Cancer Insight

ANSER PRESS

Article

OPEN ACCESS

Effect of interleukin-8 on docetaxel resistance in prostate cancer cells: insights into the role of multidrug resistance 1 protein modulation

Suleyman ILHAN [a,*]

[a] *Manisa Celal Bayar University, Faculty of Science and Letters, Biology Departmen Manisa, Türkiye*

ABSTRACT

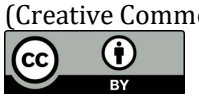
Although docetaxel treatment yields a high survival rate for prostate cancer (PCa), resistance eventually develops in many patients. Understanding the underlying mechanisms of docetaxel resistance is essential for improving treatment strategies. Cytokines, which play a role in cell signaling and immune responses, have been implicated in drug resistance mechanisms. The study revealed that interleukin-8 (IL-8) was consistently overexpressed in both docetaxel-resistant PCa cell lines. Thus, the expression levels of cytokines released from docetaxel-sensitive (PC-3- and DU-145) and resistant (PC-3/R-DU-145/R) PCa cells were compared. IL-8 was found to be commonly expressed in resistant cell lines. This finding led to the hypothesis that IL-8 could play a key role in mediating PCa cell resistance to docetaxel. IL-8 siRNA treatment increased docetaxel sensitivity in both resistant cells. To demonstrate the mechanism of IL-8-related resistance, MDR1 expression was evaluated. After IL-8 siRNA treatment MDR1 expression was reduced in both resistant cells suggesting that IL-8 regulates the docetaxel resistance of PCa cells via modulation of multidrug resistance 1 (MDR1). By expanding the knowledge of the cytokines and their effect mechanisms, novel approaches can be developed for the treatment of docetaxel-resistant prostate cancer. Further investigations into the role of IL-8 in docetaxel resistance could offer valuable insights into the development of effective treatment strategies for PCa patients.

KEYWORDS

Docetaxel resistance; Cytokine; IL-8; Prostate cancer; MDR1

*Corresponding author: Suleyman ILHAN
E-mail address: suleyman.ilhan@cbu.edu.tr

ISSN 2972-3388



Received May 20, 2023 | Accepted June 7, 2023 | Available online June 14, 2023

1. Introduction

One of the most prevalent cancers among men expected to be diagnosed is prostate cancer (PCa) [1]. Although the survival rate is high when diagnosed in the early stages, late diagnosis reduces the chance of a cure significantly [2]. Using imaging techniques and biomarkers for prognosis, improved surgical techniques and more importantly timely administration of docetaxel have improved the survival rate considerably [3]. Docetaxel is a partly-synthetic taxane analog obtained from the Western yew tree (*Taxus brevifolia*) extract [4]. It is being used as an applicable therapy for androgen-independent PCa for a long time [5, 6]. While the survival rate is high when diagnosed at early stages, a substantial number of PCa patients develop castrate-resistant prostate cancer (CRPC) in time [7]. Castrate-resistant PCa, also known as androgen-insensitive PCa, is advanced prostate cancer whose progression continues regardless of the androgen depletion treatment [8]. Despite docetaxel being recognized as one of the highly effective chemotherapeutic agents for PCa, most people eventually acquire resistance to this treatment [7]. The drug resistance of DOC limits its therapeutic use in the treatment of advanced CRPC. Therefore, an improved comprehension of the mechanisms driving DOC resistance would be extremely beneficial in terms of its clinical application.

Multiple chemotherapeutic treatments can become ineffective against malignancies due to a condition known as multidrug resistance (MDR). Several mechanisms have been linked to the growth of cancer cell resistance to chemotherapy including changes in drug metabolism and transport, increase in DNA repair and dysregulated apoptosis. The upregulation of MDR transporters is a prevalent mechanism through which cells develop MDR. These membrane transporters belong to the ABC superfamily of ATP-binding cassettes. Extensive research has identified MDR1 and MRP1 as the most frequently implicated transporters in drug resistance.

In recent studies, uncontrolled cytokine expression has emerged as a significant element in numerous drug resistance mechanisms. Despite our limited comprehension of how cytokines precisely impact drug resistance, they hold potential as valuable therapeutic targets and biomarkers. Therefore, further investigations into their roles in these contexts are urgently warranted. To enhance our understanding of cancer drug resistance mechanisms and facilitate treatment outcome predictions, it becomes crucial to uncover the associations between comprehensive cytokine profiles and drug resistance in cancer.

For this aim, in this study, comparative cytokine profiles of docetaxel-sensitive and resistant PCa cells were determined. Since IL-8 is over-expressed in both resistant cell lines, we hypothesized that IL-8 might serve as a pivotal cytokine involved in PCa cells' resistance to DOC and investigated the role of IL-8 on DOC resistance.

2. Materials and methods

2.1. Cell culture

For this research, two distinct human PCa cell lines, specifically PC-3 and DU-145, were employed. These cell lines were obtained from Interlab, Italy. The cells were cultured in RPMI 1640 medium, which was 10% heat-inactivated fetal bovine serum, 1% heat-inactivated penicillin-streptomycin, and 1% L-glutamine added as supplements. To establish DOC-resistant cell lines derived from two cell lines used, denoted as DU-145/R and PC-3/R, the cells were gradually exposed to escalating concentrations of DOC ranging from 0.1 nM to 1 nM and eventually to 10 nM over a period of 180 days. This prolonged exposure enabled the cells to acquire the ability to proliferate even in the presence of 10 nM DOC. The cell lines were cultured as monolayers in 75 cm^2 polystyrene

flasks inside a humidified incubator that is set to 37 °C and 5% CO_2. The growth rate and cellular morphologies were observed daily using a Zeiss Primovert microscope from Germany, and the cells were passaged once they reached 90% confluence.

2.2. Viability testing of cells

The MTT assay was used to determine the vitality of the cells. The cells were placed in a 96-well plate at a density of 10^4 cells per well and treated to DOC at increasing concentrations for 72 h. Following the incubation, 20 µL of the MTT solution was added to each well, and allowed to incubate at a temperature of 37 °C for 4 h. Afterward, dissolving the formazan crystals required 100 µL of dimethyl sulfoxide (DMSO), which was added after the medium was taken out of the equation. The OD values were recorded at a wavelength of 570 nm using a spectrophotometer (Tecan Infinite). Then, the IC_{50} values for both the DOC-sensitive and DOC-resistant prostate cancer cells were calculated utilizing Biosoft CalcuSyn version 2.0 software.

2.3. Cytokine profiling

A membrane antibody array (AAH-CYT-1-8, Raybiotech, GA, USA) which detects 23 cytokines was used to profile the cytokines released from DOC-sensitive and -resistant prostate cancer cells. The protocol was executed following the guidelines provided by the manufacturer. In summary, each well received a blocking buffer, which was then left there for 30 min at RT. Following the incubation, the membrane was washed twice with the array wash buffer. Next, the antibody membranes were placed in 1 mL of conditioned solution, and incubated overnight at 4 °C. After washing twice, biotinylated antibody cocktail incubation and horseradish peroxidase-conjugated streptavidin incubation steps were followed. Chemiluminescent imaging of the membrane was performed via UVP BioSpectrum® 610 Imaging System.

2.4. siRNA transfection

To prepare the IL-8 siRNA (FlexiTube Qiagen) master stock, 100 µM (100 pmol/ml) master stock was prepared by adding 50 µL of nuclease-free dH_2O to a lyophilized IL-8 siRNA (40 nmol) tube. The main stock was diluted 1:10 to obtain an intermediate stock at a concentration of 10 µM. For IL-8 siRNA transfection, $2x10^5$ cells were propagated onto 24-well plates in 500 µL media, and for 24 h the plates were incubated at 37°C. The transfection solution and siRNA solution were prepared in accordance with the directions of the manufacturer. The transfection solution was mixed with 3 µL of Lipofectamine and 50 µL of Opti-MEM. The siRNA solution was mixed 1 µL of IL-8 siRNA duplex and 50 µL of Opti-MEM. The transfection solution and siRNA solution were mixed at a ratio of 1:1 and 100 µL of the siRNA-lipofectamine agent was added to wells as drops, and plates were incubated for 48 hours. For the control of IL-8 siRNA application, IL-8 mRNA amounts in cells were investigated by the qPCR method. For the control of siRNA application in cells, 20 nM GAPDH siRNA was applied to melanoma cells and the effects of siRNA on GAPDH expression were evaluated by qPCR method. AllStars positive siRNA (PK-siRNA) and AllStars Negative Control siRNA (NK-siRNA) were used as controls.

2.5. Western blot analysis

To conduct protein isolation, the Mammalian Protein Extraction Reagent (M-PER) (Thermo) was employed. Initially, the cells were rinsed with phosphate-buffered saline and then treated with 250 µL of the M-PER reagent.

The subsequent step involved centrifuging the cells at a speed of 14,000 g for a duration of 15 minutes to obtain a total protein suspension. The Bradford technique was used to determine the protein content. Following that, proteins were isolated using polyacrylamide gel electrophoresis (PAGE) in equal proportions, and the gels were run at a voltage of 120 V for 2 hours. Subsequently, the isolated proteins were placed onto nitrocellulose membranes (Bio-Rad), utilizing an electric field of 115 V for 75 minutes. To prevent nonspecific binding, the membranes were blocked with a blocking buffer that contained 5% non-fat dried milk in TBS with 0.1% Tween20 for a duration of 1 hour. Once the blocking process was completed, the membranes were incubated overnight at 4°C with primary antibodies against IL-8 (dilution 1:500; abcam), MDR1 (dilution 1:1000; abcam), and p-p65 (dilution 1:500; abcam). After the overnight incubation with primary antibodies, secondary antibodies (dilution 1:2000) were applied for 1 hour. The membranes were then washed and images were taken via gel imager.

2.6. qRT-PCR analysis

Real-time PCR was employed to quantify the expression levels of the target genes, namely IL-8, MDR1, and GAPDH. The specific primers used in this study were designed to amplify the target genes, IL-8, MDR1, and GAPDH. The sequences of the forward and reverse primers are as follows: IL-8: Forward Primer: 5′-CAC CTC AAG AAC ATC CAG AGC T-3′; Reverse Primer: 5′-CAA GCA GAA CTG AAC TAC CAT CG-3′; MDR1: Forward Primer: 5′-CCC ATC ATT GCA ATA GCA GG-3′; Reverse Primer: 5′-TGT TCA AAC TTC TGC TCC TGA-3′; GAPDH: Forward Primer: 5′-GCACCG TCA AGG CTG AGA AC-3′; Reverse Primer: 5′-TGG TGAAGA CGC CAG TGG A-3′.

2.6.1. cDNA Synthesis

Total RNA was extracted from the target cells/tissues using a commercially available RNA extraction kit following the manufacturer's instructions. Subsequently, we used a reverse transcription kit to synthesis cDNA according to the provided protocol.

2.6.2. qRT-PCR

Real-time PCR amplification was carried out using a Light Cycler 480 (Roche) real-time PCR system. The reaction mixture was prepared using an SYBR Green-based master mix. Each reaction consisted of 1 µL cDNA template, forward and reverse primers (10 µM), and 12.5 µL SYBR Green master mix. A total of 20 µL was the reaction volume. The conditions for real-time PCR's thermal cycling amplification were as follows: initial denaturation at 95°C for 10 min, followed by denaturation at 95°C for 15 sec, annealing at 60°C for 1 min, and extension at 72°C for 40 cycles. A final extension step at 72°C was included. The quantification cycle values obtained from the qRT-PCR experiments were screened. Using the comparative Ct technique, relative expression levels of the target genes were estimated with GAPDH as the reference gene.

2.7. Statistical analysis

Dunnett's t-test and ANOVA were used to statistically analyze the data. To analyze the findings, GraphPad Prism 5 software was utilized, which allowed for the creation of informative graphs. Statistical significance was determined based on a p-value threshold of less than 0.05, indicating results that were considered statistically significant.

3. Results

3.1. Development of DOC-resistant prostate cancer cells

Doc sensitivity of DU-145/R and PC-3/R cells and their parental cells DU-145 and PC-3 cells were evaluated via calculating IC_{50} values. The IC_{50} values of DOC were 0.61 ± 0.4 and 67 ± 2.4 nM in DU-145 and DU-145/R cells, respectively, at 72 h (**Figure 1A**). The IC_{50} values of DOC were 0.57 ± 0.2 and 62 ± 2.1 nM in PC-3 and PC-3/R cells, respectively, at 72 h (**Figure 1B**). The sensitivity of both DU-145/R and PC-3/R cells to DOC was approximately 10 times lower than parental cells. Our findings confirmed that DOC-resistant PCa cells had been successfully developed. Graphs representing % cell viability versus the logarithm of concentration are presented in **Figures 1C and 1D**.

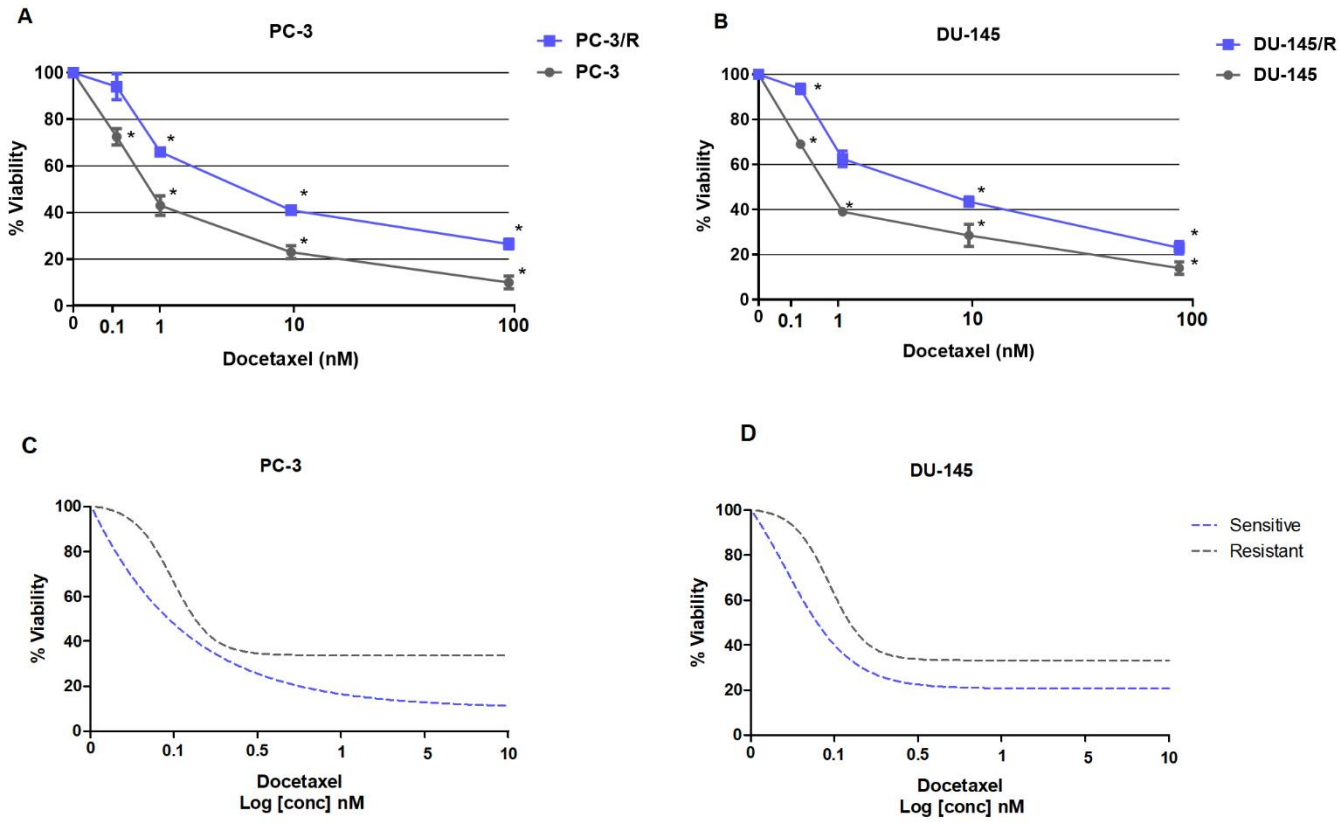


Figure 1. Cell viability of (A) PC-3, PC-3/R, (B) DU-145, and DU-145/R cells after treatment of Docetaxel at 72 h (*p<0.05). (C) and (D) Graphs representing % cell viability versus logarithm of the concentration.

3.2. IL-8 expression is significantly elevated in both DOC-resistant PCa cells

As many studies have shown that cytokines are involved in cancer cell chemoresistance, we first determined and compared the expression levels of cytokines released from DOC-sensitive (PC-3-DU-145) and DOC-resistant (PC-3/R-DU-145) prostate cancer cells (**Tables 1 and 2**). Array analyses showed that DOC-sensitive PC-3 and DU-145 PCa cells have different cytokine profiles. It was shown that DOC-sensitive PC-3 cells mainly express angiogenin, TIMP-1, TIMP-2, EGF, GROα, MCP-1 and, IL-8, however, DU-145 cells express RANTES, GROα, IL-6, MCP-1, TIMP-1, IGF-1, TIMP-2, and IL-8 angiogenic cytokines (**Table 1**). Cytokines expressed by both DOC-sensitive and -resistant cells were MCP-1, TIMP-1, GROα, TIMP-2, and IL-8. Resistant PC-3/R cells express

GROα, IGF-1, IL-6, IL-8, MCP-1, angiogenin, and VEGF, however, resistant DU-145/R cells express TIMP-1, TIMP-2, angiogenin, and IL-8 cytokines (**Table 2**). Comparative analysis of PC-3 and PC-3/R cells revealed significant differences in the expression levels of MCP-1, GROα, and IL-8 cytokines. In DU-145 and DU-145/R comparative analysis, only and TIMP-1, TIMP-2 and IL-8 cytokines were differentially expressed.

Results were also verified using qRT-PCR and western blot analysis. IL-8 expression is significantly increased in both DOC-resistant PCa cells (**Figures 2A and 2B**). As IL-8 is significantly increased in both DOC-resistant cell lines, we hypothesized that IL-8 could be an important molecule involved in the resistance of PCa cells to DOC.

Table 1. Comparison of the cytokine expression levels in DOC-sensitive PC-3 (PC-3) and DOC-resistant PC-3 (PC-3/R) cells (*p<0.05).

	Spot pixel main value ±SD	
Cytokine	PC-3	PC-3/R
GCSF	58 ± 2.5	65 ± 0.8
GM-CSF	78 ± 2.1	82 ± 1.3
GRO alpha/beta/gamma	106 ± 2.6	208 ± 3.2
GRO alpha	87 ± 2.4	228 ± 1.6*
IL-1 alpha	20.4 ± 1.8	26 ± 2.0
IL-2	25 ± 2.0	28 ± 1.8
IL-3	32 ± 1.2	38 ± 2.2
IL-5	28 ± 1.1	34 ± 0.4
IL-6	60 ± 2.1	118 ± 1.2
IL-7	30 ± 1.4	35 ± 3.7
IL-8	90 ± 2.4	320 ± 2.1*
IL-10	27 ± 0.8	29 ± 0.4
IL-13	22 ± 0.4	30 ±1.2
IL-15	23 ± 1.2	42 ± 0.2
IFN-gamma	44 ± 1.6	48 ± 1.4
MCP-1	102 ± 2.1	201 ± 2.4*
MCP-2	98 ± 1.8	106 ± 1.0
MCP-3	42 ± 0.6	50 ± 0.8
MIG	20 ± 1.2	25 ± 1.2
RANTES	80 ± 1.4	82 ± 0.6
TGF beta 1	120 ± 2.6	142 ± 1.2
TNF alpha	95 ± 2.4	102 ± 3.2
TNF beta	45 ± 2.1	52 ± 1.0

Table 2. Comparison of the cytokine expression levels in DOC-sensitive(DU-145) and DOC-resistant (DU-145/R) cells (*p<0.05).

	Spot pixel main value ±SD	
Cytokine	DU-145	DU-145/R
GCSF	25 ± 0.8	32 ± 2.6
GM-CSF	42 ± 1.2	48 ± 1.0
GRO alpha/beta/gamma	109 ± 2.0	212 ± 2.5
GRO alpha	95 ± 2.1	287 ± 3.4
IL-1 alpha	48 ± 1.4	62 ± 1.2
IL-2	35 ± 0.4	45 ± 0.2
IL-3	28 ± 2.6	36 ± 1.4
IL-5	24 ± 1.8	32 ± 1.6
IL-6	108 ± 1.2	122 ± 2.6
IL-7	26 ± 2.2	36 ± 1.2
IL-8	102 ± 2.8	325 ± 2.1*
IL-10	28 ± 3.2	32 ± 2.5
IL-13	26 ± 2.0	38 ± 0.4
IL-15	36 ± 1.0	46 ± 1.1
IFN-gamma	98 ± 0.4	232 ± 0.8*
MCP-1	102 ± 2.3	112 ± 3.1
MCP-2	45 ± 3.2	55 ± 2.1
MCP-3	49 ± 0.4	55 ± 1.5
MIG	22 ± 2.6	30 ± 2.0
RANTES	96 ± 1.2	286 ± 2.6*
TGF beta 1	87 ± 2.2	98 ± 0.6
TNF alpha	65 ± 0.8	70 ± 1.2
TNF beta	72 ± 2.1	85 ± 1.0

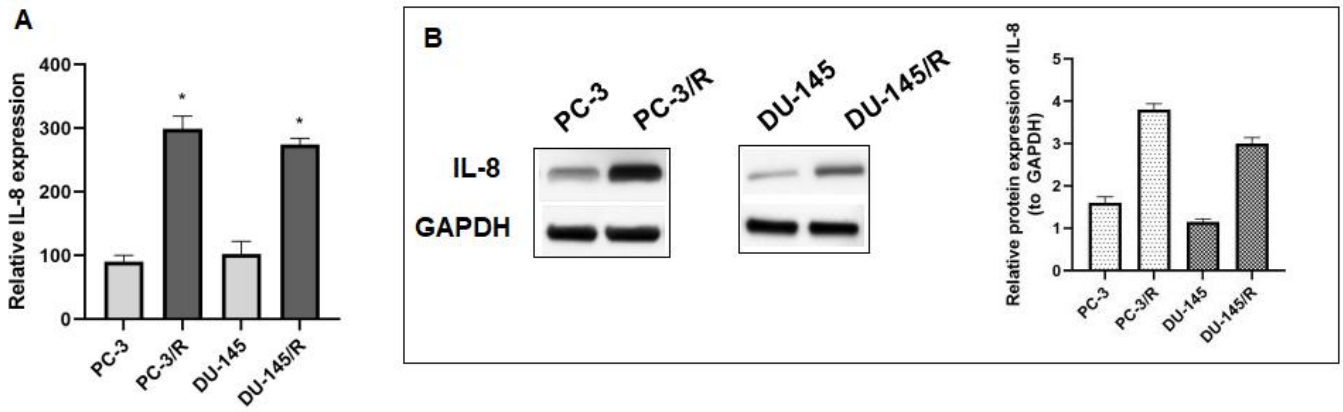


Figure 2. (A) The relative expression of IL-8 mRNA in DOC-sensitive (PC-3 and DU-145) and DOC-resistant (PC-3/R and DU-145/R) cells evaluated via qRT-PCR (*p<0.05). (B) Expression levels of IL-8 protein levels in DOC-sensitive (PC-3 and DU-145) and DOC-resistant (PC-3/R and DU-145/R) cells were detected by western blot analysis.

3.3. IL-8 is involved in the DOC resistance of PCa cells

The next step was to verify if IL-8 plays a role in the DOC-sensitivity of PCa cells. IL-8 siRNA was used to suppress its expression in PC-3/R and DU-145/R cells. IL-8 siRNA increased the DOC-sensitivity of both PC-3/R (IC_{50}: 11.5 ± 0.4 nM) and DU-145/R (IC_{50}: 15 ± 1.2 nM) cells (**Figure 3A and 3C**). Moreover, recombinant IL-8 (rIL-8) decreased the DOC sensitivity of PC-3 and DU-145 cells with the IC_{50} values 78.7 ± 2.6 nM and 84.7 ± 3.1 nM respectively ($p<0.05$) (**Figure 3B**). These data verified that IL-8 is involved in DOC resistance of PCa cells.

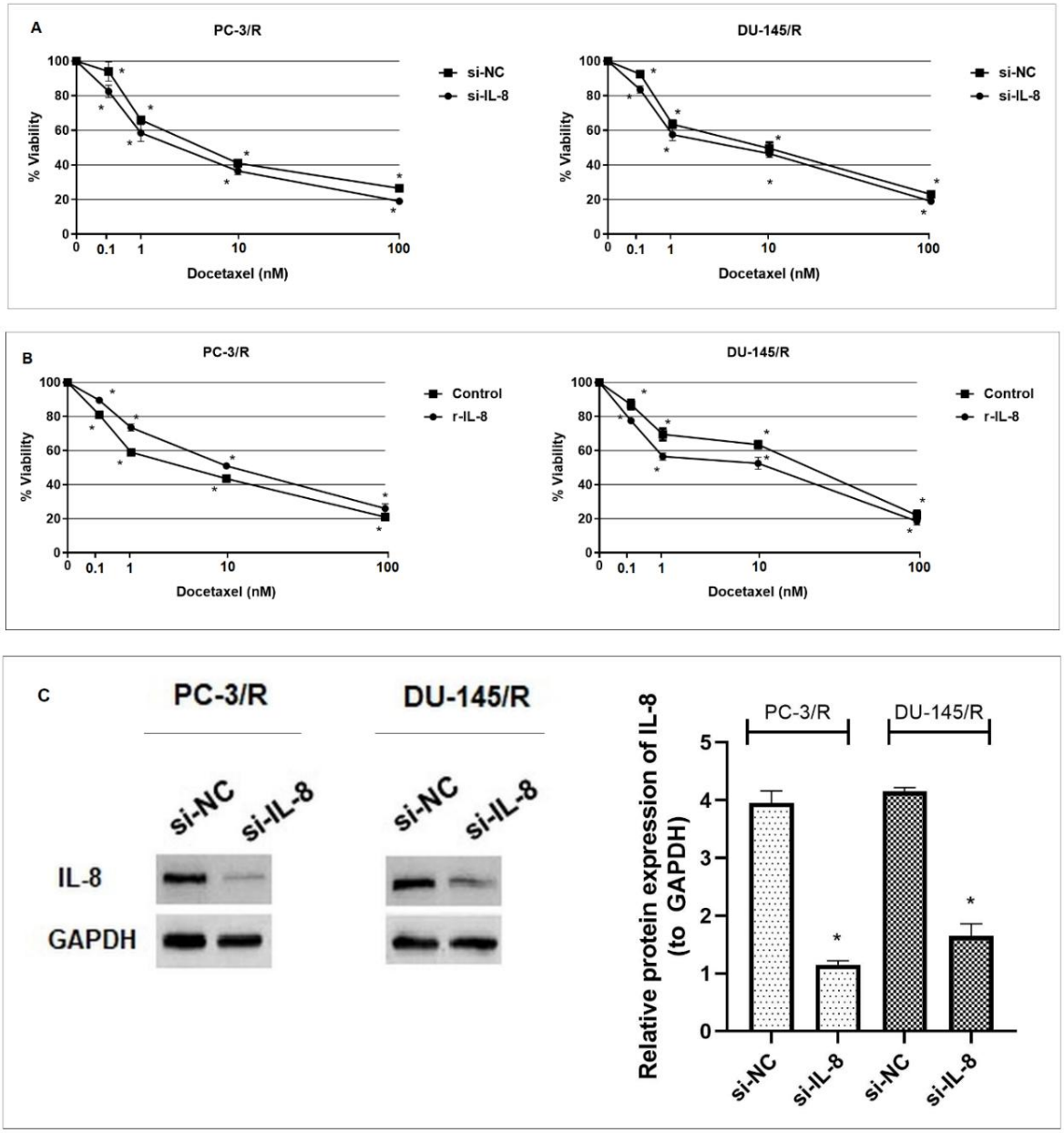


Figure 3. (A) PC-3/R and DU-145/R cells were transfected with negative control (NC) or si-IL-8 for 72 h and then further exposed to increasing concentrations of DOC for 48 h (*$p<0.05$). (B) PCa cells were pretreated with or without rIL-8 (40 nmol) for 90 min and then further exposed to increasing concentrations of DOC for 72 h (*$p<0.05$). (C) PC-3/R and DU-145/R cells were transfected with negative control (NC) or si-IL-8 for 72 h, and the expression of IL-8 was measured by western blot and quantified via Image J software.

3.4. IL-8 modulates the expression of MDR1 in PCa cells

To investigate the underlying mechanisms of IL-8-mediated DOC resistance, we examined the impact of IL-8 on the expression of MDR1 in prostate cancer (PCa) cells. Initially, the levels of MDR1 mRNA and protein were assessed in both DOC-sensitive and DOC-resistant PCa cells. Through qRT-PCR and western blot analysis, we observed a significant increase in MDR1 levels in DOC-resistant PCa cells compared to sensitive cells ($p<0.05$) (**Figure 4A**). To further elucidate the role of IL-8, siRNA targeting IL-8 was employed, which resulted in decreased expression of MDR1 mRNA and protein in both PCa cell lines (**Figure 4B**). Moreover, the introduction of recombinant IL-8 (rIL-8) led to elevated mRNA and protein levels of MDR1 in PC-3 and DU-145 cells, suggesting that IL-8 can regulate the expression of MDR1 in PCa cells (**Figures 4C and 4D**).

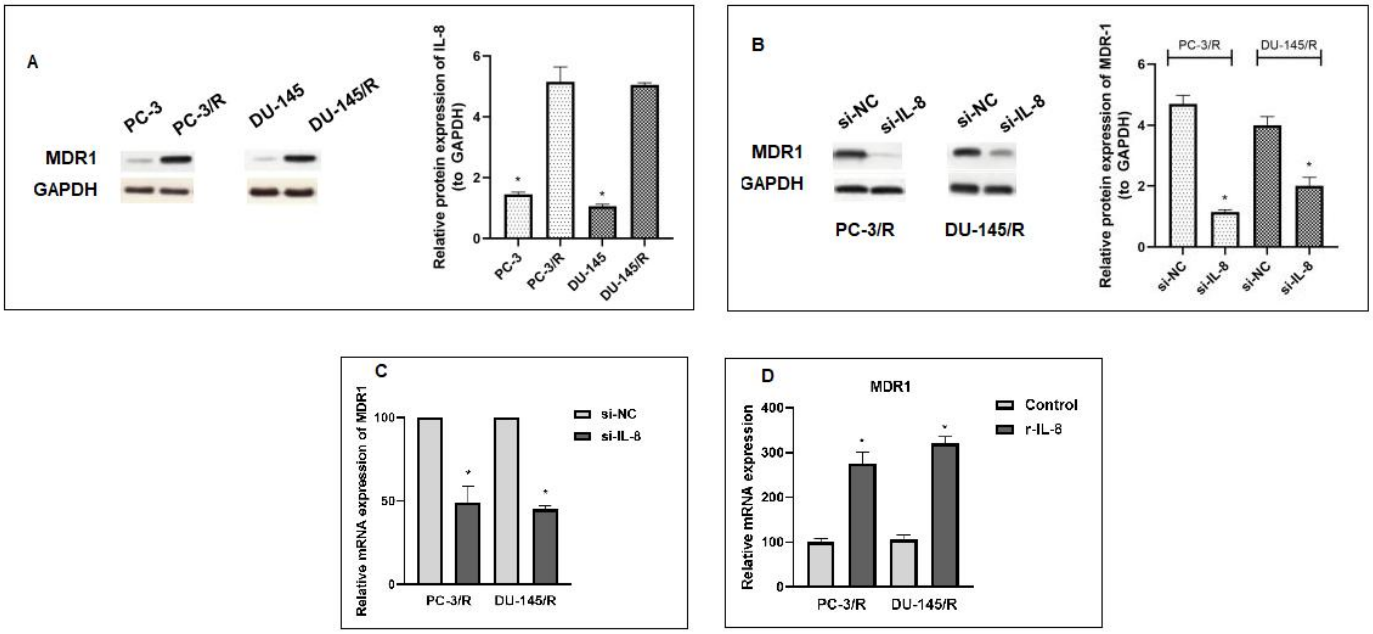


Figure 4. (A) The expression of MDR1 in PC-3/R and DU-145/R cells and their parental cells were measured by western blot analysis. (B) PC-3/R and DU-145/R cells were transfected with negative control (NC) or si-IL-8 for 72 h and then expression of MDR1 was measured by western blot analysis. (C) PC-3/R and DU-145/R cells were transfected with negative control (NC) or si-IL-8 for 72 h, and mRNA expression of MDR1 was measured by qRT-PCR analysis. (D) PC-3 or DU-145 cells were treated with or without rIL-8 (40 ng/ml) for 72 h, and mRNA expression of MDR1 was measured by qRT-PCR analysis. (*$p < 0.05$).

3.5. IL-8 regulates the transcription of MDR1 via activation of PI3K/AKT/NF-κB pathway

To elucidate the precise mechanisms underlying the IL-8-induced up-regulation of MDR1 in prostate cancer (PCa) cells, we conducted further investigations. Considering the established involvement of the PI3K/AKT/NF-κB pathway in the regulation of MDR1 expression, we explored its role in IL-8-mediated MDR1 up-regulation in PCa cells. Our results demonstrated that treatment with recombinant IL-8 (rIL-8) increased the phosphorylation of p65 in PC-3 and DU-145 cells (**Figure 5A**). Subsequently, we assessed the impact of the PI3K/AKT pathway inhibitor (LY294002, 10 μM) on the rIL-8-induced up-regulation of MDR1 mRNA in both PCa cell lines. Notably, the inhibitor effectively attenuated the rIL-8-induced up-regulation of MDR1 mRNA, as observed in **Figure 5B**. Consistent results were also observed at the protein level, as determined by western blot analysis. These findings suggest that IL-8 regulates the transcription of MDR1 in PCa cells through activation of the PI3K/AKT/NF-κB

pathway.

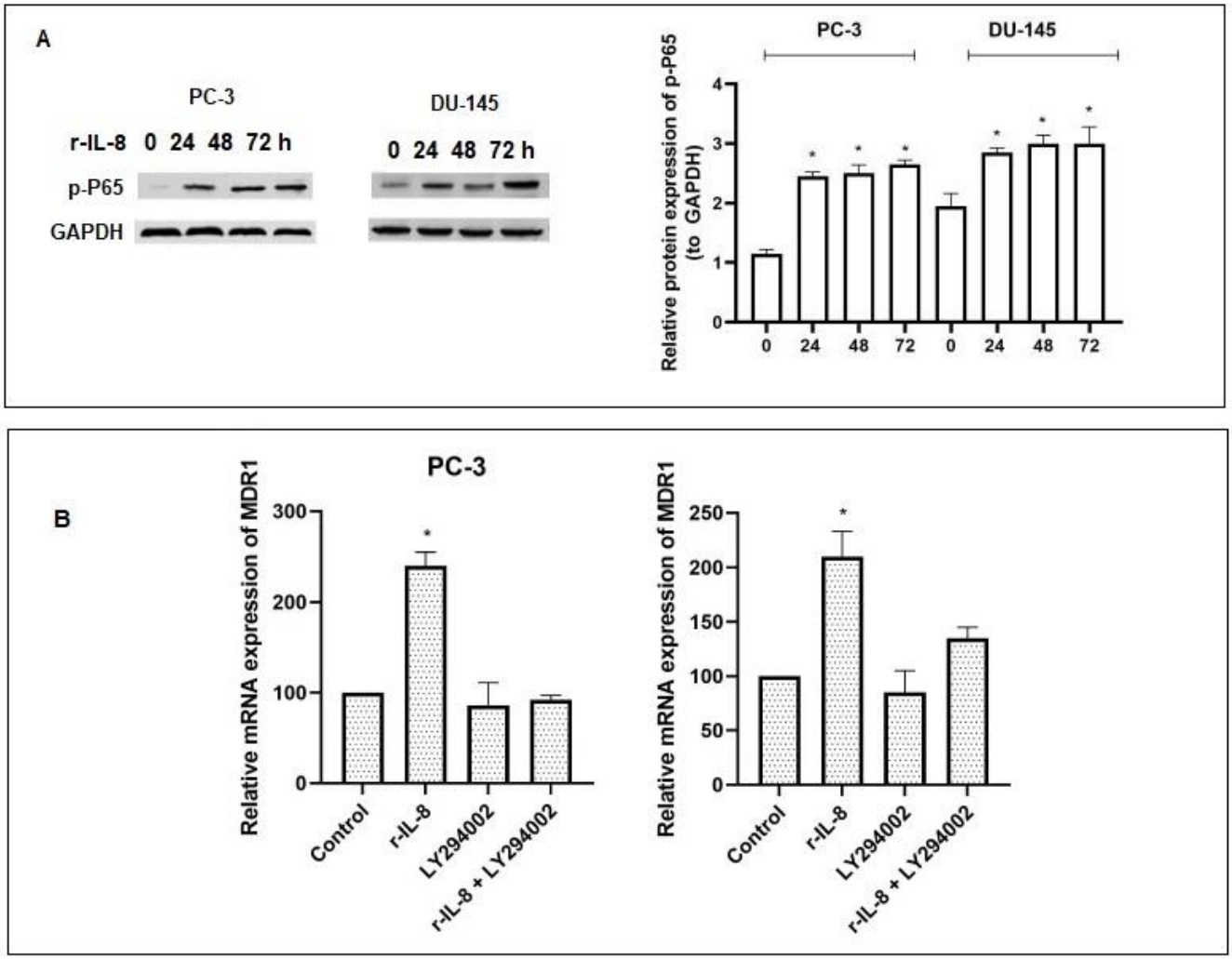


Figure 5. PC-3 and DU-145 cells were treated with rIL-8 (40 ng/ml) or si-IL-8 for 24, 48 and 72 h. (A) Protein levels were measured by western blot and quantified via Image J software. (B) The mRNA of MDR1 was measured by qRT-PCR. PCa cells were pretreated with or without LY294002 (10 μM) for 90 min and then exposed to rIL-8 (40 ng/ml) for 72 h, and the mRNA of MDR1 was measured by qRT-PCR (*p < 0.01).

4. Discussion

Human prostate carcinoma cell lines PC-3 and DU-145 are frequently used to understand the mechanisms and effects of anti-cancer agents. Both of the cell lines are androgen-receptor negative cell lines and are derived from the metastases of bone or brain, respectively [9, 10]. PC-3 cells are characterized by the absence of the p53 gene, while DU-145 cells harbor a mutant p53 gene. Previous studies have indicated that the status of p53 can influence the sensitivity of prostate cancer cells to DOC [11]. Additionally, PC-3 cells exhibit elevated levels of the anti-apoptotic protein Bcl-2 compared to DU-145 cells. Consequently, PC-3 cells demonstrate greater resistance to cytotoxic agents in comparison to DU-145 cells [12]. Along with their similarities, these cell lines differ in many ways as well [13-15], thus they provide an efficient model for the heterogeneous phenotype of prostate cancer tumors [16, 17].

Although DOC provides a successful treatment with a high survival rate for PCa, many of the patients with PCa eventually develop resistance and become not responsive to DOC treatment [18]. In response to drug

resistance challenges, numerous scientific studies have been conducted to explore a wide range of approaches aimed at overcoming resistance problems [16, 19-21]. Multiple mechanisms can contribute to docetaxel resistance, and one such mechanism involves the overexpression of the multidrug resistance 1 (MDR1) protein. MDR1 protein, also referred to as P-glycoprotein or ABCB1, is an ATP-dependent drug efflux pump that plays a crucial role in actively transporting a wide range of chemotherapeutic agents out of cancer cells. This efflux mechanism ultimately leads to a decrease in the effectiveness of these agents in combating cancer. Overexpression of MDR1 in cancer cells is a well-known mechanism of multidrug resistance [22]. Cytokines are shown to modulate the MDR process in cancer cells and they can affect the cancer treatment efficiency in different ways [23-25]. In this study, cytokines released from DOC-sensitive and resistant cells were determined via a membrane-based array. By conducting a comparative cytokine profiling analysis, notable differences in the levels of chemokine C-X-C motif ligand 1 (GROα), Interleukin 8 (IL-8), and monocyte chemo-attractant protein 1 (MCP-1) were observed between the PC-3 and PC-3R cell lines. These findings highlight significant variations in the expression of these cytokines between the two cell lines. GROα was known to endorse carcinoma cell migration, and in another study on the PC-3 cell line, it was demonstrated it may take an important part in prostate cancer progression and metastasis [26]. Monocyte chemo-attractant protein 1 (MCP-1) was similarly associated with growth and invasion capacity [27]. In contrast, when comparing DU-145 and DU-145/R cell lines, only IL-8, TIMP-1, and TIMP-2 cytokines exhibited differential expression. While tissue inhibitors of metalloproteinases (TIMPs), are known to be responsible for extracellular matrix remodeling via matrix metalloproteinases (MMPs), they function as cytokines on cell growth, apoptosis and differentiation [28]. It was shown that the deregulation of tissue inhibitors of metalloproteinases (TIMPs), acts as a cell senescent protector for tumor cells and supports metastatic potential. Disruption of TIMP function in PCa was associated with resistance to docetaxel [29]. In another study, it was demonstrated that altering the steady state between MMPs and TIMPs, increases the invasive potential of DU-145 cells [30].

As a chemokine, IL-8 with significant involvement in inflammation and immune responses. Its role extends to multiple facets of cancer progression, encompassing tumor growth, angiogenesis, invasion, and metastasis. Emerging evidence suggests that IL-8 may also contribute to chemotherapy resistance in certain types of cancer, including prostate cancer. It was also shown that IL-8 was the common cytokine found expressed in both resistant cell lines. IL-8 has been reported to be involved in the resistance of docetaxel, cisplatin and Sunitinib (Dua and He, 2018). Thus, it was considered a key player molecule in docetaxel resistance. IL-8, also referred to as chemokine (C-X-C motif) ligand 8 or CXCL8, primarily functions as a chemoattractant for granulocytes, particularly neutrophils. Additionally, IL-8 plays a significant role in facilitating phagocytosis processes. But it is also acknowledged as an effective angiogenesis promoter [31, 32]. IL-8 was shown to support tumor progression by affecting the cell survival and proliferation rate, invasion, and angiogenesis [33]. To test the theory about the relation between IL-8 and DOC resistance, resistant cell lines were exposed to IL-8 siRNA. It was found that after treatment with IL-8 siRNA DOC sensitivity was increased for both of these cells. Subsequently, to enlighten the IL-8 related resistance mechanism MDR1 expression was investigated. MDR1 is the main shortcoming in cancer treatment and essential for chemotherapy efficiency [34]. Shao et al. demonstrated the potential role of IL-8 in docetaxel resistance of estrogen-negative breast cancer cells. IL-8 was found to be a tumor-promoting cytokine. Silencing IL-8 resulted in the downregulation of cyclin D1, and the reduction of phosphorylated-Akt and NF-KB activities. However, the study was limited to ER-breast cancer cell lines. Since cancer cells show different expression patterns even in different subtypes of cancer cells, DOC resistance needs to be investigated in different subtypes modeling prostate cancer [35]. In another study, DOC resistance in prostate cancer cells was investigated via silencing NF-kB. It was determined that inhibition of NF-kB resensitized DU-145R cells to Docetaxel. Despite increased NF-kB activity, the potential role of IL-6 and IL-8 in indirect docetaxel resistance was highlighted, as

IL-6 secretion was reduced in D-resistant cell lines and was not induced by D treatment. Researchers have not been able to inhibit IL-6 and IL-8 and reveal the molecular mechanism details [36].

Here, once the DOC sensitivity was demonstrated after siRNA treatment, to provide an insight into IL-8-related resistance mechanism, Multi-Drug Resistance 1 (MDR1) expression was investigated. MDR1 expression was noticed to be high in both DOC-resistant PC-3/R- DU-145/R cell lines. Subsequently, MDR1 expression was also observed after the IL-8 siRNA treatment for both cells. It was shown that MDR1 expression levels decreased after IL-8 siRNA treatment in both PC-3/R and DU-145/R cell lines. It is known that, in prostate cancer cell lines MRP1 is closely linked with cancer progression [19]. The involvement of IL-8 with docetaxel resistance and its effects on the expression of MDR1 was also demonstrated in other studies [37]. In one of the studies with colorectal cancer cells, it was shown that IL-8 can affect the MDR1 expression levels through NF-κB phosphorylation [35]. Several NF-kB binding sites were shown in the human MDR1 promoter, suggesting that NF-kB may be mediating the IL-8 resistance mechanism. In addition, it is probable that PI3K, which is downstream of the IL-8 pathway and is associated with survival, may also play a role in the DOC resistance mechanism.

Cytokines play a central role in mediating drug resistance in cancer treatment. In the present work, differential expression of these cytokines was observed between sensitive and resistant prostate cancer cell lines, indicating their involvement in resistance mechanisms. Specifically, the investigation of angiogenic cytokines in docetaxel-resistant prostate cancer cells revealed that IL-8 is a key regulator of docetaxel resistance through modulation of MDR1. Unraveling the intricate mechanism underlying docetaxel resistance, with a focus on IL-8 and MDR1, is critical for devising strategies to overcome resistance and enhance treatment outcomes in prostate cancer patients. Targeting IL-8 signaling pathways or MDR1 expression holds promise as a potential therapeutic approach to sensitize PCa cells to docetaxel and other chemotherapy drugs. However, further research is warranted to gain a comprehensive understanding of the complex interactions among IL-8, MDR1, and docetaxel resistance in PCa, and to develop effective therapeutic interventions accordingly.

Funding statement

This research received no external funding.

Conflict of interest

The author claims that the manuscript is completely original. The author also declares no conflict of interest.

Author contributions

Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing–original draft, and writing–review & editing are conducted by solely by the first author.

References

1. Siegel, R.L., Miller, K.D., Jemal, A., (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinician* https://doi.org/10.3322/caac.21590
2. Trewartha, D., Carter, K., (2013). Advances in prostate cancer treatment. *Nature Publishing Group* 12, 823–824. https://doi.org/10.1038/nrd4068
3. Litwin, M.S., Tan, H.J., (2017). The diagnosis and treatment of prostate cancer: A review. *JAMA - Journal of the*

American Medical Association. American Medical Association, pp. 2532–2542. https://doi.org/10.1001/jama.2017.7248

4. Clarke, S.J., Rivory, L.P., (1999). Clinical pharmacokinetics of docetaxel. *Clinical Pharmacokinetics* 36, 99–114. https://doi.org/10.2165/00003088-199936020-00002
5. Gligorov, J., Lotz, J.P., (2004). Preclinical Pharmacology of the Taxanes: Implications of the Differences. *Oncologist* 9, 3–8. https://doi.org/10.1634/theoncologist.9-suppl_2-3
6. Hanauske, A.R., Degen, D., Hilsenbeck, S.G., Bissery, M.C., Von Hoff, D.D., (1992). Effects of taxotere and taxol on in vitro colony formation of freshly explanted human tumor cells. *Anticancer Drugs* 3, 121–124. https://doi.org/10.1097/00001813-199204000-00008
7. Aragon-Ching, J.B., Dahut, W.L., (2007). Chemotherapy in Androgen-Independent Prostate Cancer (AIPC): What's next after taxane progression? Cancer Therapy 5A, 151–160
8. Saad, F., Hotte, S.J., (2010). Guidelines for the management of castrate-resistant prostate cancer. *Journal of the Canadian Urological Association* 4, 380–384.
9. Stone, K.R., Mickey, D.D., Wunderli, H., Mickey, G.H., Paulson, D.F., (1978). Isolation of a human prostate carcinoma cell line (DU 145). *International Journal of Cancer* 21, 274–281. https://doi.org/10.1002/ijc.2910210305
10. Kaighn, M.E., Narayan, K.S., Ohnuki, Y., Lechner, J.F., Jones, L.W., (1979). Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Investigative Urology* 17, 16–23
11. Liu, C., Zhu, Y., Lou, W., Nadiminty, N., Chen, X., Zhou, Q., Shi, X.B., deVere White, R.W., Gao, A.C., (2013). Functional p53 determines docetaxel sensitivity in prostate cancer cells. *Prostate* 73, 418–427. https://doi.org/10.1002/pros.22583
12. Skjøth, I.H.E., Issinger, O.G., (2006). Profiling of signaling molecules in four different human prostate carcinoma cell lines before and after induction of apoptosis. *International Journal of Oncology* 28, 217–229. https://doi.org/10.3892/ijo.28.1.217
13. Nakamoto, T., Chang, C., Li, A., Chodak2, G.W., (1992). Basic Fibroblast Growth Factor in Human Prostate Cancer Cells1. *Cancer Research* 52, 571–577
14. Frønsdal, K., Saatcioglu, F., (2005). Histone deacetylase inhibitors differentially mediate apoptosis in prostate cancer cells. *Prostate* 62, 299–306. https://doi.org/10.1002/pros.20140
15. Alimirah, F., Chen, J., Basrawala, Z., Xin, H., Choubey, D., (2006). DU-145 and PC-3 human prostate cancer cell lines express androgen receptor: Implications for the androgen receptor functions and regulation. *FEBS Letters* 580, 2294–2300. https://doi.org/10.1016/j.febslet.2006.03.041
16. Erten, C., Karaca, B., Kucukzeybek, Y., Gorumlu, G., Cengiz, E., Gul, M.K., Atmaca, H., Uzunoglu, S., Karabulut, B., Sanli, U.A., Uslu, R., (2009). Regulation of growth factors in hormone- and drug-resistant prostate cancer cells by synergistic combination of docetaxel and octreotide. *BJU International* 104, 107–114. https://doi.org/10.1111/j.1464-410X.2009.08340.x
17. Jayakumar, S., Kunwar, A., Sandur, S.K., Pandey, B.N., Chaubey, R.C., (2014). Differential response of DU145 and PC3 prostate cancer cells to ionizing radiation: Role of reactive oxygen species, GSH and Nrf2 in radiosensitivity. *Biochimica et Biophysica Acta - General Subjects* 1840, 485–494. https://doi.org/10.1016/j.bbagen.2013.10.006
18. Alvin Y. Liu, (2000). Differential Expression of Cell Surface Molecules in Prostate Cancer Cells | Cancer Research. *Cancer Research* 60, 3429-3434
19. Hwang, C., (2012). Overcoming docetaxel resistance in prostate cancer: A perspective review. *Therapeutic Advances in Medical Oncology* 4, 329–340. https://doi.org/10.1177/1758834012449685
20. Cengiz, E., Karaca, B., Kucukzeybek, Y., Gorumlu, G., Gul, M.K., Erten, C., Atmaca, H., Uzunoglu, S., Karabulut, B., Sanli, U.A., Uslu, R., (2010). Overcoming drug resistance in hormone-and drug-refractory prostate cancer cell line, PC-3 by docetaxel and gossypol combination. *Molecular Biology Reports* 37, 1269–1277.

https://doi.org/10.1007/s11033-009-9501-y

21. Ganju, A., Yallapu, M.M., Khan, S., Behrman, S.W., Chauhan, S.C., Jaggi, M., (2014). Nanoways to overcome docetaxel resistance in prostate cancer. *Drug Resistance Updates*, Churchill Livingstone, pp. 13–23. https://doi.org/10.1016/j.drup.2014.04.001
22. Bhangal, G., Halford, S., Wang, J., Roylance, R., Shah, R., Waxman, J., (2000). Expression of the multidrug resistance gene in human prostate cancer. *Urologic Oncology* 5, 118–121. https://doi.org/10.1016/S1078-1439(99)00055-1
23. Soto-Ortiz, L., (2016). A cancer treatment based on synergy between anti-angiogenic and immune cell therapies. *Journal of Theoretical Biology* 394, 197–211. https://doi.org/10.1016/j.jtbi.2016.01.026
24. Dudek, A.Z., Mahaseth, H., (2005). Circulating Angiogenic Cytokines in Patients with Advanced Non-Small Cell Lung Cancer: Correlation with Treatment Response and Survival. *Cancer Investigation* 23, 193–200. https://doi.org/10.1081/CNV-200055949
25. Neufeld, G., Kessler, O., (2006). Pro-angiogenic cytokines and their role in tumor angiogenesis. *Cancer and Metastasis Reviews.* https://doi.org/10.1007/s10555-006-9011-5
26. Reiland, J., Furcht, L.T., McCarthy, J.B., (1999). CXC-chemokines stimulate invasion and chemotaxis in prostate carcinoma cells through the CXCR2 receptor. *Prostate* 41, 78–88. https://doi.org/10.1002/(SICI)1097-0045(19991001)41:2<78::AID-PROS2>3.0.CO;2-P
27. Lu, Y., Cai, Z., Galson, D.L., Xiao, G., Liu, Y., George, D.E., Melhem, M.F., Yao, Z., Zhang, J., (2006). Monocyte chemotactic protein-1 (MCP-1) acts as a paracrine and autocrine factor for prostate cancer growth and invasion. *Prostate* 66, 1311–1318. https://doi.org/10.1002/pros.20464
28. Ries, C., (2014). Cytokine functions of TIMP-1. *Cellular and Molecular Life Sciences*. Springer, pp. 659–672. https://doi.org/10.1007/s00018-013-1457-3
29. Guccini, I., Revandkar, A., D'Ambrosio, M., Colucci, M., Pasquini, E., Mosole, S., Troiani, M., Brina, D., Sheibani-Tezerji, R., Elia, A.R., Rinaldi, A., Pernigoni, N., Rüschoff, J.H., Dettwiler, S., De Marzo, A.M., Antonarakis, E.S., Borrelli, C., Moor, A.E., Garcia-Escudero, R., Alajati, A., Attanasio, G., Losa, M., Moch, H., Wild, P., Egger, G., Alimonti, A., (2021). Senescence Reprogramming by TIMP1 Deficiency Promotes Prostate Cancer Metastasis. *Cancer Cell* 39, 68-82.e9. https://doi.org/10.1016/j.ccell.2020.10.012
30. Powell, W.C., Knox, J.D., Navre, M., Grogan, T.M., Kittelson, J., Nagle, R.B., Bowden, G.T., (1993). Expression of the Metalloproteinase Matrilysin in DU-145 Cells Increases Their Invasive Potential in Severe Combined Immunodeficient Mice. *Cancer Research* 53 (2)
31. Hébert, C.A., Baker, J.B., (1993). Interleukin-8: A review. *Cancer Investigation* Informa Healthcare, pp. 743–75 https://doi.org/10.3109/07357909309046949
32. Matsushima, K., Baldwin, E.T., Mukaida, N., (1992). Interleukin-8 and MCAF: Novel Leukocyte Recruitment and Activating Cytokines (Part 2 of 2), in: Interleukins: *Molecular Biology and Immunology*. KARGER, Basel, pp. 251–265. https://doi.org/10.1159/000319091
33. Campbell, L.M., Maxwell, P.J., Waugh, D.J.J., (2013). Rationale and means to target pro-inflammatory interleukin-8 (CXCL8) signaling in cancer. *Pharmaceuticals* 6, 929-959. https://doi.org/10.3390/ph6080929
34. Kadkol, H., Jain, V., Patil, A.B., (2019). Multi drug resistance in cancer therapy-an overview. *Journal of Critical Reviews* 6, 1–6.
35. Shao, N., Chen, L.H., Ye, R.Y., Lin, Y., Wang, S.M., (2013). The depletion of Interleukin-8 causes cell cycle arrest and increases the efficacy of docetaxel in breast cancer cells. *Biochemical and Biophysical Research Communications* 431, 535–541. https://doi.org/10.1016/j.bbrc.2013.01.022
36. Codony-Servat, J., Marín-Aguilera, M., Visa, L., García-Albéniz, X., Pineda, E., Fernández, P.L., Filella, X., Gascón, P., Mellado, B., (2013). Nuclear factor-kappa B and interleukin-6 related docetaxel resistance in castration-resistant prostate cancer. Prostate 73, 512-21. https://doi.org/10.1002/pros.22591

37. Du, J., He, Y., Li, P., Wu, W., Chen, Y., Ruan, H., (2018). IL-8 regulates the doxorubicin resistance of colorectal cancer cells via modulation of multidrug resistance 1 (MDR1). *Cancer Chemotherapy and Pharmacology* 8, 1111–1119. https://doi.org/10.1007/s00280-018-3584-x