTT-3’ (reverse), and for GAPDH, 5’-GCTGAGTACGTCTGAGTC-3’ (forward) and 5’-GGGCGAGATGATGACCC-3’ (reverse). The PCR reaction was carried out in a volume of 25 μL using iQ SYBR Green Supermix (Bio-Rad Laboratories) on Myiq Single-Color Real-Time PCR Detection System (Bio-Rad Laboratories). Expression of GADD45α and of the reference gene TBP in tissues was done by real-time reverse transcription-PCR (RT-PCR) on an ABI 7900 instrument (Applied Biosystems) using assay systems supplied by the same company, as described (15).

**Western blotting.** Whole cell lysates were prepared using the M-PER Mammalian Protein Extraction Reagent (Pierce). Protein concentrations of the lysate were determined using bicinchoninic acid protein assay reagent (Pierce). For Western blot analysis, the proteins were resolved on a 4% to 20% SDS-PAGE gel and blotted onto a nitrocellulose membrane. The blot was then probed with rabbit anti-human GADD45α antibody (1:200 dilution; Santa Cruz Biotechnology), detected with antirabbit immunoglobulin-horseradish peroxidase (GE Healthcare Bio-Sciences Corp.), and visualized with SuperSignal West Femto Maximum Sensitivity Substrate kit (Pierce). To ensure equal loading in all the lanes, the blot was stripped

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**Figure 1.** GADD45α expression and methylation in prostate cancer cells. A, RT-PCR analysis. cDNA synthesized from RNA was amplified by real-time PCR. Levels of GADD45α were normalized to that of GAPDH. Inset, Western blotting. Whole cell lysates were prepared from Du145, LNCaP, and PC3 cells and resolved on a 4% to 20% SDS polyacrylamide gel and blotted onto a nitrocellulose membrane. The blot was probed with anti-GADD45α antibody and detected by enhanced chemiluminescence (ECL). To ensure equal loading of the samples, the blot was stripped and reprobed with anti-tubulin antibody. B, bisulfite genomic sequencing analysis of the proximal promoter region. DNA extracted from cells was bisulfite treated and amplified using primers flanking the proximal promoter region. The PCR product was run on a 3% agarose gel and purified using Wizard SV gel and PCR clean-up system (Promega). The PCR product was sequenced using Thermosequenase Radiolabeled Terminator cycle sequencing kit (USB) and run on a 7 mol/L urea/5% polyacrylamide gel. The gel was dried and analyzed using Phosphiomager. Arrows, CpGs. CpGs in the GADD45α proximal promoter region are unmethylated in all three cell lines. C, bisulfite genomic sequencing analysis of the 5’ CpG region. The 4 CpGs at positions –737, –723, –706, and –690 (not shown) with respect to the transcription start site are methylated in Du145 and LNCaP, whereas they were unmethylated in PC3. D, methylation was quantitated using Quantity One software (Bio-Rad).
Figure 2. Reactivation of GADD45α after treatment with DNMT inhibitors. A, bisulfite sequencing showing demethylation of CpGs in the GADD45α 4 CpG region. Du145 cells were treated with 0.5 μmol/L 5-azacytidine (AzadC) or 1 μmol/L 5-azacytidine (AzaC) for 72 h. Control cells were left untreated. Bisulfite-treated DNA was amplified using primers flanking the 4 CpG region. The PCR product was run on a 3% agarose gel and purified using Wizard SV gel and PCR clean-up system (Promega). The PCR product was sequenced using Thermosequenase Radiolabeled Terminator cycle sequencing kit (USB). B, methylation was quantitated using Quantity One software (Bio-Rad). C, quantitative RT-PCR. RNA extracted from the drug-treated cells was reverse transcribed and amplified by real-time PCR. Levels of GADD45α mRNA were normalized to that of GAPDH. D, Western blot analysis. Whole-cell lysates prepared from these cells were resolved on a 4% to 20% SDS polyacrylamide gel and blotted onto a nitrocellulose membrane. The blot was probed with anti-GADD45α antibody and detected by ECL. To ensure equal loading of the samples, the blot was stripped and reprobed with anti-actin antibody.

Results

GADD45α expression in prostate cancer cells correlates inversely to methylation of 5′ 4 CpG region. Transfection of...
GADD45\(\alpha\) has been shown to induce apoptosis in Du145 and PC3 prostate cancer cell lines (11); hence, we explored the role of GADD45\(\alpha\) as a potential target for chemotherapy. We examined GADD45\(\alpha\) expression in the three prostate cancer cell lines, LNCaP, Du145, and PC3, by real-time RT-PCR and Western blotting analyses. Compared with Du145, we found that LNCaP expressed similarly low levels (\(P = 0.756\)) whereas PC3 showed significantly elevated levels of GADD45\(\alpha\) expression (17-fold, \(P = 0.029\); Fig. 1A).

Because promoter methylation is commonly associated with silencing of gene expression, we examined the methylation status of the GADD45\(\alpha\) promoter region in Du145, PC3, and LNCaP prostate cancer cells by bisulfite genomic sequencing. CpG islands in the proximal promoter region were unmethylated in all three cell lines (Fig. 1B). However, unlike known patterns of methylation of CpG islands, an atypical methylation pattern was observed. Similar to an earlier study in breast cancer cells (23), only 4 CpGs located \(~700\) bases upstream of the transcription start site were methylated in Du145 and LNCaP whereas they were unmethylated in PC3 (Fig. 1C). The average methylation of 5 CpGs was 71%, 84%, and 27% in Du145, LNCaP, and PC3, respectively (Fig. 1D). Levels of expression of GADD45\(\alpha\) in these cell lines correlated inversely with the methylation levels of CpGs in the 5’ region.

**Treatment with DNMT inhibitors reactivates GADD45\(\alpha\) expression in Du145 cells.** Several in vitro and in vivo studies have shown that treatment with inhibitors of DNMTs results in DNA demethylation and subsequent reactivation of otherwise silenced genes (24–26). To confirm the role of methylation of the 5’ 4 CpG region in transcriptional repression of GADD45\(\alpha\), we analyzed the expression of GADD45\(\alpha\) after treatment of Du145 cells with DNMT inhibitors, 5-azacytidine and 5-azadeoxycytidine. Levels of GADD45\(\alpha\) mRNA and protein levels of GADD45\(\alpha\) increased significantly after drug treatment (Fig. 2C and D), concomitant with demethylation of the 4 CpGs (Fig. 2A and B). When compared with untreated Du145 control, we observed a 7-fold increase in expression of GADD45\(\alpha\) in cells treated with 5-azacytidine (\(P = 0.049\)) and an 11-fold increase with 5-azadeoxycytidine (\(P = 0.000\)). Similar results were observed in LNCaP cells when treated with DNMT inhibitors (data not shown). These results support the role of methylation in repressing GADD45\(\alpha\) gene expression in Du145 and LNCaP cells.

![Figure 3](https://example.com/fig3.png)

**Figure 3.** Quantitation of methylation of CpGs in the 4 CpG region of GADD45\(\alpha\) promoter in prostate cancer tissues and BPH tissues. A, DNA extracted from tissues was bisulfite treated and amplified using primers flanking the 4 CpG region and sequenced as described before. PCT, prostate cancer tissues. Arrows, CpGs. Methylation of CpGs was quantitated using Quantity One (Bio-Rad). Percent methylation was calculated using the formula C/C + T × 100. B, mean and SD of percent methylation in 22 prostate cancer patients and 10 BPH patients. The difference between the means is statistically significant, \(P = 0.001\). C, representative MS-SNuPE analysis of methylation of GADD45\(\alpha\) 4 CpG region (CpG site 2 shown) in prostate cancer and benign prostate tissues. D, correlation of expression of GADD45\(\alpha\) with methylation of the 4 CpG region. Levels of GADD45\(\alpha\) expression normalized to TBP levels were correlated with average methylation of CpG sites 1, 2, and 3 in the 5’ 4 CpG region.
Methylation of GADD45α 5’ 4 CpG region is a frequent event in prostate cancer and correlates inversely with gene expression in primary prostate cancer and benign prostate tissues. Previous results showed that GADD45α was down-regulated in prostate cancer compared with benign prostate tissues (15). However, only proximal promoter methylation was evaluated and this region was found to be unmethylated in both cancer tissues and benign specimens. To determine if methylation of GADD45α 5’ 4 CpG region is present in prostate tumors, we compared the methylation of GADD45α 4 CpG region in 22 prostate cancer tissues and 10 BPH tissues by bisulfite sequencing (Fig. 3A). We found a significant increase in methylation of this 4 CpG region in prostate cancer tissues compared with BPH (mean of 47% in prostate cancer tissues versus 26% in BPH, P = 0.001; Fig. 3B). Methylation of proximal promoter region was evaluated in randomly selected samples. These showed lack of methylation in proximal promoter despite methylation of the 4 CpG region (data not shown). Hence, atypical methylation of GADD45α 4 CpG region is present in primary prostate cancer tissues and is not confined to prostate cancer cell lines.

To correlate GADD45α expression with methylation of the 5’ 4 CpG region, we examined methylation in five prostate cancer tissues with high GADD45α expression, four prostate cancer tissues with low GADD45α expression, and five benign prostate tissues from the previous study (15). All five benign tissues were found to have high GADD45α expression. MS-SSNuPE quantitation showed that methylation in each of 3 CpGs is significantly higher in tumor samples (average methylation of 3 CpGs, 52%) than in normal tissue samples (average methylation of 3 CpGs, 24%; P < 0.0001; Fig. 3C, data for CpG2 shown). Methylation of GADD45α 5’ 4 CpG region correlated inversely with gene expression in combined analysis of all tissue samples (n = 14, Pearson’s correlation coefficient = −0.537, P = 0.047; Fig. 3D). These results were also independently confirmed by bisulfite sequencing (see Supplementary Fig. S1).

Transcriptional repression of GADD45α occurs via interaction of MeCP2 with its methylated promoter. Interaction of transcriptional repressor proteins with methylated DNA either directly by the methyl-binding domain (MBD) or indirectly by
interaction with other proteins contributes to transcriptional repression (27–29). To elucidate the mechanism of transcriptional silencing of GADD45x in prostate cancer cells, we analyzed the binding pattern of MeCP2 to the methylated 5′ 4 CpG region upstream of GADD45x promoter in Du145 and LNCaP cells using chromatin immunoprecipitation assay. MeCP2 was found to interact with the methylated 4 CpG region in Du145 cells (mean percent binding: 310% normalized to no-antibody control, \( P = 0.002 \)) in Du145 (Fig. 4A) but not in LNCaP cells (data not shown). In contrast, no binding of MBD2 to this region was observed in Du145 cells (data not shown).

It has been reported that knockdown of MeCP2 by siRNA transfection restores the expression of epigenetically silenced genes (30). To examine the role of Mcp2 in methylation-mediated transcriptional regulation of GADD45x in Du145 cells, we carried out transient knockdown of Mcp2 using a Mcp2 siRNA vector in Du145 cells and examined the changes in the expression pattern of GADD45x. GADD45x expression in Du145 Mcp2− cells was increased by ~2-fold compared with wt Du145 cells, suggesting that Mcp2 interaction with the GADD45x 5′ region has functional significance (data not shown). Consistent with chromatin immunoprecipitation analysis, transient transfection of MBD2 siRNA did not alter the gene expression levels in Du145 cells (data not shown). No enhancement of GADD45x expression was observed in LNCaP cells on stable knockdown of Mcp2 (data not shown). This was consistent with the chromatin immunoprecipitation analysis data that showed that Mcp2 does not bind to the GADD45x 4 CpG region in these cells (data not shown).

We further confirmed these findings by generating a stable Du145 Mcp2− cell line in which Mcp2 was knocked down by transfection with pSuppressor/Mcp2 siRNA plasmid. Reduced expression of Mcp2 in Du145 Mcp2− cells was observed by immunofluorescence staining with anti-Mcp2 antibody and also by Western blotting analysis, thus confirming the stable knockdown of Mcp2 in these cells (Fig. 4B). Consistent with these results, we found that the binding of Mcp2 to the GADD45x promoter is reduced in Du145 Mcp2− cells (mean of 310% in Du145 wt versus 47% in Mcp2−, \( P = 0.002; \) Fig. 4C). Further, the levels of GADD45x increased by ~3-fold in Du145 Mcp2− cells as compared with Du145 wt cells (\( P = 0.035; \) Fig. 4D) although methylation of the 4 CpGs remained unaltered (data not shown).

To elucidate the mechanism of GADD45x repression in LNCaP cells, we examined interaction of other MBDs using chromatin immunoprecipitation assay. We observed that interaction of MBD2 with GADD45x promoter and stable depletion of MBD2 by siRNA vector resulted in enhanced expression of GADD45x gene (data not shown).

Recombinant GADD45x expression results in increased sensitivity of Du145 cells to docetaxel. Up-regulation of GADD45x is a key event in docetaxel-induced apoptosis (10, 11, 31). Because GADD45x is down-regulated in Du145 cells, we hypothesized that expression of GADD45x would enhance sensitivity to docetaxel. Du145 cells were transiently transfected with pCMV/GADD45x or mock transfected with empty pCMV vector. Western blotting analysis confirmed the expression of GADD45x in these GADD45x-transfected cells (Fig. 5A). Increased apoptosis as measured by Annexin V/propidium iodide staining was observed in Du145 cells expressing GADD45x as compared with mock-transfected cells when treated with docetaxel for 72 hours (47% versus 31%, \( P < 0.001 \)) as well as when left untreated (mean of 12% versus 4%, \( P = 0.003; \) Fig. 5B and C). Cells expressing GADD45x showed increased apoptosis when treated with docetaxel compared with untreated cells (47% versus 12%, \( P < 0.001 \)). The mean difference in apoptosis between GADD45x−expressing and mock-transfected cells was greater in cells treated with docetaxel (mean difference, 16%) than in untreated cells (mean difference, 8%; \( P < 0.001 \)).
augmenting docetaxel-mediated apoptosis in Du145 prostate cancer cells.

Up-regulation of GADD45α by pretreatment with 5-azacytidine increases sensitivity of Du145 cells to docetaxel. Combination treatments can improve the antitumor activity of chemotherapeutic drugs (32). We hypothesized that induction of GADD45α expression by 5-azacytidine pretreatment would increase sensitivity of Du145 cells to docetaxel chemotherapy. We performed time and dose response kinetics in Du145 cells to docetaxel and 5-azacytidine (Fig. 6A and B). Based on these results, we chose the subcytotoxic dose of 1 μmol/L 5-azacytidine for pretreatment of the cells. As shown in Fig. 6C, we observed an increased sensitivity to docetaxel treatment. Annexin V/propidium iodide staining followed by flow cytometry showed that cells pretreated with 5-azacytidine showed higher apoptosis with docetaxel than previously untreated cells. The mean apoptotic fraction was 12% in 5-azacytidine–treated cells versus 4% in untreated cells (P = 0.006) and 25% in 5-azacytidine pretreated cells + docetaxel versus 16% in untreated cells + docetaxel (P = 0.001). Similar results were obtained with fluorescein di-O-acetate cell viability assay (Fig. 6D). The additive effect of 5-azacytidine and docetaxel is likely a result of up-regulation of GADD45α by 5-azacytidine treatment. We also examined the ability of 5-azacytidine to enhance sensitivity to docetaxel treatment of LNCaP cells. The results were similar to those with Du145 cells (data not shown).

Discussion

This article describes epigenetic regulation of GADD45α in prostate cancer cells and its potential role in docetaxel sensitivity. The key findings in this article are as follows. GADD45α expression in prostate cancer cells correlates inversely with an atypical CpG methylation upstream of transcription start site. Methylation of GADD45α occurs more frequently in prostate cancer tissues as compared with BPH tissues. Methylation of the 5′4 CpG region correlates inversely with GADD45α expression in prostate cancer and benign prostate tissues. Transcriptional repression of GADD45α in Du145 cells involves interaction of MeCP2 with
the 5′ 4 CpG region of the gene. Induction of GADD45α expression by down-regulation of MeCP2 or by treatment with DNMT inhibitors leads to enhanced sensitivity of Du145 cells to docetaxel chemotherapy.

Although methylation-mediated inactivation of genes is attributed to methylation of dense CpG islands in the proximal promoter region (1–3, 6, 33, 34), a few examples to the contrary exist. Early evidence that sparse methylation could lead to transcriptional repression arose from the observation that in vitro methylation of human γ-globin and mouse α-globin sequences lacking CpG clusters resulted in loss of gene expression. Similar results were obtained when the human α-globin promoter with dense CpG clusters was methylated to a lower density (35). Atypical DNA methylation was described in the case of the APAF1 gene, which is transcriptionally repressed in malignant melanoma. No methylation could be detected in its core promoter region. However, gene expression was restored by treatment with DNMT inhibitors, suggesting that methylation affects Cpgs located outside of the promoter region (36). Conventional techniques to identify epigenetically regulated genes include MS-PCR and bisulfite sequencing of CpG islands located in the proximal promoter region of the gene. Often, these approaches do not address sparsely populated Cpgs that are situated far upstream of the transcription start site. Another approach to identify epigenetically regulated genes is use of expression arrays to detect genes that are induced by 5-azacytidine. Using this strategy, GADD45α methylation outside of the proximal promoter region was detected in breast cancer (23). In the previous study, although GADD45α repression was observed in prostate cancer, evaluation of the proximal promoter region failed to detect methylated CpG dinucleotides (15). Consistent with these results, we did not detect methylation in the CpG-rich proximal promoter region in the three prostate cancer cell lines analyzed. Instead, gene expression was regulated by methylation of 4 CpGs upstream of the proximal promoter region. This pattern of methylation is atypical because it occurs in a sparsely distributed CpG region situated far away from the actual promoter sequence. Moreover, these 4 CpGs are not contained within any recognized response element. We observed increased levels of methylation of GADD45α 4 CpG region in prostate cancer tissues compared with BPH tissues. Further, methylation levels of GADD45α 4 CpG region inversely correlated with gene expression in prostate tissues, suggesting the functional significance of methylation of the 4 CpG region in regulation of GADD45α expression. Therefore, methylation analysis of CpG dinucleotides away from the proximal promoter region may also be important for genes suspected to be repressed by methylation.

Epigenetic suppression of transcription involves proteins that bind specifically to methylated DNA, in particular MBD proteins. Transcriptional regulation by MBDs occurs by binding to methylated DNA, thus acting as a “locking” mechanism stabilizing gene repression caused by other components of chromatin modification systems (29). MeCP2, the archetypical MBD protein, has been shown to play a role in epigenetic transcriptional repression mechanism (37). MeCP2 binds to methylated CpGs of the IκBα promoter leading to the formation of a transcriptional repression complex that silences expression of the gene in myofibroblasts. Knockdown of MeCP2 led to enhanced IκBα promoter activity and restoration of mRNA and protein expression in these cells (30). In Du145 cells, GADD45α was found to be transcriptionally regulated via binding of MeCP2 to the methylated GADD45α 5′ 4 CpG region. Transcriptional repression of the gene was alleviated by knocking down MeCP2. Knockdown of MBD2, however, did not restore GADD45α expression in these cells. The specificity of binding of MeCP2 to sparsely methylated GADD45α sequence in Du145 could be explained by the fact that MeCP2 binds to single methylated CpG whereas MB2/MeCP1 requires densely methylated CpG islands for binding (27). We found that the effect of MeCP2 knockdown on GADD45α expression was cell line specific because in LNCaP cells, there was no interaction of MeCP2 with GADD45α 5′ 4 CpG region and, hence, no effect of MeCP2 knockdown on GADD45α expression. Conversely, in LNCaP cells, MBD2 interaction with GADD45α promoter was observed and depletion of MBD2 by siRNA vector resulted in enhanced expression of GADD45α gene. It has been postulated that a gene could be associated with different MBDs in different cells (29). Moreover, one of the models for gene regulation by MBDs proposes a random interaction of MBDs, thus accounting for the functional redundancy of these proteins (29). This explains why there are few instances of loss of a specific MBD protein resulting in over-expression of corresponding target genes.

Docetaxel is the only known chemotherapeutic agent shown to improve survival in patients with hormone-refractory prostate cancer as established by randomized controlled trials and therefore approved by the Food and Drug Administration for this use (38, 39). It has been known that docetaxel binds to microtubules inhibiting cancer cell proliferation (40). However, the precise molecular mechanisms for inhibiting cancer cell growth by docetaxel have not been fully elucidated. Resistance to chemotherapy, especially to docetaxel, has presented itself as a major obstacle in treatment of advanced prostate cancer. Currently, there is no standard of care defined for such patients. There is a continuous search for new treatment regimens to improve the efficacy of chemotherapeutic agents. Evidence from literature suggests that disruption of the apoptotic pathway seems to be a major mechanism of uncontrolled cell proliferation as well as resistance to chemotherapeutic agents (1). Gene expression profiling revealed that treatment of cells with docetaxel increased GADD45α expression, thus indicating GADD45α as a key element in docetaxel-mediated apoptosis (14). Consistent with this observation, we found that sensitivity to docetaxel was enhanced in Du145 cells expressing GADD45α as compared with mock-transfected cells. Du145 cells treated with 5-azacytidine before docetaxel treatment showed increased sensitivity to docetaxel treatment likely due to increased GADD45α expression. Our results suggest that combination treatment with a DNMT inhibitor and docetaxel may improve the efficacy of docetaxel in treatment of metastatic prostate cancer.

Disclosure of Potential Conflicts of Interest
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References

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