Role of DNA Methylation in Cabazitaxel Resistance in Prostate Cancer

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Abstract. *Background/Aim: Cabazitaxel is an approved second-line treatment for docetaxel-refractory metastatic castration-resistant prostate cancer. However, the median time to progression on cabazitaxel is 2.8 months. We aimed to determine whether DNA methylation plays a role in cabazitaxel resistance. Materials and Methods: DU145 cells, resistant to docetaxel and cabaxitaxel (DU145 10DRCR), were generated from cells resistant to 10 nM docetaxel (DU145 10DR). The effect of pre-treatment with 5-azacytidine was determined with regards to cabazitaxel sensitivity. Gene expression profiling was carried-out on DU145 10DR, DU145 10DRCR and DU145 10DRCR treated with 5-azacytidine. Results: Pre-treatment of cells with 5-azacytidine resulted in enhanced sensitivity to cabazitaxel. Gene expression profiling identified a subset of genes that may be regulated by DNA methylation. Conclusion: Our results indicate that DNA methylation of pro-apoptotic and cell-cycle regulatory genes may contribute to cabazitaxel resistance and pre-treatment with 5-azacytidine may restore sensitivity to cabazitaxel in prostate cancer cells.*

Prostate cancer is the most commonly diagnosed malignancy and the second leading cause of cancer-related death in American men. There will be an estimated 220,800 new prostate cancer cases and 27,540 estimated prostate cancer-related deaths in the United States in 2015 (19). After an initial response period, metastatic prostate cancer progresses to castration resistance. Most prostate cancer-related deaths occur in patients with metastatic castration-resistant prostate cancer (CRPC). The median survival in patients with metastatic CRPC is only 12-18 months. Based on results from two randomized control studies (RCTs), TAX327 and SWOG-99-16 (13, 22), the Food and Drug Administration (FDA) approved the use of docetaxel in combination with prednisone for the treatment of metastatic CRPC in 2004. However, after an initial response to docetaxel, approximately 80% of patients demonstrate PSA relapse within 12 months and median time to progression is approximately 6 months (13). For over 6 years, there were no other treatment options for patients who progressed, on or after docetaxel chemotherapy. In 2010, a novel taxane, cabazitaxel was approved as a second-line chemotherapy treatment in these patients by the FDA. However, the median time to progression on cabazitaxel is 2.8 months (2). The molecular mechanisms of cabazitaxel resistance are presently not fully understood. One possible mechanism may involve epigenetic silencing of pro-apoptotic genes and genes involved in cell-cycle regulation.

We have previously shown that growth arrest and DNA damage inducible-alpha (*GADD45a*), a pro-apoptotic gene, is frequently inactivated by methylation in prostate cancer and contributes to docetaxel sensitivity (15). Furthermore, our results from the Phase I study on azacitidine, docetaxel and prednisone in treatment of metastatic CRPC who progressed on or after docetaxel chemotherapy showed that this combination is well-tolerated and shows an exciting response in a recently completed Phase I study in patients with prior docetaxel treatment (20). In the present study, we aimed to identify pathways of resistance to cabazitaxel and determine whether epigenetic gene silencing contributes to cabazitaxel resistance in prostate cancer cells.

Materials and Methods

Cell culture. DU145 prostate cancer cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were routinely cultured in RPMI-1640 medium (Mediatech, Manassas, VA, USA) supplemented with 10% fetal bovine serum, 2 mM glutamine (Invitrogen, Carlsbad, CA, USA) and 100 μg/ml penicillin-streptomycin (Invitrogen) in a humidified incubator at

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Key Words: Epigenetics, prostate cancer, DNA methylation, drug resistance.

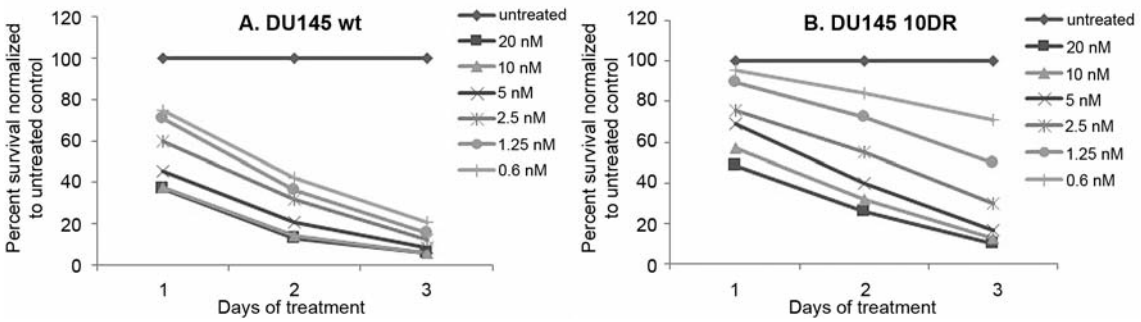

Figure 1. *Comparison of sensitivity of DU145 wt and DU145 10DR cells to cabazitaxel. DU145 wt and DU145 cells resistant to 10 nM docetaxel (DU145 10DR) were seeded in 96-well plates and treated under varying concentrations of cabazitaxel ranging from 0.6 nM to 20 nM for 72 h. Control cells were left untreated. Cell viability was assayed by Cell-titer blue assay.*

37˚C with 5% CO_2. Docetaxel and 5-azacytidine were procured from LC laboratories (Woburn MA, USA) and Sigma Aldrich (St. Louis MO, USA) respectively. Cabazitaxel was provided by sanofi-aventis (Bridgewater, NJ, USA).

Drug-resistant cells. DU145 cells resistant to 10 nM docetaxel (DU145 10DR) were obtained by culturing in docetaxel in a dose-escalating manner. After cells sensitive to the drug were no longer present and the surviving DU145 cells had re-populated the flask and continued to divide through four passages, the concentration of drug in the medium was increased. This was continued in a step-wise manner until a final concentration of 10 nM docetaxel was reached. DU145 10DR cells were maintained in medium containing 10 nM docetaxel. Using a similar strategy, DU145 10DR cells resistant to cabazitaxel (DU145 10DRCR) were also generated. These cells were maintained in medium containing 10 nM docetaxel and 10 nM cabazitaxel.

Drug treatment. Wild-type and drug-resistant DU145 cells were treated with different concentrations of cabazitaxel and cell viability was measured 72 h following treatment using Cell Titer Blue (Promega, Madison, WI, USA). Cells were treated with different concentrations of 5-azacytidine for 72 h, after which RNA was extracted. For combination treatment, wild-type and drug-resistant cells were seeded in 96-well plates and treated with 5-azacytidine for 72 h followed by treatment with cabazitaxel for 72 h. Cell viability was measured after 24, 48 and 72 h using Cell Titer Blue.

Cell viability assay. Cells were incubated with RPM1 medium containing Cell Titer Blue for 5 h and fluorescence ($560_{Ex}/590_{Em}$) was measured in a Synergy HT multi-task plate reader (Biotek, Winooski, VT, USA).

RNA extraction. RNA was extracted from cells using the Masterpure RNA purification kit (Epicentre, Madison, WI, USA) as per the manufacturer's instructions and reverse transcribed using MMLV Reverse Transcriptase (USB Corporation, Cleaveland, OH).

Gene expression profiling. Gene expression profiling in DU145 10DRCR and DU145 10DR with and without 5-azacytidine treatment was permormed using the Illumina HumanHT-12 Expression BeadChip (Illumina, San Diego, CA, USA). Differential gene expression analysis was performed using the Partek Genomic

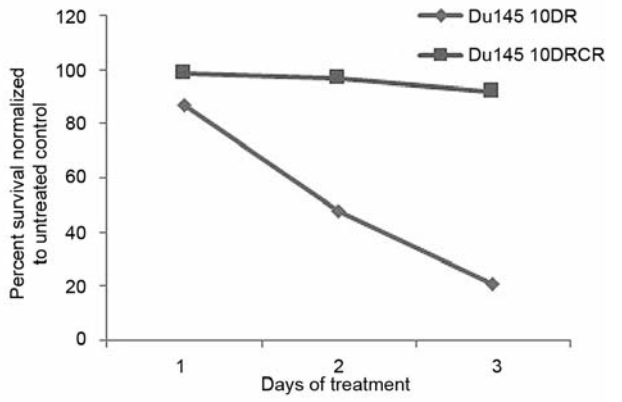

Figure 2. *Evaluation of resistance of DU145 10DRCR cells to cabazitaxel. DU145 cells resistant to docetaxel (DU145 10DR) and DU145 10DR cells resistant to 10 nM cabazitaxel (DU145 10DRCR) were seeded in 96 well plates and treated with 10 nM cabazitaxel for 72 h. Control cells were left untreated. Cell viability was assayed by Cell-titer blue assay.*

Suite v.6.6 software (St. Louis, MO, USA). Only probes with a detection *p*-value <0.5 for at least one sample were selected for analysis. After subtraction of background, data were normalized using the quantile method. Values <0.01 were converted to 0.01 to avoid deletion to zero. Differentially expressed probes were identified by performing Analysis of Variance (ANOVA).

Results

DU145 cells resistance to docetaxel and cabazitaxel. Firstly, we evaluated the sensitivity of DU145 wild-type (wt) and DU145 cells resistant to 10 nM docetaxel (DU145 10DR) to cabazitaxel. DU145 wt cells were found to be more sensitive to cabazitaxel compared to DU145 10DR cells (Figure 1). Next, we generated DU145 10DR cells resistant to cabazitaxel (DU145 10DRCR) by culturing cells in cabazitaxel in a dose-escalating manner. We then evaluated

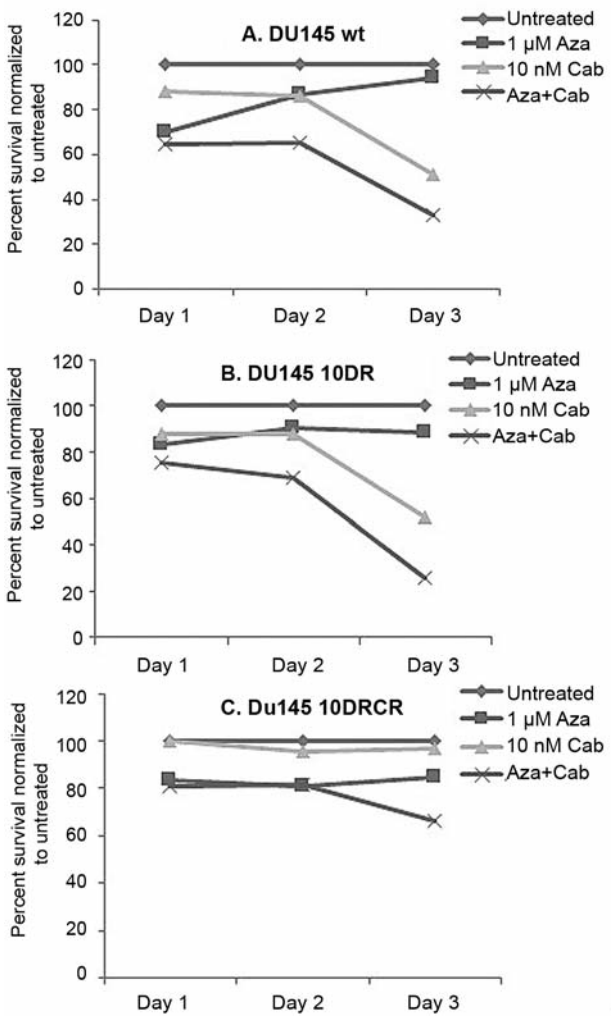

Figure 3. *Pre-treatment with 5-azacytidine enhances sensitivity to cabazitaxel in DU145 cells. DU145 wild-type cells (DU145 wt Panel A), DU145 cells resistant to docetaxel (DU145 10DR Panel B) and DU145 10DR cells resistant to 10 nM cabazitaxel (DU145 10DRCR Panel C) were seeded in 96-well plates and treated with 1 μM 5-Azacytidine for 72 h followed by 10-nM cabazitaxel for 72 h. Control cells were left untreated. Cell viability was assayed by Cell-titer blue assay 24, 48 and 72 h after cabazitaxel treatment.*

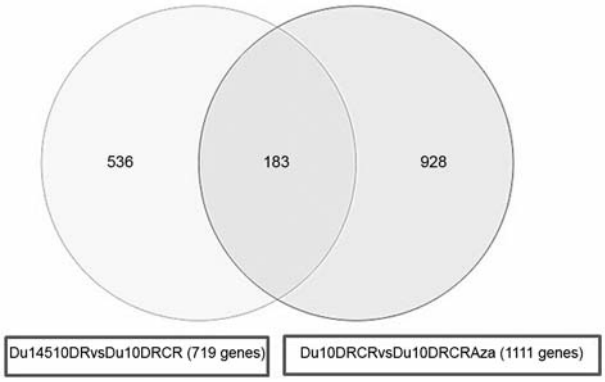

Figure 4. *Venn diagram showing epigenetically-regulated genes in DU14510DRCR cells compared to DU14510DR cells. Gene expression profiling in DU145 10DRCR and DU145 10DR with and without AzaC treatment were done using the Illumina HumanHT-12 Expression BeadChip. The intersect comprising of 183 genes represents genes that are potentially epigenetically regulated that is derived from the overlap of genes having reduced expression in DU145 10DRCR cells compared to DU145 10DR cells (719 genes) and the genes whose expression is increased upon azacitidine treatment in DU145 10DRCR cells (1,111 genes).*

the sensitivity of DU145 10DRCR cells to cabazitaxel compared to that of DU145 10DR cells. After 72 h of treatment with 10 nM cabazitaxel, there was 8% cell death in DU145 10DRCR cells compared to 80% cell death in DU145 10DR cells (Figure 2).

Pre-treatment with azacitidine reverses resistance to cabazitaxel in cabazitaxel-resistant prostate cancer cells. DU145 wild-type and drug-resistant cells were seeded in 96-well plates and treated with 1 μM 5-azacytidine for 72 h followed by treatment with cabazitaxel for 72 h. Cell viability was measured after 24, 48 and 72 h using Cell Titer Blue. Pre-treatment with 5-azacytidine resulted in increased cytotoxicity of cabazitaxel. DU145 wt cells treated with a combination of 5-azacytidine and cabazitaxel showed 18.43% increase in cell death compared to cells treated with 10 nM cabazitaxel alone. DU145 10DR cells treated with a combination of 5-azacytidine and cabazitaxel showed 26.19% increase in cell death compared to cells treated with 10 nM cabazitaxel with no 5-azacytidine pre-treatment. DU145 10DRCR cells treated with 5-azacytidine alone showed 21% cell death compared to cells that were left untreated. DU145 10DRCR cells pre-treated with 5-azacytidine showed a 31% increase in cell death when treated with 10 nM cabazitaxel compared to cells with no pre-treatment (Figure 3). This shows that pre-treatment with 5-azacytidine enhances sensitivity to cabazitaxel and reverses resistance to cabazitaxel, to some extent, in DU145 10DRCR cells.

Gene expression profiling to identify epigenetically-regulated genes in cabazitaxel-resistant cells. To identify the genes that are epigenetically regulated and may contribute to cabazitaxel resistance, gene expression profiling in DU145 10DRCR and DU145 10DR with and without 5-azacytidine treatment was performed using the Illumina HumanHT-12 Expression BeadChip. Differential gene expression analysis was performed using Partek Genomic Suite v.6.6 software. Probes filtered for False Discovery Rate (FDR)-corrected *p*-value <0.05 and fold change >1.5 and <-1.5 were used to generate the Venn Diagram (Figure 4). The intersect,

Table I. *List of genes potentially regulated by DNA methylation that may contribute to cabazitaxel resistance in Du145 prostate cancer cells.*

Gene	Description	KEGG Pathways	Fold change DU145 10DRCR *vs.* DU145 10DR	Fold change DU145 10DRCR *vs.* DU145 10DRCR+Aza
DVL1	Dishevelled, dsh homolog 1 (Drosophila)	Cancer, Wnt Signalling pathway	–2.11	2.67
TRAF1	TNF receptor-associated factor 1	Cancer, Small cell lung cancer	–2.27	2.00
BIRC3	Baculoviral IAP repeat containing 3	Cancer, NOD-like receptor signaling pathway, small cell lung cancer, focal adhesion, toxoplasmosis	–1.68	1.65
PDGFB	Platelet-derived growth factor beta polypeptide	Cancer, MAPK signaling pathway, glioma, melanoma, cytokine-cytokine receptor interaction, prostate cancer, gap junction, focal adhesion	–3.96	1.76
CDK6	Cyclin-dependent kinase 6	Cancer, small cell lung cancer, glioma, p53 signaling pathway, melanoma, non-small cell lung cancer, pancreatic cancer, cell cycle	–2.1	2.2
IL8	Interleukin 8	Cancer, NOD-like receptor signaling pathway, cytokine-cytokine receptor interaction, toll-like receptor signaling pathway, bladder cancer, hepatitis C, epithelial cell signaling in *H. pylori* infection, rheumatoid arthritis, amoebiasis	–4.18	3.24
EGFR	Epidermal growth factor receptor	Cancer, MAPK signaling, glioma, melanoma, cytokine-cytokine receptor interaction, calcium signaling, prostate cancer, gap junction, focal adhesion, bladder cancer, hepatitis C, non-small cell lung cancer, epithelial cell signaling in *H. pylori* infection, pancreatic cancer, adherens junction, GnRH signaling	–1.56	1.69
LAMB3	Laminin, beta 3	Cancer, small cell lung cancer, focal adhesion, toxoplasmosis, amoebias	–4.15	1.57
ATF4	Activating transcription factor 4 (tax-responsive enhancer element B67)	MAPK signaling, prostate cancer, protein processing in endoplasmic reticulum, GnRH signaling, neurotrophin signaling	–1.59	3.41
GADD45A	Growth arrest and DNA-damage-inducible, alpha	MAPK signaling, p53 signaling, cell cycle	–1.55	1.59
DUSP5	Dual specificity phosphatase 5	MAPK signaling	–1.93	2.2
DDIT3	DNA-damage-inducible transcript 3	MAPK signaling, protein processing in endoplasmic reticulum	–1.6	4.47
DUSP1	Dual specificity phosphatase 1	MAPK signaling	–2.64	3.73
CXCL2	Chemokine (C-X-C motif) ligand 2	NOD-like receptor signaling, cytokine-cytokine receptor interaction	–1.51	4.58
TNFAIP3	Tumor necrosis factor, alpha-induced protein 3	NOD-like receptor signaling	–2.2	1.92
PSAT1	Phosphoserine aminotransferase 1	Glycine, serine and threonine metabolism, metabolic pathways	–1.8	3.35
PHGDH	Phosphoglycerate dehydrogenase	Glycine, serine and threonine metabolism, metabolic pathways	–3.18	5.01
PSPH	Phosphoserine phosphatase	Glycine, serine and threonine metabolism, metabolic pathways	–1.5	1.7
GFPT1	Glutamine--fructose-6-phosphate transaminase 1	Alanine, aspartate and glutamate metabolism, metabolic pathways	–1.53	2.48
B3GALNT1	Beta-1,3-N-acetylgalactosaminyl-transferase 1 (globoside blood group)	Metabolic pathways	–1.86	2.2
MTHFD2	Methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase	Metabolic pathways	–1.7	3.47
ASNS	Asparagine synthetase (glutamine-hydrolyzing)	Alanine, aspartate and glutamate metabolism, metabolic pathways	–2.52	4.56

Table I. *Continued*

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Gene	Description	KEGG Pathways	Fold change DU145 10DRCR *vs*. DU145 10DR	Fold change DU145 10DRCR *vs*. DU145 10DRCR+Aza
AMY2B	Amylase, alpha 2B (pancreatic)	Metabolic pathways, pancreatic secretion	–236.1	116.8
GCLC	Glutamate-cysteine ligase, catalytic subunit	Metabolic pathways	–2.41	1.78
ITPKA	Inositol-trisphosphate 3-kinase A	Calcium signaling, metabolic pathways	–1.64	2.06
BST1	Bone marrow stromal cell antigen 1	Metabolic pathways, calcium signaling, nicotinate and nicotinamide metabolism, pancreatic secretion	–8.51	3.32
NT5E	5'-nucleotidase, ecto (CD73)	Metabolic pathways, nicotinate and nicotinamide metabolism		–2.21.58
CARS	Cysteinyl-tRNA synthetase	Aminoacyl-tRNA biosynthesis	–1.55	2.75
MARS	Methionyl-tRNA synthetase	Aminoacyl-tRNA biosynthesis	–1.6	2.88
IARS	Isoleucyl-tRNA synthetase	Aminoacyl-tRNA biosynthesis	–1.69	2.12
PMAIP1	Phorbol-12-myristate-13-acetate-induced protein 1	p53 signaling	–1.99	2.24
TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	Cytokine-cytokine receptor interaction	–1.58	1.69
PTGER1	Prostaglandin E receptor 1 (subtype EP1), 42kDa	Calcium signaling	–2.24	2.63
TUBB2B	Tubulin, beta 2B class IIb	Gap junction, pathogenic *E. coli* infection, phagosome	–13.73	14.61
LY96	Lymphocyte antigen 96	Toll-like receptor signaling, toxoplasmosis, pathogenic E.coli infection,	–3.20	1.59
TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein	Toll-like receptor signaling	–1.58	1.51
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa	Hepatitis C	–1.64	2.25
ERN1	Endoplasmic reticulum to nucleus signaling 1	Protein processing in endoplasmic reticulum	–1.57	1.69
SNAI2	Snail homolog 2 (Drosophila)	Adherens junction	–3.30	3.24
SLC3A2	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	Protein digestion and absorption	–1.72	3.53
SLC1A5	Solute carrier family 1 (neutral amino acid transporter), member 5	Protein digestion and absorption	–1.78	2.67
CTSL1	Cathepsin L1	Rheumatoid arthritis, phagosome	–1.83	2.34
ZNF274	Zinc finger protein 274	Neurotrophin signaling pathway	–1.72	2.36
FOSL1	FOS-like antigen 1	Osteoclast differentiation, Wnt signaling	–2.09	2.37
FHL2	Four and a half LIM domains 2	Osteoclast differentiation	–1.73	1.82

comprising of 183 genes represents genes that are potentially epigenetically regulated and is derived from the overlap of genes that have reduced expression in DU145 10DRCR cells compared to DU145 10DR cells (719 genes) and genes whose expression is increased upon azacitidine treatment in DU145 10DRCR cells (1,111 genes). Transcripts from the intersect of the Venn Diagram were imported into WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt/) and KEGG pathways enriched (FDR<0.1) in the list of transcripts were identified (Table I).

We observed that pre-treatment with 5-Azacytidine enhances sensitivity of DU145 10DRCR cells to cabazitaxel indicating the contribution of methylation-mediated regulation of genes in cabazitaxel resistance in these cells. Through gene expression profiling, we identified 183 epigenetically regulated genes in cabazitaxel resistance. Pathway analysis showed that these genes were involved in MAPK signaling, p53 signaling, GnRH signaling, Gap junction, cytokine-cytokine receptor interaction, focal adhesion, cell cycle, Wnt signaling pathways *etc*.

Discussion

To our knowledge, this is the first report on cabazitaxel resistance in prostate cancer cells. To date, there exist only published reports on genes involved in docetaxel resistance in prostate cancer. We found that pre-treatment of DU145 10DRCR cells with 5-azacytidine enhances sensitivity to cabazitaxel. This result suggests that DNA methylation-mediated silencing of genes may play a role in resistance of

DU145 cells to cabazitaxel. Previous studies from our lab have demonstrated the role of epigenetic silencing of pro-apoptotic and tumor suppressor genes in development of resistance to chemotherapeutic drugs. We showed that GADD45a, a gene involved in apoptosis and cell cycle regulation, plays a role in docetaxel sensitivity in DU145 prostate cancer cells. GADD45a is silenced by DNA methylation in DU145 cells as well as in prostate cancer tissues. Up-regulation of GADD45a either by recombinant gene expression or by treatment with 5-azacytidine resulted in increased sensitivity to docetaxel chemotherapy (15). Following this, we conducted a phase I/II clinical trial to check the safety and efficacy of 5-azacytidine, docetaxel and prednisone in patients with docetaxel refractory metastatic castration resistant prostate cancer. Our results showed that this combination is active in these patients (20). In a recently published study, we showed that GADD45a is frequently methylated in serum of prostate cancer patients compared to patients with benign prostatic disease and can be a useful marker in distinguishing benign from prostate cancer patients (17). We have also demonstrated the role of TMS1, a pro-apoptotic gene, in sensitivity of bladder cancer cells (16) and breast cancer cells (5) to chemotherapeutic agents.

Our results indicated that DU145 wt cells were more sensitive to cabazitaxel compared to DU145 10DR cells. For instance, at a concentration of 0.6 nM, we observed 80% cell death in DU145 wt cells compared to only 29% cell death in DU145 10DR cells. Similarly, when treated with 5 nM cabazitaxel, we found 92% cell death in DU145 wt cells compared to 83% in DU145 10DR cells. Hence, there seems to be an inherent resistance to cabazitaxel in docetaxel-resistant cells compared to DU145 wt. Since docetaxel and cabazitaxel are both taxane drugs, possible mechanism of the cross-resistance could be the involvement of same pathways and genes in sensitivity to docetaxel and cabazitaxel.

Gene expression profiling revealed potential genes and pathways involved in cabazitaxel resistance in DU145 cells. Although we found several genes that were over-expressed and under-expressed in DU145 10DR CR cells compared to parent DU145 10DR cells, we primarily focused on genes that were regulated by DNA methylation. For this reason, we analyzed the overlap of genes having reduced expression in DU145 10DRCR cells compared to DU145 10DR cells (719 genes) and the genes whose expression is increased upon azacitidine treatment in DU145 10DRCR cells (1,111 genes). We found 183 genes that were potentially epigenetically regulated based on this analysis. Out of these KEGG pathways were identified for 45 genes. The pathways included prostate cancer, MAPK signaling, metabolism, p53 signaling, gap junction, toll-like receptor signaling, Wnt signaling *etc*. DVL1, the human homolog of the Drosophila dishevelled gene (dsh) is a cytoplasmic mediator of the Wnt/b-catenin signaling pathway, that is critical for embryonic development, stem-cell maintenance, and oncogenesis (24). DVL cascade is related to apoptosis in several cell types, and is linked to the aberrant activation of Wnt/b-catenin signaling (23). It has also been shown that DVL1 contributes to cyclosporine-induced apoptosis in cardiomyoblast cells (26). Our results showed that DVL1 is re-activated by azacitidine treatment in DU145 10DRCR cells indicating that DNA methylation may be a possible mechanism of regulation of DVL1 and may contribute to cabazitaxel resistance in DU145 cells. DVL1 has been reported to be methylated in ovarian cancer and increased methylation is associated with an increased risk of disease progression and poor response (1). Another gene of interest is CDK6, that encodes a member of the cyclin-dependent protein kinase (CDK) family, which are known to be important regulators of cell-cycle progression (4). This kinase is a catalytic subunit of the protein kinase complex that is important for cell-cycle G_1 phase progression and G_1/S transition. This kinase has been shown to phosphorylate, and thus regulate the activity of the tumor suppressor protein Rb (10). Sun *et al*. reported that down-regulation of CDK6 results in cell-cycle arrest in lung cancer cells (21). Consistent with this, Huang *et al*. showed that selective and reversible inhibition of CDK4/CDK6 inhibits proliferation and enhances bortezomib-induced cytotoxic killing of cancer cells and suggested that reversible inhibition of CDK4/CDK6 in sequential combination therapy, thus, represents a novel mechanism-based cancer therapy (6). However, a recent study showed that overexpression of CDK6 causes p53-dependent apoptosis (8). Since *CDK6* expression is restored by treatment with 5-azacytidine indicating that the gene may be down-regulated by CpG methylation and may confer cabazitaxel resistance in DU145 cells. *IL8* and *TUBB2B* have been found to be under-expressed in docetaxel-resistant DU145 and PC3 cells and may have a role in docetaxel resistance in these cells (11). Interestingly, we present indirect evidence that *IL8* and *TUBB2B* may be regulated by DNA methylation in cabazitaxel-resistant cells indicating that they may confer cross-resistance to docetaxel and cabazitaxel. De Larco *et al*. showed that *IL8* gene expression is regulated by methylation of two CpG sites upstream in the gene in breast cancer. Contrary to the common epigenetic paradigm in which methylation of promoter CpG islands silences gene expression, the authors found that increased methylation of these 2 sites resulted in overexpression of IL8 (3).

Another gene that may confer cross-resistance to docetaxel and cabazitaxel is *GADD45a*. As mentioned above, we have previously shown the role of *GADD45a* in docetaxel sensitivity in prostate cancer (15, 16). Other genes of interest include *ATF4, DDIT3, DUSP5* and *DUSP1* that play a role in MAPK signaling and may play role in apoptosis in response to cabazitaxel treatment. *DDIT3* is hypermethylated in A2780 ovarian cancer cells and thought to contribute to cisplatin

resistance (25). It has been shown that silencing of *DUSP5* by promoter hypermethylation causes increased maintenance of phosphorylated ERK1/2, drives cell proliferation and contributes to gastric carcinogenesis (18). Khor *et al*. showed that *DUSP1* was hypermethylated in oral squamous cell carcinoma compared to normal tissues and was a potential diagnostic, prognostic and therapeutic target (9). PMAIP1 is a pro-apoptotic member of the Bcl-2 protein family. The expression of PMAIP1 is regulated by the tumor suppressor p53 and has been shown to be involved in p53-mediated apoptosis (12). Our results showed that PMAIP1 may have a role in cabazitaxel sensitivity in prostate cancer. Interestingly, Putnik *et al*. found that although PMAIP1 expression increased with treatment of breast cancer cells with 2-azadeoxycytidine, the promoter was not methylated in untreated cells (14). This suggested that 5-aza-deoxycytidine regulates the expression of these genes either *via* de-methylation of other methylated DNA regions, such as CpG shores, shelves and open seas (7), or indirectly, through de-methylation of other genes. We have previously reported a similar finding on GADD45a in prostate cancer. The promoter region of *GADD45a* is unmethylated. However, gene expression is regulated by the methylation of 4 CpGs situated ~700 bp upstream of the transcription start site.

Our results indicate that one of the mechanisms of cabazitaxel resistance in prostate cancer is methylation-mediated silencing of tumor suppressor and pro-apoptotic genes. Furthermore, resistance to cabazitaxel can be reversed by treatment with a de-methylating agent such as 5-Azacytidine. Further studies on genes identified in the present study may lead to a better understanding of mechanisms of cabazitaxel resistance in prostate cancer. Although the scenario of management of metastatic castration-resistant prostate cancer has changed considerably over recent years with the availability of several treatment options, patients eventually stop responding to these treatments. A clinical trial to test the efficacy of combination treatment with 5-azacytidine and cabazitaxel may be useful as an alternative option for these patients.

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Conflicts of Interest

The Authors have no conflicts of interest to disclose.

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