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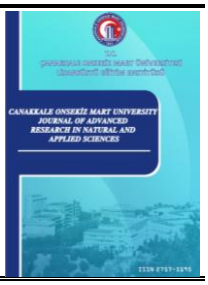

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Effects of IL-6 and TNF-α Cytokines on Cell Proliferation in Androgen Dependent/Independent Prostate Cancer Cell Lines

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Abstract − Prostate cancer is the second most common types of cancer among men worldwide. Prostate cancer, caused by abnormal and uncontrolled growth of the cells that make up the prostate tissue, is second only to related deaths in men. Cytokines, which have a significant impact on the response of the immune system, play an active role in the development of prostate cancer. This study determined the effect of proinflammatory cytokines, IL-6 and TNF-α on the proliferation of prostate cancer (PCα) cell lines. MTT test was used for the effect of cytokines applied at different doses and hours on cell viability. According to our results, IL-6 cytokine caused a high increase in proliferation in androgen-dependent LNCaP cells, while androgen-independent PC-3 cells showed different proliferative effects in time and dose-dependent manner. TNF-α cytokine had a negative effect on the proliferation of androgen-dependent LNCaP cells, while it increased the proliferation level of androgen-independent PC-3 cells. These results show that the effects of cell lines-on the proliferation of cells are different depending on their androgen sensitivity, which will be used in different cytokinetic studies to determine the inflammatory response to treat prostate cancer. It is especially important to consider this condition during the advancement of prostate cancer treatment strategies.

Keywords − *Cytokine, IL-6, prostate cancer, proliferation, TNF-α*

1. Introduction

Prostate cancer is the second most common cancer in men, according to data from the World Health Organization for 2020 [1]. The prostate gland, part of the male reproductive system, is a malignant tumor structure that occurs by the uncontrolled and abnormal proliferation of cells [2]. The greatest advances in the identification and treatment of prostate cancer were accelerated by the discovery of prostate-specific antigen (PSA) from the mid-1970s onwards. A relationship has been established between the follow-up of PSA from patient serums and the prognosis of patients with cancer [3]. Prostate cancer, a highly complex disease that exhibits heterogeneity at pathological, genomic, and molecular levels, is a multi-stage process that can begin with carcinoma and then turn into castration-resistant prostate cancer (CRPC), and finally continue with a metastatic prostate tumor [4]. Normal prostate tissues need androgen and androgen receptors (AR) for their development. Suppressing the AR pathway is often a common method for treating prostate cancer. Although surgical intervention and radiation for localized prostate cancer are effective treatment options, suppression of the AR pathway remains the first treatment option in metastatic prostate cancer [5].

As with many types of cancer, cytokines have a highly significant effect on prostate cancer. Cytokines directly or indirectly regulate the growth, invasiveness, and metastasis of tumor cells. They affect tumor formation by

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inducing angiogenesis in the cancer microenvironment. It has been determined by studies that many different cytokines play an effective role as regulators in prostate cancer metastasis [6]. These studies have found that IL-6 plays a role in the mechanism of prostate cancer, due to the presence of a receptor of IL-6 cytokine in the prostate cancer cell lines [7]. Another cytokine tumor necrosis factor alpha (TNF-α), which is actively involved in cancer metastases, is a multifunction proinflammatory cytokine [8]. Studies have shown that TNF-α regulates many critical processes of tumor progression, such as DNA damage, oncogenic activation, and tumor metastasis [9]. TNF-α levels in the serum of prostate cancer patients have been reported in studies showing a positive correlation with the prognosis of the disease [10].

Localized and metastatic prostate cancers could be androgen-dependent or -independent. The presence or absence of androgen differs in prostate cancer treatment processes. On the other hand, IL-6 and TNF-α are prostate cancer inflammatory cytokines and have effects on cancer processes [11]. Androgen-dependent and androgen-independent prostate cancers especially differ in their responses to chemotherapeutic agents in clinical processes [12].

Considering the androgen-dependent and androgen-independent prostate cancer, the aim of this study is to comparatively analyze the effects of IL-6 and TNF-α, which are also important for prostate cancer, on cell proliferation. Therefore, in our study, the effects of IL-6 and TNF-α cytokines on androgen-dependent (LNCaP) and the androgen-independent (PC-3) PCα cell lines were investigated at different doses (10 ng/mL, 20 ng/mL, and 40 ng/mL) and at different administration times (24, 48, and 72 hours). In this study, considering that the effects of cytokines on cells could be different at different concentrations, it is planned to evaluate the effects of cytokine application at various concentrations on cells.

2. Materials and Methods

2.1. Cell Culture

Androgen-dependent LNCaP and androgen-independent PCα cells, PC-3 cells, were used for the study. RPMI-1640 (Gibco) was used for the LNCaP cell line and DMEM High Glucose (Gibco) mediums were used for PC-3 cells for the growing conditions. 10% Fetal Calf serum (FCS) was added to this medium containing glutamine. 1 mL of LNCaP and PCα cells are suspension stored in a -80 °C refrigerator was taken from -80 °C and the cell brought to the culture laboratory. After the cell suspension is thawed at 37 °C, then 6 mL of medium. Dissolved cells in a centrifuge at 1000 rpm for 5 min. centrifuged. After centrifugation, the supernatant is discarded, and the remaining pellet is taken into 10 mL medium by pipetting. The cells were incubated in 37°C incubators with 5% CO_2. Cell cultures with flask occupancy above 80% were regularly passaged [13].

2.2. Cell Viability Assay

In experimental studies, cell counts were performed with trypan blue and a hemocytometer counter to use adequate amounts of cells. For this process, the cells removed from the flasks were centrifuged for 5 minutes at 1000 rpm and then dissolved to become homogeneous in the 10 mL medium containing 10% FCS. To distinguish between living and dead cells, the 10 µL of cell mix was incubated at room temperature for 5 minutes with an equal volume of trypan blue (1:1 dilution rate). This process eventually calculated the number of living cells in mL by counting living cells. (Total live cells/mL = Hemocytometer count result x 2 x 10^4) [14].

2.3. Cytokine Treatment

8,000 cells per well were plated out into 96-well plates. The cells were incubated over-night to attach to the surface. After O/N incubation, the medium of the cells was refreshed with DMEM containing 0.1% BSA. After 1 hour, IL-6 (Peprotech, 200-06) and TNF-α (Peprotech, 300-01A) cytokines were administered with final concentrations of 10 ng/mL, 20 ng/mL, and 40 ng/mL per well. Plates were incubated for 24, 48, and 72 hours at 37°C with 5% CO_2 [14].

2.4. MTT

At the end of the time points, MTT dye was performed into each well of 96 well plates and cells were incubated for 4 hours at 37°C in an environment containing 5% CO_2. After the incubation, the medium was removed, adding 200 µL isopropanol containing 4mM HCI to each well. Each well was measured by a UV spectrophotometer at the wavelength of 550 nm and the results were analyzed in the program [15].

2.5. Statistical Analyses

All experimental sets were run in 3 replicates and the data obtained were statistically analyzed with GraphPad Prism 8.0.2 (GraphPad Software, San Diego, CA, USA). Statistical values were stated as mean ± SD, and the results were evaluated with the one-way ANOVA method. Situations, where it is $p \leq 0,05$ in the assessment, have been considered meaningful.

3. Results and Discussion

Proinflammatory cytokines were thoroughly investigated due to their different roles in prostate cancer, such as proliferation, apoptosis, migration, invasion, and regulation of angiogenesis. One of these cytokines, IL-6, is an upregulated cytokine in prostate cancer [16]. The positive or negative effects of IL-6 on the proliferation of cancer cells can be explained by differences in the activation of serum or autocrine cycles [17]. Most information on the regulation of the cell cycle by IL-6 in prostate cancer was obtained from studies with LNCaP cells. However, our study investigated the effect of cytokines on the proliferation of the cell using androgen-independent and dependent cell lines. At the same time, prostate cancer cells were exposed to different doses in different time periods, and the effect of cytokine on cell proliferation was determined to be time and dose dependent. It has been determined that some inflammatory agents used in androgen-independent prostate cancer cell lines have effects on IL-6 and TNF-α cytokines at different doses [18]. It has been reported that different chemotherapeutic agents trigger oxidative stress, apoptosis, and inflammation in a dose-dependent manner in prostate cancer cell lines [19].

In our study, IL-6 and TNF-α cytokines were applied to androgen-dependent LNCaP and androgen-independent PC-3 PCα cell lines for 24, 48, and 72 hours, with a final concentration of 10 ng/mL, 20 ng/mL, and 40 ng/mL percentage (%) viability were calculated based on cell lines.

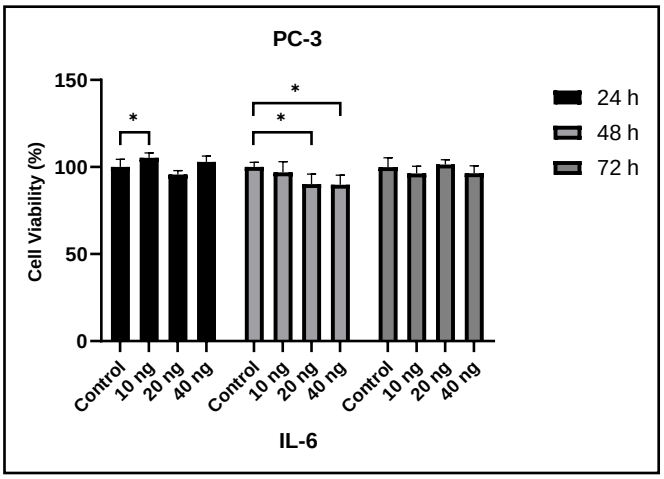

Figure 1. The effect of IL-6 cytokines on PC-3 cell viability (p>0.05(Not significant), p≤0.05(*Significant), p≤0.01(**Very significant), p≤0.001(***Very significant))

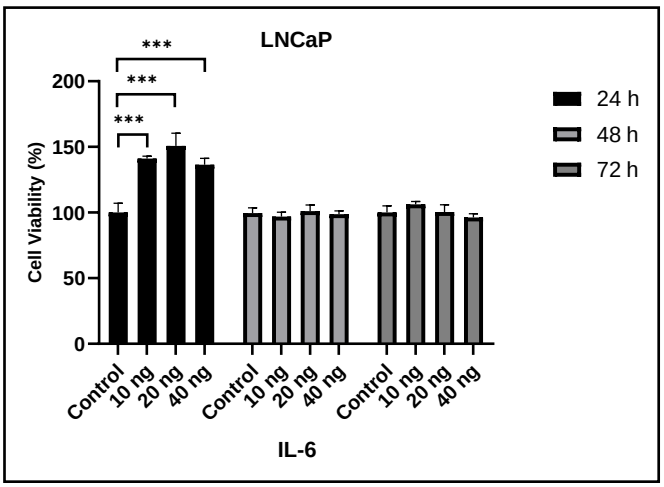

Figure 2. The effect of IL-6 cytokines on LNCaP cell viability (p>0.05(Not significant), p≤0.05(*Significant), p≤0.01(**Very significant), p≤0.001(***Very significant))

The results from the first part of our study showed statistically significant results in the androgen-independent cell line administered IL-6 cytokine for 24 and 48 hours. According to these results, the proliferation level in PC-3 cells increased in the first 24 h, but only 48 h hours cell proliferation decreased time and dose-dependent manner. However, while increases or decreases in cell proliferation have been determined for 72 h, statistically significant results have not been achieved (Figure 1).

In LNCaP cells, which have an androgen-dependent cell line, IL-6 is applied with same doses in different times. The first 24 h of the experiment showed that the level of cell proliferation increased by approximately 50%. Depending on the cytokine doses, the proliferation in LNCaP cells increased at a statistically significant rate. In the case of 48- and 72-hours experimental groups, no statistically significant results were achieved in terms of cell proliferation (Figure 2). Our results indicate that IL-6 cytokine led to the proliferation of the cell lines depending on the cell's androgen sensitivity. Our results confirm that the proliferation of IL-6 cytokine in the prostate cancer cell line increases the proliferation rate in cells that may have an androgen response [20,21]. In the literature, in hormone-independent prostate cancer, HER-2/neu tyrosine kinase is stated to have modulated the signal of the androgen receptor [22]. Another study showed that androgen absence induces N-cadherin, increasing the metastasis of prostate cancer [23]. Studies show that IL-6 cytokine organizes androgen

receptor activity and PCα cell growth supports differences in proliferation in androgen dependent/independent cell lines [24-26].

Some studies report that TNF-α cytokine acts as inductive or suppressive in the development of different types of cancer [27]. Studies with TNF-α cytokine implies that it has an important role in the advance processes of prostate cancer [28,29]. The high levels of TNF-α cytokine were identified in serums of patients whose prostate cancer prognosis was progressing poorly [10]. It is also thought that there is a study showing that TNF-α inhibits androgen dependence in prostate cancer. TNF-α cytokine may take a role in the start of an androgen-independent condition in prostate cancer, in this case an androgen-independent process can be initiated [30].

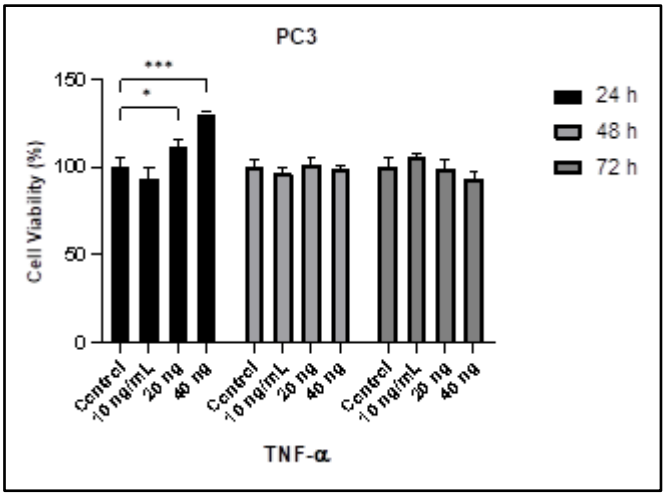

Figure 3. The effect of TNF-α cytokines on PC-3 cell viability (p>0.05(Not significant), p≤0.05(*Significant), p≤0.01(**Very significant), p≤0.001(***Very significant))

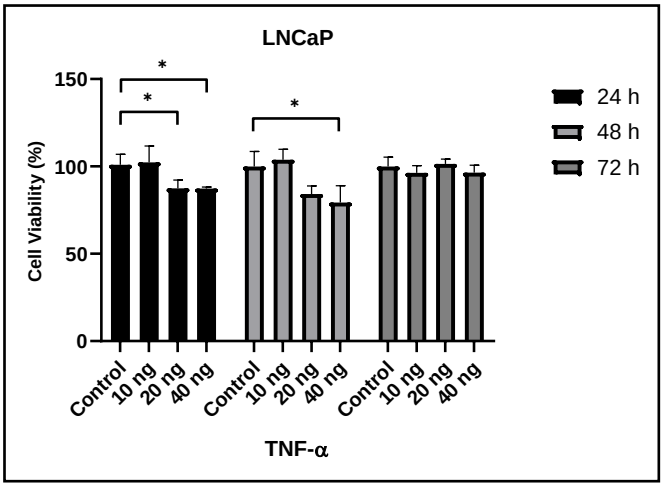

Figure 4. The effect of TNF-α cytokines on LNCaP cell viability (p>0.05(Not significant), p≤0.05(*Significant), p≤0.01(**Very significant), p≤0.001(***Very significant))

In our study, TNF-α cytokine caused the proliferation in the first 24 h in the androgen-independent PC-3 cells depending on the dose. Statistically meaningful the level of proliferation has been obtained at 20 ng/mL and 40 ng/mL of TNF-α. No statistically significant results were achieved for 48 and 72 hours (Figure 3). Similarly, when TNF-α cytokines are applied to androgen-dependent cell line, LNCaP cells at the same time and doses, the cell proliferation level decreases with 20 ng/mL and 40 ng/mL doses for 24 h and 40 ng/mL for 48 h (Figure 4). In LNCaP cells, there is no meaningful change for 72 hours of cytokine application in terms of proliferation level. Studies in literature have found that TNF-α suppresses androgen sensitivity in an androgen-dependent

prostate cancer cell line and induces the AR-mediated transcriptional activation of the TGF-beta signal [31,32]. Differences in the proliferation levels of cells were determined based on differences in the dose and time differences. Statistically meaningful results were not achieved, especially during 72 hours in all cell lines and in different cytokines applied. This is attributed to the fact that cytokines have lost their effectiveness during long incubation times. Cytokines administered have been confirmed to have different effects on cell proliferation based on cells' androgen sensitivity levels [33].

4. Conclusion

This study showed that proinflammatory cytokines applied to prostate cancer cell lines, which are androgen dependent/independent, had different effects on the proliferation of cells. The IL-6 cytokine applied to our study significantly increased the proliferation in the androgen-dependent LNCaP cells, but the proliferation in the androgen-independent PC-3 cell line varies depending on the cytokine application dose and time points. In the first 24 hours of a cytokine application to PC-3 cells, the proliferation of the cell increased while in the 48 hours, the proliferation decreased in the cytokine application. TNF-α cytokines used for the same doses and time points have been determined to reduce proliferation in androgen-dependent LNCaP cells but increase proliferation in the androgen-independent PC-3 cell lines. As a result, IL6 and TNF-α cytokines show differential effects on cell proliferation in androgen-dependent and androgen-independent prostate cancer cell lines at different concentrations and time intervals.

Author Contributions

The first author collected data and performed the experiment and wrote the paper. The second author performed data analysis and interpretation. The third author planned the analysis and wrote the article. All authors edited the paper and read and approved the final version. This paper is derived from the first author's doctoral dissertation supervised by the third author.

Conflicts of Interest

All the authors declare no conflict of interest.

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