

# Possibility of paclitaxel to induce the stemness-related characteristics of prostate cancer cells

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## Abstract

**Background.** Drug resistance poses a crucial problem in the treatment of prostate cancer. Recent studies have shown that chemotherapy agents may cause cancer cells to acquire stem cell-like properties, resulting in drug resistance and, eventually, treatment failure.

**Objectives.** To evaluate whether long-term paclitaxel exposure causes an increase in the stem cell-like properties of prostate cancer cells.

**Materials and methods.** Paclitaxel-resistant PC-3 cells were generated from parental PC-3 cells by treating them with increasing concentrations of paclitaxel. The expression levels of the stem cell markers NANOG, C-MYC, CD44, and ABCG2 were evaluated using quantitative real-time polymerase chain reaction (RT-qPCR). A sphere formation assay was performed to test the potential of the cells to behave as stem cells, and a wound healing assay was carried out to evaluate migration ability of the cells.

**Results.** The expression levels of C-MYC and NANOG were significantly higher in paclitaxel-resistant PC-3 cells compared to the parental PC-3 cells. However, there was no significant increase in the expression of CD44 or ABCG2. In addition, the sphere-forming capacity and migration ability of resistant PC-3 cells were increased.

**Conclusions.** The results of the current study indicate that paclitaxel exposure may increase the stem cell-like properties of PC-3 prostate cancer cells.

**Key words:** prostate cancer, cancer stem cell, paclitaxel, taxane resistance

## Cite as

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## Background

Prostate cancer is the 2<sup>nd</sup> most common cancer and one of the leading causes of cancer death among men worldwide.<sup>1</sup> Generally, the first treatment choice for advanced prostate cancer patients is androgen deprivation therapy (ADT). Although patients tend to respond to ADT at first, treatment resistance develops in the majority of individuals, and the disease is termed castration-resistant prostate cancer (CRPC) at this stage. Taxanes (paclitaxel, abiraterone, docetaxel) – a class of microtubule stabilizing agents – causes cell cycle arrest at the G2/M phase and apoptosis.<sup>2</sup> Taxane-based treatment provides a good initial response and an increased survival in CRPC patients. However, almost all patients develop resistance over time, and the clinical use of these drugs is severely limited. Therefore, elucidating the mechanisms of taxane resistance is critical for the development of improved treatment strategies, favorable prognosis and prolonged survival in CRPC patients.<sup>2,3</sup> Despite current data on possible resistance mechanisms (such as increased activation and expression of efflux transporters, reactivation of the androgen receptor pathway, impairment in the apoptotic pathway, cytokine and chemokine induction, changes in the structure and function of microtubules, upregulation of stress survival proteins) and new drugs targeting these mechanisms, CRPC is known to cause death in many patients.<sup>2,4–6</sup> Therefore, other mechanisms causing taxane resistance need to be investigated.

Recent data indicate that tumors are composed of heterogeneous cell populations and most of these cells have a limited self-renewal capacity. On the other hand, a small group of cells within the heterogeneous tumor tissue has been reported to have a high self-renewal capacity, the ability to transform into different cancer cells, and tumor initiation properties. These are known as cancer stem cells (CSCs).<sup>5,7</sup> The CSCs form cellular spheroids in serum-free and non-adherent conditions, and have differential gene expression profiles, as well as enhanced epithelial-to-mesenchymal transformation (EMT) properties. High levels of expression for genes that encode cell surface receptors (such as *CD44* or *CD133*), transcription factors associated with pluripotency and other stemness characteristics (i.e., *NANOG*, *OCT4*, *SOX2*, *C-MYC*), and proteins associated with drug resistance (i.e., *ABCG2* and *ABCB1*) are all the examples of differential gene expression profiles in CSCs.<sup>7,8</sup> An embryonic transcription factor – *NANOG* – is highly expressed in CSCs, and shows a lower expression in differentiated tissues. It plays a key role in CSCs gaining properties such as self-renewal, pluripotency, stemness, metastasis, invasiveness, angiogenesis and drug resistance, with the help of proteins such as WNT, *OCT4*, *SOX2*, and Hedgehog.<sup>9</sup>

A transcription factor *C-MYC* plays an important role in processes such as the cell cycle, cell growth, proliferation, differentiation and apoptosis, as well as tumorigenesis, because it regulates the expression of many genes. Recent

studies have revealed that *C-MYC* effects the self-renewal capacity of cells and is one of the most important core markers for stem cells.<sup>10,11</sup> The *CD44* is a cell surface receptor. Hyaluronic acid, osteopontin and many other ligands bind to this receptor to mediate processes such as metastasis, invasion and migration. It is one of the most commonly used cell surface receptors in identifying prostate cancer stem cells.<sup>12</sup> The *ABCG2* is one of the ABC family efflux proteins and is responsible for removing drugs and harmful substances from cells. This receptor is responsible for the development of the resistance to many different drugs, and is defined as a stem cell marker for side populations in many types of tumors, including prostate cancer.<sup>13</sup>

Recent studies also suggest that CSCs may be responsible for chemotherapy and radiotherapy resistance in several cancer types, including prostate cancer.<sup>3,14,15</sup> An emerging hypothesis about CSCs and chemotherapy resistance argues that cancer non-stem cells may acquire stemness properties when they are treated with chemotherapy, and that this process may result in chemotherapy resistance.<sup>7,16</sup>

Some studies have reported an increase in the number of chemotherapy- or radiotherapy-resistant cancer cell groups that exhibit stem-like characteristics, as well as features of EMT.<sup>3,17,18</sup> In studies examining the relationship between prostate CSCs and taxane resistance, researchers have isolated CSCs from prostate cancer cell lines and have reported that those cells were resistant to taxanes. However, these studies failed to reveal whether taxanes affected the stem cell-like characteristics of prostate cancer cells. To the best of our knowledge, the present study is the first attempt to investigate whether a long-term exposure to paclitaxel affects the stem cell characteristics of prostate cancer cells.

## Objectives

The aim of this study is to evaluate whether there is an increase in the stem cell-like properties of PC-3 prostate cancer cells following treatment with paclitaxel for a prolonged period. Therefore, the expression of the CSC markers *CD44*, *C-MYC*, *NANOG*, and *ABCG2*, as well as sphere formation and wound healing capabilities, were compared between parental and paclitaxel-resistant PC-3 cell lines (PC-3-R).

## Materials and methods

### Cell culture and the establishment of paclitaxel-resistant cell lines

PC-3 androgen-independent cells (CRL-1435™; American Type Culture Collection (ATCC), Manassas, USA) were cultured in RPMI 1640 Media (Biological Industries, Beit HaEmek, Israel), containing 10% heat-inactivated fetal bovine serum (FBS; Biological Industries), 1% L-glutamine

(Biological Industries), penicillin (100 U/mL), and streptomycin (100 µg/mL). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

PC-3-R cells were generated from parental PC-3 cells by treating them with increasing concentrations of paclitaxel (Sigma–Aldrich, St. Louis, USA). Parental PC-3 cells were incubated for 24 h, and then treated with 5 nM of paclitaxel for 48 h. Then, the culture media was replaced with fresh media without paclitaxel, and the cells were incubated until they reached 80–90% confluency. The same protocol was repeated with gradually increasing concentrations of paclitaxel (5 nM each time) until 100 nM paclitaxel-resistant and viable PC-3 cells (PC-3-R) could be obtained. Cytotoxicity tests were conducted, and the viability of PC-3 and PC-3-R cells was confirmed under an inverted microscope.

## Evaluating the viability and the number of the cells

A trypan blue stain was used to determine the number of viable cells. The cell resistance level and cytotoxic effects of paclitaxel in PC-3 and PC-3-R cells were assessed using a MTT assay. Resistant and parental cells were inoculated in 96-well plates at a density of  $5 \times 10^3$  cells per well (for each dose, triplicate wells were used) and incubated for 24 h. Afterwards, the cells were treated with increasing concentrations of paclitaxel (1.6–600 nM) and incubated for 72 h. After incubation, a MTT test was conducted and the absorbances were measured using a microplate reader at a wavelength of 570 nM.

## Quantitative real-time polymerase chain reaction (RT-qPCR)

The total RNA extraction from cells was conducted using a High Pure RNA Isolation Kit (Roche Diagnostics, Basel, Switzerland). A transcript First Strand cDNA Synthesis Kit (Roche Diagnostics) was used to obtain cDNA samples from 1 µg of total RNA. The expression levels of genes were quantified using a Lightcycler 480 (Roche Diagnostics). Suitable probes and gene specific primers were designed by the Universal ProbeLibrary (UPL) Assay Design Center. Each sample was quantified in triplicate, and the mean value was used for further calculations. The B-actin was used to normalize the target gene expressions. Relative changes in the amount of mRNA were calculated based on  $\Delta\Delta CT$ . The following primers were used for each target gene:

### B-ACTIN

forward 5'- CCCAGCACAATGAAGATCAA -3'  
reverse 5'- CGATCCACACGGAGTACTTG -3'

### NANOG

forward 5'- ACAGGTGAAGACCTGGTTCC -3'  
reverse 5'- TTGCTATTCTTCGGCCAGTT -3'

### CD44

forward 5'- TCCAACACCTCCCAGTATGACA -3'  
reverse 5'- GGCAGGTCTGTGACTGATGTACA -3'

### C-MYC

forward 5'- TCCACCTCCAGCTTGTACCT-3'  
reverse 5'- TGAGAGGGTAGGGGAAGACC-3'

### ABCG2

forward 5'- TGGCTTAGACTCAAGCACAGC-3'  
reverse 5'- TCGTCCCTGCTTAGACATCC-3'

## Sphere formation assay

PC-3 and PC-3-R cells were inoculated onto 96-well ultra-low attachment plates at a density of 750 cells per well in serum-free DMEM/F12 (Thermo Fisher Scientific, Waltham, USA), supplemented with 20 ng/mL EGF (Sigma–Aldrich), B27 (1:50; Invitrogen, Waltham, USA), 0.4% bovine serum albumin (BSA; Sigma–Aldrich), and 4 mg/mL insulin (Sigma–Aldrich). Fresh stem cell media was added every 3–4 days. The cultures were incubated at 37°C in a humidified incubator containing 5% CO<sub>2</sub>. The sphere growth was monitored for 14 days and the number of spheres was counted on days 7 and 14. Six wells were inoculated for both PC-3 and PC-3-R cells. The assay was conducted in triplicate.

## Wound healing assay

PC-3 and PC-3-R cells were inoculated in six-well plates at a density of  $5 \times 10^3$  cells per well and incubated in order to reach a confluency of 90% in a complete medium. The complete medium was removed, and cells were starved in a serum-free medium for 24 h. Following the formation of cell monolayers, an artificial wound on the monolayer was scratched with a sterile 200 µL pipette tip. The cells were then washed with phosphate-buffered saline (PBS) and cultured in complete media. The assay was conducted in triplicate. Cells migrating into the wound were photographed at 0 h, 8 h, and 24 h using a camera attached to an inverted microscope.

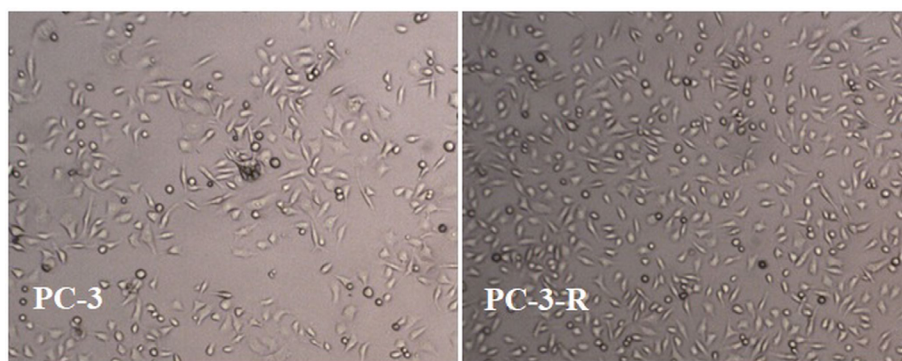
## Statistical analysis

Statistical analyses were conducted using SPSS v. 15.0 (SPSS Inc., Chicago, USA). The data are expressed as the mean  $\pm$  standard error (SE) and were analyzed using the Mann–Whitney U test. The statistical significance of mRNA expression levels was analyzed using REST 2009 v. 2.0.13 (Qiagen, Hilden, Germany). The values of  $p < 0.05$  were deemed statistically significant.

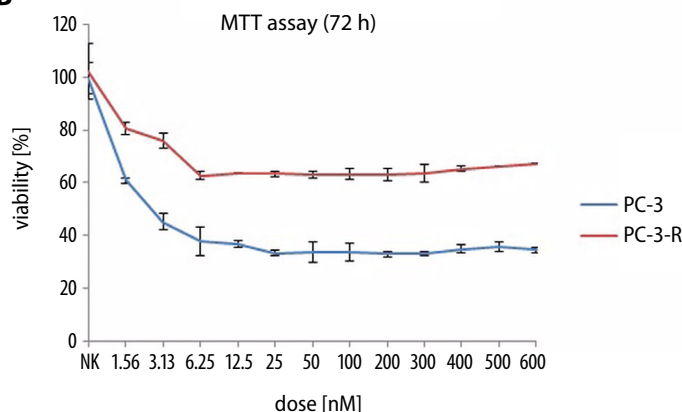
## Results

### Development of paclitaxel resistance

The PC-3-R cells were obtained by treating the parental cells with increasing concentrations of paclitaxel over a period of several months. The proliferation performance

**A**

**Fig. 1.** A. Images of PC-3 and PC-3-R cells at 100 nM paclitaxel concentration (at  $\times 40$  total magnification); B. Cytotoxic effect of paclitaxel against PC-3 and PC-3-R cells in MTT assay. The MTT test was conducted in triplicate. Data are shown as mean  $\pm$  standard error (SE)

**B**

of PC-3-R cells at the 100 nM paclitaxel level was higher than the PC-3 cells (Fig. 1A). A MTT test was conducted to assess the cytotoxic effect of paclitaxel in the PC-3 and PC-3-R cells. The  $IC_{50}$  dose of PC-3-R cells was higher ( $>600$  nM) than the PC-3 cells (2.65 nM; Fig. 1B).

### Expression of stem cell markers in the PC-3 and PC-3-R cells

The expressions of several stem cell markers, including *CD44*, *C-MYC*, *NANOG*, and *ABCG2* in PC-3 and PC-3-R cells were analyzed. The expression levels of *NANOG* and *C-MYC* genes significantly increased with fold changes of 15.5 ( $p = 0.0130$ , 95% confidence interval (95% CI) = 12.930–18.259) and 3.28 ( $p = 0.0400$ , 95% CI = 2.802–3.909) in PC-3-R cells, respectively, when compared to PC-3 cells. There was no significant difference in the expression levels of *ABCG2* (fold change 1.69,  $p = 0.0650$ , 95% CI = 1.647–1.727) and *CD44* (fold change 1.12,  $p = 0.0630$ , 95% CI = 1.051–1.226), between resistant and parental cell lines (Fig. 2; Table 1).

### Sphere-forming capacity of PC-3 and PC-3-R cells

The sphere formation assay was applied to PC-3 and PC-3-R cells in order to find out whether there was an increase in the stem-like cell population with high sphere forming

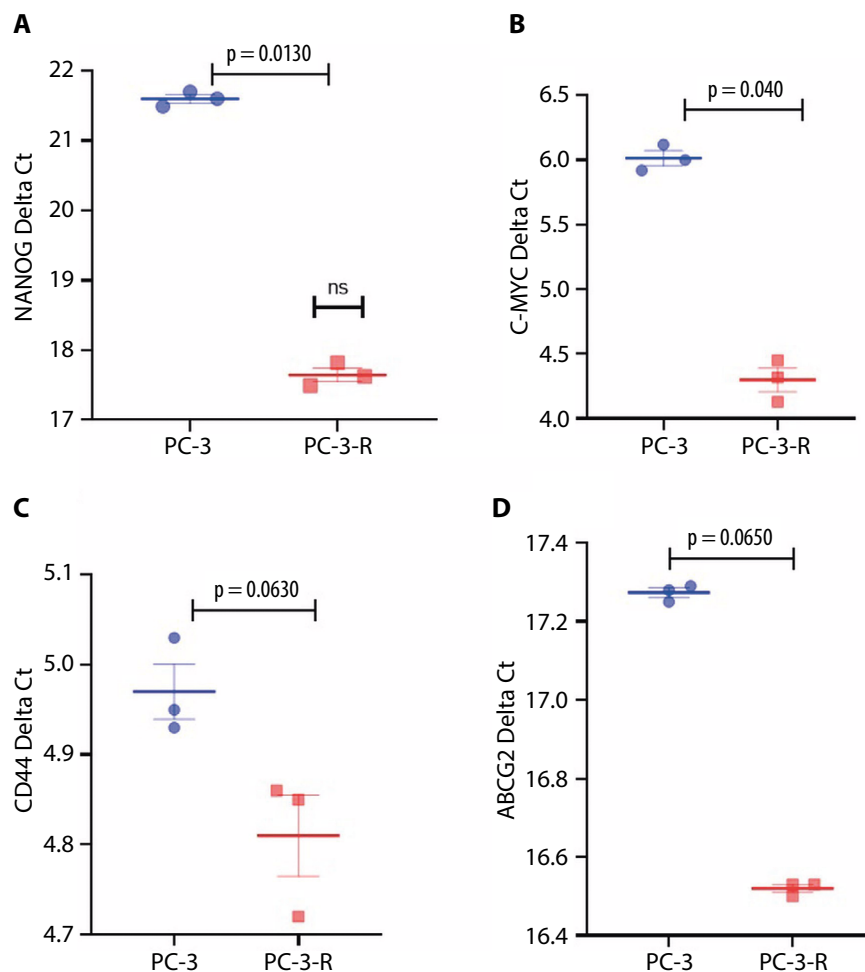
ability in the PC-3-R cell line (compared to parental PC-3 cells). The results showed that the number of spheres was higher in the PC-3-R cells than in the PC-3 cells, and the difference between the groups was statistically significant both on day 7 ( $p = 0.0130$ ,  $z = -2.4810$ ), and day 14 ( $p = 0.0450$ ,  $z = -2.0010$ ; Fig. 3; Table 1).

### Wound healing assay

The ability of cell migration to serve as an indicator of the stemness-like phenotype was assessed using a wound healing assay. The PC-3-R cells healed wounds better than the PC-3 cells. The rate of wound closure at 8 h for PC-3R cells was 65%, whereas it was only 35.4% for PC-3 cells. At 24 h, the wound had closed at the rate of 100% in PC-3R cells and at 54.5% in the PC-3 cells. The differences in wound closure rates between both groups at 8 h ( $p = 0.0500$ ,  $z = -1.964$ ) and 24 h ( $p = 0.0370$ ,  $z = -2.0870$ ) were statistically significant (Fig. 4; Table 1).

## Discussion

Recent studies have suggested that chemotherapeutics may cause cancer cells to gain stem cell properties, resulting in drug resistance.<sup>19,20</sup> An increased expression in several genes has been reported in CSCs. These genes



**Fig. 2.** Expressions of stem cell markers in PC-3 and PC-3-R cells. The expression analysis was conducted in triplicate. A. The NANOG  $\Delta$ Ct values in PC-3 and PC-3-R cells; B. The C-MYC  $\Delta$ Ct values in PC-3 and PC-3-R cells; C. The CD44  $\Delta$ Ct values in PC-3 and PC-3-R cells; D. The ABCG2  $\Delta$ Ct values in PC-3 and PC-3-R cells. Statistical significance of mRNA expression levels were analyzed using REST 2009 v. 2.0.13 (Qiagen, Hilden, Germany)

**Table 1.** Sphere numbers, wound healing rates and relative expression of genes observed in PC-3 and PC-3-R cells

Compared parameters	PC-3 n = 3	PC-3-R n = 3	p-value	z-value or 95% CI
Sphere number on day 7	4.3 $\pm$ 0.48	9.60 $\pm$ 1.83	0.0130	–2.4810
Sphere number on day 14	2 $\pm$ 0.45	3.4 $\pm$ 0.46	0.0450	–2.0010
Wound healing at 8 h [%]	35.4 $\pm$ 5.6	54.5 $\pm$ 3.2	0.0500	–1.964
Wound healing at 24 h [%]	65 $\pm$ 2.8	100 $\pm$ 0	0.0370	–2.0870
C-MYC $\Delta$ Ct	6.02 $\pm$ 0.06	4.3 $\pm$ 0.09	0.0400	2.802–3.909
NANOG $\Delta$ Ct	21.60 $\pm$ 0.06	17.64 $\pm$ 0.1	0.0130	12.930–18.259
CD44 $\Delta$ Ct	4.97 $\pm$ 0.03	4.81 $\pm$ 0.04	0.0630	1.051–1.226
ABCG2 $\Delta$ Ct	17.27 $\pm$ 0.01	16.52 $\pm$ 0.01	0.0650	1.647–1.727

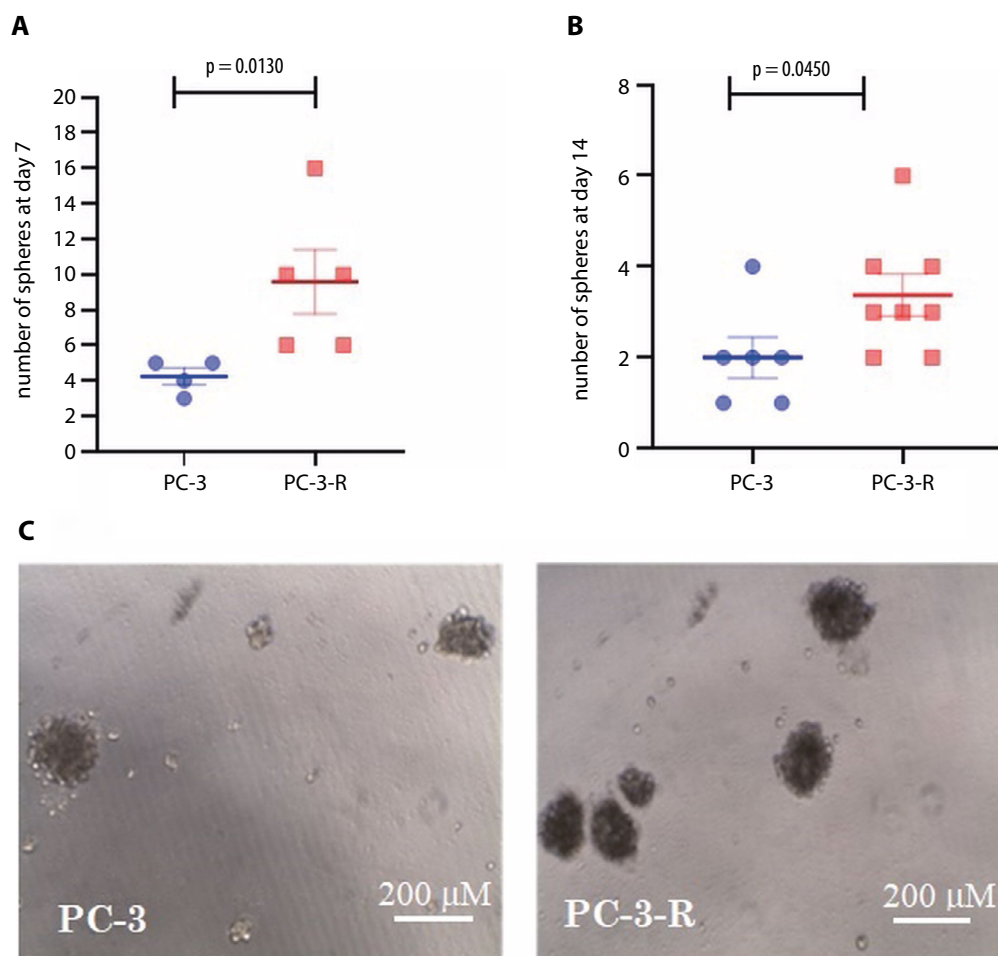
Relative expression of genes is expressed as  $\Delta$ Ct, where  $\Delta$ Ct = Ct target gene – Ct B-actin for each sample. The lower the  $\Delta$ Ct values, the higher the gene expression. Data are shown as mean  $\pm$  standard error (SE). Mann–Whitney U test was used to analyze the data between the groups. The statistical significance of mRNA expression levels was analyzed using REST 2009 v. 2.0.13 (Qiagen, Hilden, Germany). All experiments were conducted triplicate.

encode pluripotency-related proteins, cell surface markers and transporter proteins. In addition, CSCs have increased EMT and migration capabilities.<sup>15,21–23</sup> Another characteristic of these cells is the capability to form spheres in serum-free medium.<sup>24</sup> The aim of this study was to evaluate whether repeated paclitaxel treatment increased the stem cell-like properties of prostate cancer cells. To this end, the expression of genes related to stem cell properties (*NANOG*, *C-MYC*, *CD44*, *ABCG2*), as well as sphere formation

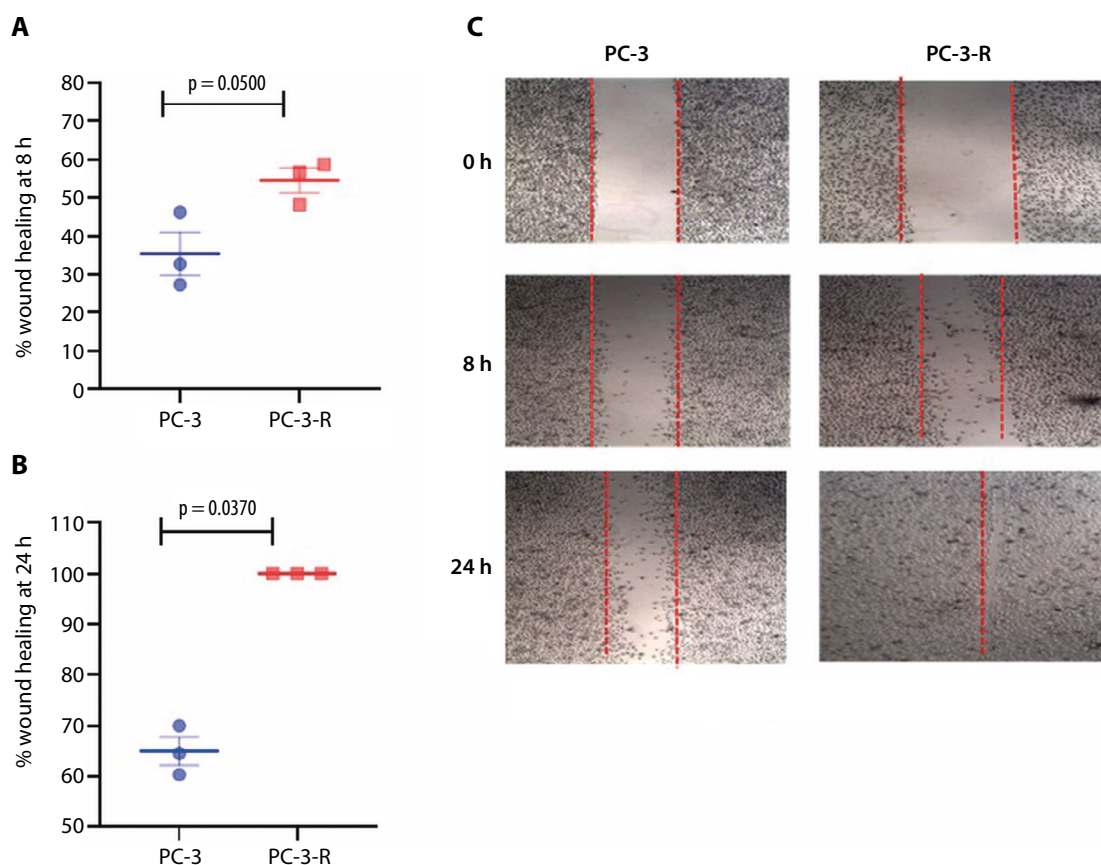
and migration capabilities, were compared between PC-3 and PC-3-R cells.

The NANOG protein is encoded by the *NANOG1* gene localized to chromosome 12, spanning 4 exons and 3 introns.<sup>25</sup> Embryonic stem cells have complexes or gene regulation networks containing various transcription factors (*NANOG*, *Oct4*, *SOX2*, *KLF4*, *C-MYC*, *SLUG*, *ESRRB*, *UTF1*, *TET2*, and *GLIS1*) that help maintain self-renewal and pluripotency. These complexes regulate the expression





**Fig. 3.** A. The number of spheres in PC-3 and PC-3-R cells on day 7; B. The number of spheres in PC-3 and PC-3-R cells on day 14; C. Representative micrographs of spheres formed by PC-3 and PC-3-R cells (at  $\times 100$  total magnification). Sphere formation assay was conducted in triplicate. Mann-Whitney U test was used to analyze the data between the groups



**Fig. 4.** A. Wound healing rates of PC-3 and PC-3-R cells at 8 h; B. Wound healing rates of PC-3 and PC-3-R cells at 24 h; C. Representative images of wound healing experiments (at  $\times 40$  total magnification). Mann-Whitney U test was used to analyze the data between the groups. The wound healing test was conducted in triplicate

of proteins in signaling pathways, such as Wnt, BMP4 and TGF- $\beta$ , to maintain pluripotency and self-renewal. On the other hand, these complexes interact with various repressors, such as the NuRD complex, REST and co-REST, to inhibit differentiation. NANOG plays a key role in these complexes. It activates 2 other crucial proteins, SOX2 and OCT4, and regulates the expression of many downstream proteins.<sup>26</sup> Data from various studies suggest that NANOG has oncogenic properties, such as increasing the migration and invasion capabilities of cancer cells, and that this is achieved through its roles in the formation and maintenance of CSCs.<sup>27</sup>

*C-MYC* is an oncogene localized to chromosome 8. It plays important roles in many processes, including the regulation of the cell cycle, proliferation, metabolic reprogramming, and cellular survival in cancer cells. Recent studies have revealed that *C-MYC* induces dedifferentiation through the downregulation of lineage-specific transcription factors. *C-MYC* downregulates *GATA3* and *ESR1* (the master regulator genes of cell differentiation) by binding to their cis-regulatory elements. Hence, oncogenic and epigenetic reprogramming induced by *C-MYC* protein leads cells to acquire CSC-like properties.<sup>28</sup>

The present study demonstrated that the expression of *NANOG* and *C-MYC* increased significantly in resistant PC-3 cell lines to which paclitaxel had been applied (Fig. 2). Moreover, compared to parental cells, an increase in sphere formation abilities (Fig. 3) and wound healing properties (Fig. 4) was detected in PC-3-R cells. Many studies have investigated the effects of various chemotherapeutics on stem cell characteristics in different types of cancers. For example, Martins-Neves et al. applied increasing concentrations of doxorubicin, cisplatin and methotrexate to osteosarcoma cell lines and evaluated the expression of pluripotency related markers (*KLF4*, *NANOG*, *SOX2*, *OCT4*). They reported that *KLF4*, *NANOG* and *SOX2* expressions increased in the cell line to which doxorubicin was applied, that *NANOG*, *KLF4* and *OCT4* expressions increased in the cell line to which cisplatin was applied, and that the expressions of *KLF4* and *OCT4* had increased in the cell line to which methotrexate was applied.<sup>19</sup> When the findings of the current study are evaluated in the light of those obtained by Martins-Neves et al., the entire body of data suggests that different chemotherapeutics stimulate the expressions of different genes related to stem cell characteristics, thus leading to an increase in the stem-like features in various ways. The majority of the studies suggesting that chemotherapeutics can increase the stem cell-like properties of cancer cells show that *NANOG* expression increases.<sup>8,19,29,30</sup> The other research has reported that *NANOG* plays a key role in the regulation of stem cell properties, and that it activates many genes in downstream signaling pathways.<sup>26</sup> Considering all these findings, the increase in *NANOG* expression, *C-MYC* expression, sphere formation, and migration capability in paclitaxel-resistant prostate cancer cells (compared to parental

cells) observed in the current study suggests that paclitaxel may induce the stem cell-related features of PC-3 prostate cancer cells.

A study by Yoshiyama et al. reported that – in addition to the capability of forming spheres – *NANOG*, *C-MYC* and *SOX2* expressions increased significantly in zoledronate-resistant non-small cell lung cancer and osteosarcoma cell lines, compared to the parental cell lines.<sup>8</sup> Similarly, Wiechert et al. found that CSC-related properties such as increased *NANOG*, *SOX2* and *OCT4* expressions, as well as in vivo tumor-forming abilities were induced in ovarian cancer cells that were resistant to cisplatin.<sup>29</sup> Liu et al. also reported that neoadjuvant chemotherapy induced the stem-like characteristics of breast cancer cells in both mice and humans. It has been shown that neoadjuvant chemotherapy caused an increase in monocyte chemoattractant proteins (MCPs), which then resulted in elevated expressions of *NANOG* and *SOX9*, together with an increased sphere-forming capability.<sup>30</sup> Cajigas-Du Ross et al. performed transcriptomic profiling using RNA sequencing in the docetaxel-resistant and parental PC-3 and Du145 prostate cancer cells. Their results indicated that 75% of the top 25 genes that are upregulated in the docetaxel-resistant cells are associated with stem cell characteristics (e.g., *NES*, *TSPAN8*, *DPPP*, *DNAJC12*, and *MYC*).<sup>5</sup> Similarly to the current study, that study was performed on prostate cancer cells (PC-3 and Du145). Moreover, gene expression levels were compared between cells resistant to docetaxel, which is also a taxane (like paclitaxel), and parental cells. In this study, *MYC* was among the top 25 genes to exhibit increased expression, which supports the data of the current study. In light of the aforementioned research, it was observed that some genes related to the stem cell characteristics (such as *NANOG*, *SOX2*, *OCT4*, *C-MYC*, and *ABCG2*) were upregulated in cells resistant to chemotherapeutics. Furthermore, these genes block apoptosis and cause drug resistance. They are also associated with stem cell features such as pluripotency and self-renewal, suggesting a close relationship between the increase in CSC characteristics and drug resistance. Our findings that *NANOG* and *C-MYC* expressions are upregulated in PC-3-R cells support this idea. Additionally, the increased sphere-forming capacity and migration capability of the PC-3-R cells indicate an increase in stem cell properties.

The *ABCG2* is located on chromosome 4 and consists of 16 exons. *ABCG2* is a member of the family of ATP binding cassette (ABC) transporters, which pump endogenous and exogenous harmful compounds (such as drugs) out of the cell. Many drugs commonly used in cancer chemotherapy are among the substrates of *ABCG2*. This gene was first cloned from doxorubicin-resistant MCF-7 breast cancer cells; hence, it is also known as breast cancer resistance protein (BCRP). Increased expression of *ABCG2* is thought to cause both active extracellular removal of chemotherapy agents and resistance to chemotherapy. This gene is also

highly expressed in stem cell subpopulations. ABCG2 is thought to inhibit cell death in stem cell populations, and to maintain stem cell homeostasis under extreme stress. It is also believed to be associated with both the stem cell phenotype and drug resistance.<sup>31</sup>

The CD44 is a single polypeptide chain cell surface receptor that is encoded by the *CD44* gene. The *CD44* gene is located on chromosome 11 and has 19 exons. Alternative splicing of exons 6 to 15 of the *CD44* gene leads to the formation of multiple CD44 isoforms, while their removal produces the standard *CD44* transcript. This protein acts as a receptor for hyaluronan and many other extracellular matrix components. It also acts as a cofactor for various growth factors and cytokines. Therefore, CD44 is thought to be a signaling platform that processes and transfers multiple signals to membrane-associated cytoskeleton proteins or the nucleus, thereby regulating the expression of many genes involved in differentiation, cell migration, proliferation, adhesion, EMT, and survival. For example, many cell surface receptors such as EGFR, TGF-BRI, c-MET, ErbB2, and oncogenic signaling pathways become activated when hyaluronan binds to CD44. Increasing evidence suggests that CD44 is a critically important regulator of CSC properties (e.g., self-renewal, tumor initiation, chemoresistance, and metastasis).<sup>32–34</sup>

There was no significant increase in *ABCG2* and *CD44* expressions in paclitaxel-resistant PC-3 cells in the current study (Fig. 2). The lack of change in *ABCG2* expression is possibly related to the fact that paclitaxel may not be an ABCG2 substrate.<sup>35</sup> Martins-Neves et al. evaluated the expressions of the multidrug resistance transport proteins ABCB1 and ABCG2 in osteosarcoma cell lines to which increasing concentrations of doxorubicin, cisplatin and methotrexate were applied. The expressions of these genes increased in doxorubicin-applied cells, whereas the same effect was not observed in cisplatin-applied and methotrexate-applied cells.<sup>19</sup> Similar to the current study, no increase was observed in *ABCB1* and *ABCG2* expressions in cell lines treated with cisplatin and methotrexate in the study of Martins-Neves et al. This may be because paclitaxel, cisplatin and methotrexate are not the substrates of ABCG2 or ABCB1, or that they are excreted in different ways.

Several studies have reported that CD44-negative cells can transform into CD44-positive cells, or that CD44-negative cells may display stem cell-like characteristics.<sup>36,37</sup> One such a study reported that CD44-negative PC-3 cells spontaneously converted into CD44-high cells expressing a different *CD44* isoform in stem cell medium – which was more invasive and had higher clonogenic and self-renewal potential than *CD44*-high cells expressing the standard isoform.<sup>38</sup> In the present study, there may be an increase in the expression of *CD44* isoforms different from the standard one observed in PC-3-R cells.

The present study evaluated the effects of paclitaxel, a chemotherapeutic from the taxane group, on the stem

cell properties of prostate cancer cells, and filled a gap in the literature on this subject. The current study indicated that paclitaxel can cause a heightened sphere-forming and migration capability in prostate cancer cells, in addition to an increased expression of *NANOG* and *C-MYC*. In light of these findings, it was thought that targeting CSCs together with taxanes and the use of therapeutic agents regulating the expression of stem cell phenotype-related genes (such as *NANOG* and *C-MYC*) will minimize treatment failures due to taxane resistance. Pouyafar et al. showed that natural compounds such as resveratrol can increase the mesenchymal endothelial transformation rate and transdifferentiation of CSCs. On the other hand, they can also reduce the resistance of cells to chemotherapeutics by modulating GALNT11 synthesis and autophagy signals.<sup>39</sup> To overcome taxane resistance, natural compounds or different treatment agents that target CSCs could be exploited.

## Limitations

The present study has several limitations. First, the expressions of other genes related to stem cell characteristics were not evaluated. Second, the changes in gene expression have not yet been confirmed with western blot analysis. Third, other methods that analyze stem cell populations (e.g., using a flow cytometer to measure aldehyde dehydrogenase activity) were not used.

## Conclusions

Repeatedly applying paclitaxel to prostate cancer cell lines is thought to increase the stem cell-like properties of the cells since this is one of the mechanisms causing drug resistance. The combined use of therapies targeting CSCs with classical chemotherapy may help patients overcome drug resistance and thus, may improve their treatment. Future studies evaluating the expressions of different genes related to stem cell characteristics and using additional protein expression analyses and flow cytometry techniques may prove beneficial in supporting these conclusions.

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