Original Article

Fucoidan Inhibits Prostate Cancer Growth Through Modulation of Different Cell Deaths

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Abstract

Background: Docetaxel (DOC) is the main chemotherapeutic agent for the treatment of advanced metastatic prostate cancer. Docetaxel shows anticancer effects by preventing the depolymerization of microtubules in the cell, therefore preventing cell division. However, the low survival effect of docetaxel has prompted researchers to search for novel therapeutic agents. Fucoidan (FUC) is a sulfated polysaccharide derived from brown algae. It has many bioactivities which makes fucoidan a promising anticancer agent. In this study, the potential anti-tumorigenic and preventive effects of fucoidan with or without docetaxel in prostate cancer were investigated by analyzing different cell death modalities. **Methods:** The *in-vivo* six groups (n = 8) were conducted; preventive (Pt), docetaxel treated after preventive (Pt-D), control, fucoidan (FUC), docetaxel (DOC), and FUC and DOC (FUC+DOC) combination. Apoptotic, necroptotic, and autophagic cell death-related protein expressions were assessed in tumor tissues by using immunohistochemical staining. Oxidative stress-related lipid peroxidation, glutathione peroxidase, and glutathione levels were also determined in tumor tissues. **Results:** Although apoptotic, necroptotic, and autophagic cell deaths were significantly induced in agent-treated groups compared to the control. Apoptotic cell death was more significantly induced in FUC and FUC+DOC-treated groups. Necroptotic cell death was increased considerably by inducing MLKL protein expression in all treatment groups. In the FUC, Pt, and DOC groups, LC3A/B expressions were significantly increased. DOC, FUC+DOC, and Pt-D treatments caused a significant increase in Beclin-1 expression. Oxidative stress-related MDA, GPX, and GSH levels significantly decreased with FUC treatment. The anti-tumorigenic effects of FUC and DOC were also demonstrated through tumor size reduction. **Conclusion:** According to the findings of this study, FUC inhibited tumor growth temporally and dimensionally, especially in preventive applications. FUC and FUC+DOC combinations in both treatment groups showed anti-tumorigenic effects. The results of this study suggest that fucoidan is a promising anticancer agent against prostate cancer. FUC can be considered as a preventive or treatment agent in prostate cancer therapy with DOC. Further studies are needed to fully elucidate the mechanism of action of fucoidan in metastatic prostate cancer.

Keywords: *Cell death, docetaxel, fucoidan, xenograft prostate cancer model*

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Introduction

Prostate cancer is the second most common malignancy in men worldwide. Docetaxel is the first-line chemotherapy agent in advanced-stage prostate

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cancer, although treatment may change according to the histological type and stages of the disease.[1,2]

DOC, derived from *Taxus bacata*, is an anticancer agent, that inhibits cell proliferation. It is known to induce microtubule bundle formation to lead cells to apoptosis.[2,3] Moreover, DOC shows anti-proliferative effects through autophagic cell death in prostate cancer, both *in vitro* and *in vivo*. Besides, in a study, DOC triggered necroptotic cell death in PC-3 prostate cancer cells which are tolerant to acidity.[4] However, recently it has been reported that the contribution of DOC to the survival in both early-diagnosed and advanced-stage patients, was not statistically significant.[5] Therefore, there is an urgent need for alternative treatment agents for patients with advanced-stage prostate cancer.

Fucoidan is a sulfated polysaccharide derived from brown algae and various marine invertebrates. Its bioactivity may vary due to the species it was derived from. Anticancer effects of fucoidan types, including the one derived from *Fucus vesiculosus (FUC)*, were shown in various malignancies.[6,7] Fucoidan shows anticancer effects by inhibiting angiogenesis, metastasis, invasion, and disrupting the cell cycle.[6-9] The examination of the effects of fucoidan in the PC-3 human metastatic prostate cancer cell line revealed that fucoidan caused apoptotic cell death.[10] Fucoidan suppresses migration by decreasing matrix metalloproteinase-9 (MMP-9) secretion.[11] It was indicated that fucoidan exerts its anticancer effect by inducing intrinsic and extrinsic apoptotic pathways, decreasing mitochondrial membrane potential.[8,12] Fucoidan with anticancer agents has the potential to increase synergistically anti-tumor efficiency. It was demonstrated that the anticancer effects of fucoidan were caused by supporting immune responses. In addition, it was stipulated that it has a potential protective effect against the adverse effects of chemotherapeutics by balancing free oxygen radicals.[12] A study showed that fucoidan-coated-doxorubicin nanoparticles increased the uptake of doxorubicin in breast cancer cells. Therefore, fucoidan stands as an important agent due to its anticancer and efficiency-enhancing effects in combination with chemotherapeutic agents.[13,14]

A study has shown that fucoidan was applied together with DOC and doubled the efficiency of DOC in DU-145 prostate cancer cells.[15] Also, fucoidan does not show any harmful effects on healthy prostate cells.[6,15] However, up to date, it has not been investigated whether fucoidan in combination with DOC would have potential anti-tumorigenic effects in the prostate cancer xenograft model. This study is the first study to investigate whether fucoidan, alone or in combination with DOC, has both preventive and therapeutic effects by the modulation of apoptotic, necroptotic, and autophagic cell death mechanisms in the xenograft prostate cancer model. The aim of this study, the potential preventive and anti-tumorigenic effects of fucoidan with or without docetaxel have been examined by assessing different cell death mechanisms in the xenograft prostate cancer model.

Material-Methods

For the xenograft prostate cancer model, male nude mice 5–6 weeks old, with an average weight of 22–25 g, obtained from Dokuz Eylul University Faculty of Medicine Experimental Animals Research Laboratory (DEÜTFDHAL), were housed in cages ventilated with individual HEPA filters. Mice were kept at room temperature (22 ± 2 ºC) and in a 12-hour light/dark environment throughout the study and were fed with sterile pellet mouse chow and were allowed to access sterile water.

Ethical permission was obtained for experimental animals in the study from the Multidisciplinary Laboratory Animals Ethics Committee of Dokuz Eylul University on 10.07.2018 with Protocol No 34/2018. Forty-eight animals were used in the study and randomly divided into six groups. The preventive and therapeutic efficacy of fucoidan in prostate cancer was evaluated in two different models. Groups in the preventive application model were Pre-treatment (n = 8) and Pre-treatment + DOC (n = 8), while the treatment groups were Control (n = 8), FUC (n = 8), DOC (n = 8), FUC+DOC (n = 8).

The animals were sacrificed at the end of the experiment, and tumor tissues were collected.

Xenograft prostate cancer model

Cultured DU-145 prostate cancer cells[16] (2×10^6/100 µl) were injected subcutaneously (sc) into the dorsal flank of male nude mice (5–6 weeks old). Tumor volume was measured every other day for 10–14 days with a caliper. Mice (n = 32) whose tumors reached 60–80 mm^3 were randomized to four study groups (without preventive groups (n = 16)). Tumor volume (V) was calculated using the formula; V = ½ (Length × Width^2).[17] Following the experimental procedure, all animals were sacrificed and tissues were collected and used for biochemical and immunohistochemical analysis.

Preventive groups; Pt, Pt-D

Pt: To investigate the preventive effect of FUC; mice were treated with 20 mg/kg[18] FUC every other day for three weeks via oral gavage. Then, DU-145 cells were injected, and mice were monitored for tumor development (60–80 mm^3) was followed for 28 days.

Pt-D: To investigate the preventive effect of FUC; mice were treated with 20 mg/kg FUC every other day for three weeks via oral gavage. Then, DU-145 cells were injected. After tumor development was observed, mice were treated with only DOC (10 mg/kg)[19] every other day for three weeks intraperitoneally (ip), and the effectiveness of DOC against tumor development was assessed.

Treatment groups; Control, FUC, DOC, FUC+DOC

Control (n = 8): Normal saline (NS) (100 uM) was administered ip every other day for three weeks after tumor development (60–80 mm^3) was determined by injection of DU-145 cells (2 × 10^6/mL).

FUC (n = 8): 20 mg/kg FUC was administered via oral gavage (og) every other day for three weeks after tumor development (60–80 mm^3) was determined by injection of DU-145 cells (2 × 10^6/mL).

DOC (n = 8): 10 mg/kg DOC was administered ip every other day for three weeks after tumor development (60–80 mm^3) was determined by injection of DU-145 cells (2 × 10^6/mL).

FUC+DOC (n = 8): 10 mg/kg DOC (ip) and 20 mg/kg FUC (og) were administered via og every other day for three weeks after tumor development (60–80 mm^3) was determined by injection of DU-145 cells (2 × 10^6/mL).

Immunohistochemical examination of cell death-related protein expressions in tumor tissues

Primary antibodies used in immunohistochemical (IHC) analysis of paraffin sections of tumor tissues for the assessment of cell death protein expressions were as follows; for apoptosis; Active Caspase-3, Caspase-8, Caspase-9, Bcl-2, for necroptosis; RIP1, RIP3, MLKL, for autophagy; Beclin-1 and LC3A/B.[20]

First, the primary antibody was bound to the specific antigen, then the antibody–antigen complex was bound to the second antibody with the help of a conjugation enzyme. Lastly, in the presence of a substrate and a chromogen, this enzyme was turned into a colored deposit at the antibody–antigen binding site. These complex structures were shown under a light microscope (Leica). All areas were scanned to count at least 1000 cells and the expression rate was determined as a percentage (%) of positive cells.

Biochemical analysis of oxidative stress in tumor tissues

Oxidative stress in tumor tissues was biochemically analyzed with Enzyme-Linked Immunosorbent (ELISA) kits (Biovision) of oxidative stress-associated parameters; malondialdehyde (MDA), glutathione peroxidase (GPx), and glutathione (GSH) according to manufacturers' instructions.

For this purpose, tumor tissues were homogenized with the lysis buffer contained in each kit in an ultrasonic homogenizer (TissueLyser II, Qiagen, Germany) and each kit procedure was applied. With the Bicinchoninic acid assay (BCA) Protein Assay Kit (K813-2500, Biovision, USA), according to the protein amounts of the tissues.

Lipid peroxidation (malondialdehyde (MDA)) Colorimetric/Fluorometric Assay Kit (Biovision, USA) was expressed as nmol/g protein, Glutathione Peroxidase Activity Colorimetric Assay Kit (Biovision, USA) was expressed as nmol/g protein, and Glutathione Colorimetric Assay Kit (Biovision, USA) was expressed as μmol/g protein.

Statistical analysis

The F-test was used to assess the normality of the distribution of the parameters. Data were represented as mean ± standard deviation. All statistical analyses were performed using the SPSS 26.0 software program (Chicago, USA). Differences were tested to compare with the *Kruskal–Wallis'* test. $P < 0.05$ was considered statistically significant.

Results

Tumor volume changes

The three-dimensional measurements of the tumor tissues were recorded before the initiation and after the termination of treatment and converted to a graph shown in Figure 1.

Mean tumor volume changes per group followed as; Control: 0.83 mm^3, Pt: 0.38 mm^3, FUC: -0.23 mm^3, FUC+DOC: −0.15 mm^3, DOC: −0.22 mm^3, Pt-D: −0.17 mm^3 It was significantly decreased of all groups' tumor volume compared to the control group ($P < 0.05$). Tumor formation was much slower in the Pt and Pt-D groups

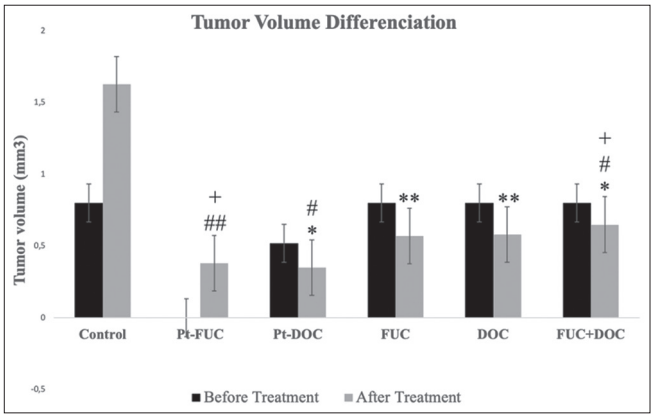

Figure 1: The tumor volume of all groups decreased significantly compared to the control group ($P < 0.05$). Compared to the control group $^{*}P < 0.05$ $^{**}P < 0.001$, compared to the FUC group $^{\#}P < 0.05$ $^{\#\#}P < 0.001$, compared to the DOC group $^{+}P < 0.05$ $^{++}P < 0.001$

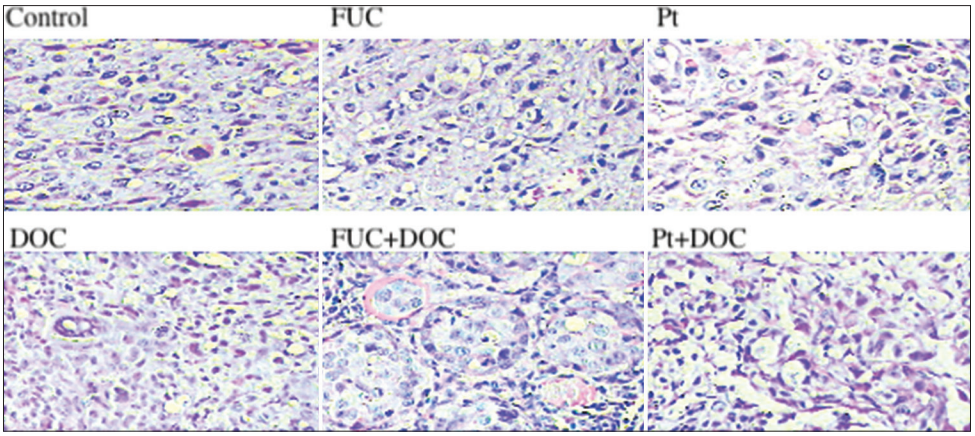

Figure 2: Images of histomorphologic structures of tumor tissues stained with hematoxylin–eosin captured at 20x magnification are shown

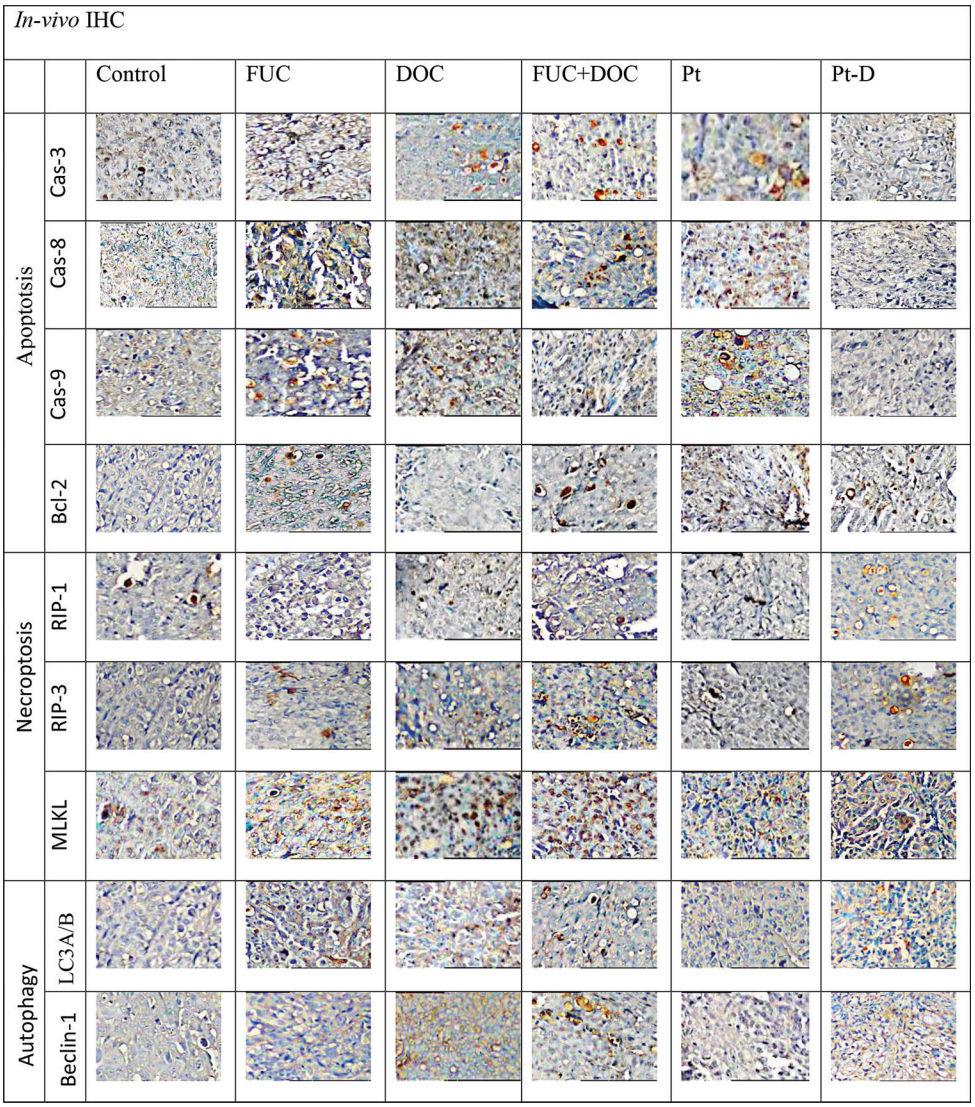

Figure 3: Images of immunohistochemically stained tumor tissues captured at 20x magnification by light microscopy are shown. Figures show Caspase-3, Caspase-8, Caspase-9, Bcl-2, RIP-1, RIP-3, MLKL, LC3A/B ve Beclin-1 protein expressions, respectively

compared to the others ($P < 0.05$). In the preventive groups of Pt and Pt-D, tumorigenesis has been shown to affect tumor growth significantly ($P < 0.001$).

Hematoxylin–eosin staining results

The tumor samples of the control group were characterized by hyperproliferation of luminal epithelial cells and active

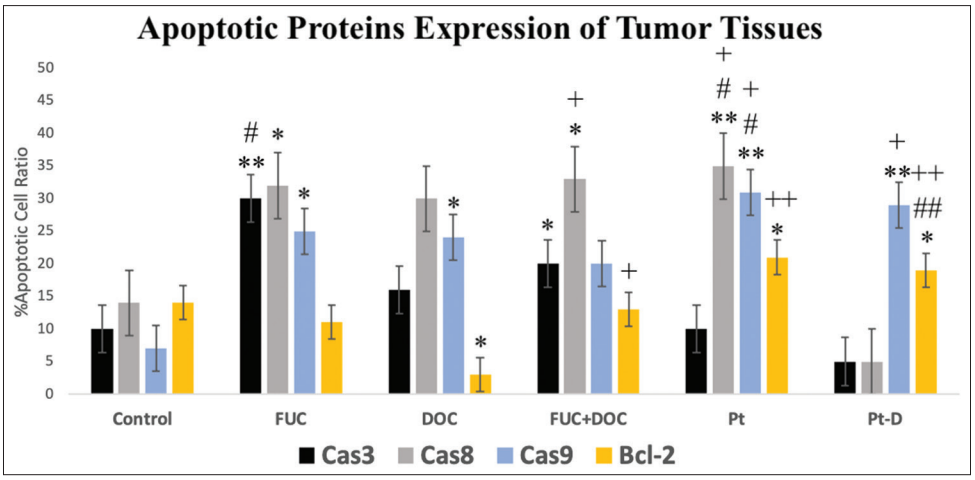

Figure 4: The graphic demonstrates the results of the quantitative assessment of apoptotic proteins by the IHC method in tumors obtained from the xenograft prostate cancer model. Black bars indicate Active Caspase-3 protein expression; Control group: 10%, FUC group: 30%, Pt group: 10%, DOC group: 16%, FUC+DOC combination group: 20%, and Pt-D group: 5%. Cas-3 expression was significantly increased in the FUC, FUC+DOC combination groups. Gray bars indicate Caspase-8 protein expression; Control group: 14%, FUC group: 32%, Pt group: 38%, DOC group: 30%, FUC+DOC combination group: 33%, and Pt-D group: 5%. Cas-8 expression was significantly increased in the FUC, FUC+DOC combination, and Pt groups. Blue bars indicate Caspase-9 protein expression; Control group: 7%, FUC group: 25%, Pt group: 31%, DOC group: 24%, FUC+DOC combination group: 20%, and Pt-D group: 29%. Caspase-9 expression was significantly increased in the FUC, DOC, Pt, and Pt-D groups. Yellow bars indicate Bcl-2 protein expression; Control group: 14%, FUC group: 11%, Pt group: 21%, DOC group: 3%, FUC+DOC combination group: 13%, and Pt-D group: 19%. A significant increase was shown in the Pt and Pt-D groups, while a significant decrease was shown in the DOC group. Compared to the control group $^{*}P < 0.05$ $^{**}P < 0.001$, compared to the FUC group $^{\#}P < 0.05$ $^{\#\#}P < 0.001$, compared to the DOC group $^{+}P < 0.05$ $^{++}P < 0.001$

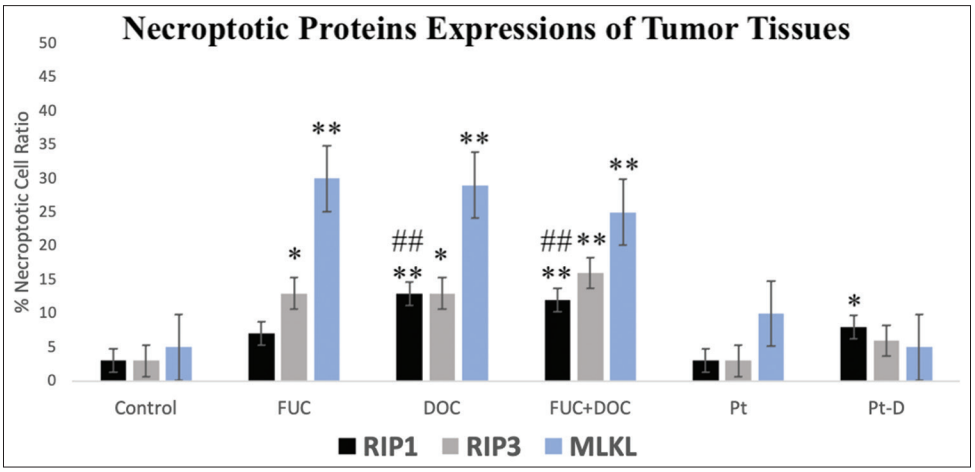

Figure 5: The graphic demonstrates the results of the quantitative assessment of necroptotic proteins by the IHC method in tumors obtained from the *in-vivo* xenograft prostate cancer model. Black bars indicate RIP1 protein expression; Control group: 3%, FUC group: 7%, Pt group: 3%, DOC group: 13%, FUC+DOC combination group: 12%, and Pt-D group: 8%. There was a significant increase in RIP1 expression in the DOC, FUC+DOC combination, and Pt groups. Gray bars indicate RIP3 protein expression; Control group: 3%, FUC group: 13%, Pt group: 3%, DOC group: 13%, FUC+DOC combination group: 16%, and Pt-D group: 6%. There was a significant increase in RIP3 protein expression in the FUC, DOC, and FUC+DOC combination groups. Blue bars indicate MLKL protein expression; Control group: 5%, FUC group: 30%, Pt group: 10%, DOC group: 29%, FUC+DOC combination group: 25%, and Pt-D group: 5%. There was a significant increase in RIP1 expression in the FUC, DOC, and FUC+DOC combination groups. Compared to the control group $^{*}P < 0.05$ $^{**}P < 0.001$, compared to the FUC group $^{\#}P < 0.05$ $^{\#\#}P < 0.001$, compared to the DOC group $^{+}P < 0.05$ $^{++}P < 0.001$

stroma was shown. Mitotic activity was decreased with treatment in all groups except for the control. There was a decrease in tumor development in the treatment groups with a reduction of cell infiltration and active stroma compared to the control group [Figure 2].

Immunohistochemical staining results

Necroptotic cell death in tumor tissue and Autophagic cell death in tumor tissue

Active Caspase-3 expression was analyzed in tumor tissues for apoptotic cell death. Caspase-3 expressions followed as; control 10%, FUC 30%, Pt 10%, DOC 16%, FUC+DOC 20%, and Pt-D 5%. A significant increase in apoptotic cell death was shown in the FUC+DOC and FUC groups compared to the control group ($P < 0.011$, $P < 0.001$, respectively). Caspase-8 protein expression in tumors followed as; control 14%, FUC 32%, Pt 35%, DOC 30%, FUC+DOC 33%, and Pt-D 5%. A statistically significant increase in Caspase-8

protein expression was shown in the FUC, FUC+DOC, and Pt groups compared to the control ($P < 0.046$, $P < 0.003$, $P < 0.001$, respectively). Caspase-9 protein expression followed as; control 7% FUC 25%, Pt 31%, DOC 24%, %20 FUC+DOC, and Pt-D 29. There was a significant increase in Caspase-9 protein expression in the DOC, FUC, Pt, and Pt-D groups compared to the control ($P < 0.046$, $P < 0.003$, $P < 0.001$, $P < 0.001$, respectively). Bcl-2 protein expression in tumor tissues followed as; control 14%, FUC 11%, Pt 21%, DOC 3%, FUC+DOC 13%, and Pt-D 19%. A statistically significant change was found in DOC, Pt-D, and Pt groups compared to the control group. While a significant increase in Pt and Pt-D groups was shown, a decrease in the DOC group was detected ($P < 0.046$, $P < 0.046$, $P < 0.003$, respectively) [Figures 3 and 4].

Necroptotic cell death in tumor tissue

RIP1 protein expressions in tumor tissues followed as; control 3%, FUC %7, Pt 3%, DOC 13%, FUC+DOC 12%, and Pt-D 8%. A statistically significant difference was in the DOC, FUC+DOC combination, and Pt-D groups compared to the control group ($P < 0.003$, $P < 0.001$, $P < 0.011$, respectively). RIP3 protein expression in tumor tissues followed as; control 3%, FUC 13%, Pt 3%, DOC 13%, FUC+DOC 16%, and Pt-D 6%. RIP3 protein expression was significantly increased in the FUC, DOC, and FUC+DOC groups compared to the control group ($P < 0.002$, $P < 0.002$, $P < 0.001$, respectively). MLKL protein expression in tumor tissues followed as; control 5%, FUC 30%, Pt 10%, DOC 29%, FUC+DOC 25%, and Pt-D 5%. MLKL protein level was increased in the FUC, DOC, and FUC+DOC groups compared to the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$, respectively) [Figures 3 and 5].

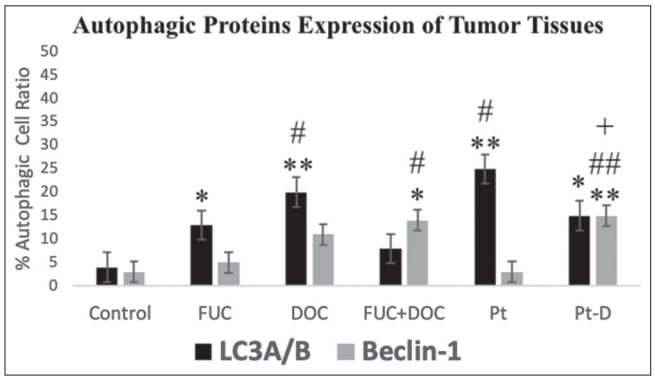

Figure 6: The graphic demonstrates the results of the quantitative assessment of autophagic proteins by the IHC method in tumors obtained from the in-vivo xenograft prostate cancer model. Black bars indicate LC3A/B protein expression; Control group: 4%, FUC group: 13%, Pt group: 25%, DOC group: 20%, FUC+DOC combination group: 8%, and Pt-D group: 15%. FUC, DOC, Pt, and Pt-D groups show a significant increase in the LC3A/B protein expression. Gray bars indicate Beclin-1 protein expression; Control group: 3%, FUC group: 5%, Pt group: 3%, DOC group: 11%, FUC+DOC combination group: 14%, and Pt-D group: 15%. A significant increase in Beclin-1 protein was detected in the FUC+DOC combination and Pt-D groups. Compared to the control group $^{*}P < 0.05$ $^{**}P < 0.001$, compared to the FUC group $^{\#}P < 0.05$ $^{\#\#}P < 0.001$, compared to the DOC group $^{+}P < 0.05$ $^{++}P < 0.001$

Autophagic cell death in tumor tissue

LC3A/B protein expression in tumor tissues followed as; control 4%, FUC 13%, Pt 25%, DOC 20%, FUC+DOC 8%, and Pt-D 15%. There was a significant increase in LC3A/B protein in the FUC, Pt, DOC, and Pt-D groups compared to the control group ($P < 0.046$, $P < 0.001$, $P < 0.001$, $P < 0.003$, respectively). Beclin-1 protein expressions followed as; control 3%, FUC 5%, Pt 3%, DOC 11%, FUC+DOC 14%, and Pt-D 15%. A statistically significant increase in Beclin-1 expression was shown in the FUC+DOC and Pt-D groups compared to the control group ($P < 0.011$ $P < 0.001$, respectively) [Figures 3 and 6].

Biochemical analysis results

A high MDA level was found in the control group. MDA levels in the Pt group were similar to the control group ($P > 0.05$). A significant decrease was shown in the FUC, FUC+DOC combination, DOC, and Pt-D groups compared to the control group [Figure 7A] ($P < 0.001$, $P < 0.001$, $P < 0.003$, $P < 0.046$, respectively). GPx activity in the DOC group was similar to the control group. A statistically significant increase in GPx activity was found in the Pt, Pt-D, and FUC groups compared to the control group. GPx activity was also increased in the

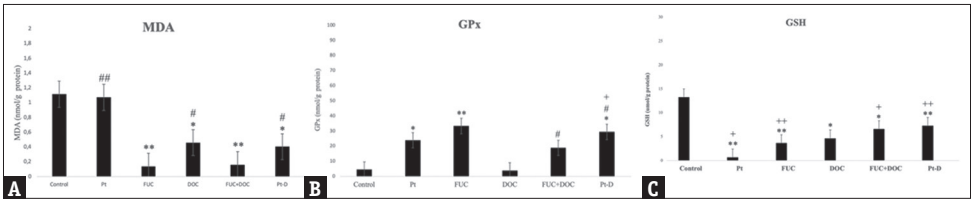

Figure 7: Graphic A demonstrates the MDA values of the groups. Graphic B demonstrates the GPx values of the groups, Graphic C demonstrates the GSH values of the groups. Significant decreases in the MDA levels in the FUC, DOC, FUC+DOC combination, and Pt-D groups were observed. Significant increases in the GPx activity in the FUC, Pt, and Pt-D groups were shown compared to the control group. The enzyme activity was also increased in the FUC+DOC combination group compared to the control group. There was a significant decrease in total GSH levels in the Pt, FUC, DOC, and FUC+DOC combination groups. Compared to the control group $^{*}P < 0.05$ $^{**}P < 0.001$, compared to the FUC group $^{\#}P < 0.05$ $^{\#\#}P < 0.001$, compared to the DOC group $^{+}P < 0.05$ $^{++}P < 0.001$

FUC+DOC group compared to the control group ($P < 0.046$, $P < 0.003$, $P < 0.001$, respectively) [Figure 7B]. When all groups were compared to the control group. There was a significant decrease in total GSH levels in Pt, FUC, DOC, and FUC+DOC groups. GSH values of all groups are shown in [Figure 7C] ($P < 0.001$, $P < 0.001$, $P < 0.003$, $P < 0.046$, respectively).

Discussion

Chemotherapy is the best treatment option for patients with metastatic prostate cancer. DOC has been widely used as the first-line treatment.[2] Although DOC cannot provide the desired outcome in the survival of metastatic prostate cancer patients, it is still accepted as the best chemotherapeutic agent.[5] Therefore, there is a need for new agents that can potentiate the effect of DOC or be an alternative to it.

It is known that most chemotherapeutic agents exert their effects by driving cancer cells into apoptosis. Although both autophagic and necroptotic effects of DOC have been demonstrated in prostate cancer in experimental studies, they mostly have focused on the apoptotic and autophagic effects of DOC.[21,22] However, the potential necroptotic effect of DOC in the prostate cancer xenograft model has not been studied.

It has been reported in studies that fucoidan from *Fucus vesiculosus*, due to its high sulfate structure, has a high anticancer effect in different cancer types.[23] Besides its anticancer effects, fucoidan-related studies revealed that samples from different sources showed different bioactivities.[24-26] Fucoidan has been shown to cause apoptotic cell death in prostate cancer cells.[27] It is also stated in studies that fucoidan may play a regulatory role in terms of autophagic cell death in cancer cells.[28] However, to date, it has not been evaluated whether fucoidan triggers necroptotic cell death in cancer, especially prostate cancer cells.

Autophagy plays a role in cancer treatment. It can also produce a double-sided effect; lethal in aggressive tumors or supporting survival by increasing the expression of stress molecules.[29] It has been reported that DOC may also have dual effects in terms of autophagy.[30] It has been indicated that DOC induces autophagy by increasing LC3 and p62 protein expression levels in metastatic PC-3 prostate cancer cells.[30] In this study, it was also shown that DOC treatment triggered autophagic cell death through increased expression of both LC3A/B and Beclin-1 protein in tumor tissues. The results of this study are consistent with the study, showing that autophagic cell death was also triggered by DOC.[30]

It has been reported in a limited number of studies that DOC can induce necroptosis in prostate cancer.[31-33] According to a study, the expression level of necroptosis-associated RIP3 protein is decreased in both prostate cancer cell lines and prostate cancer patient samples due to increased SIRTs expression.[33] Reducing RIP3 protein supports the emergence of necroptotic cell death in prostate cancer as an important target in terms of both disease progression and treatment.[31,33] Compatible with the results of this study, DOC is once again demonstrated to be a very important treatment agent, especially for the treatment of prostate cancer, by triggering necroptotic cell death in prostate cancer.

It has been reported in studies that fucoidan causes apoptosis in prostate cancer cells.[34,35] The administration of fucoidan in DU-145 prostate cancer cells was shown to induce apoptosis *in-vitro* by increasing the expressions of BAX, cleaved-PARP, cleaved-Caspase 9, and dose-dependently reduced the expression levels of Bcl-2, p-AKT, p-PI3K, p-p38, and p-ERK. In the same study, the usage of 5 and 10 mg of fucoidan in a xenograft prostate cancer model showed an antitumoral effect and a decreased tumor volume by inducing apoptotic cell death.[34,35] In the preventive group of this study, it was determined that fucoidan caused apoptosis, especially with the increase in Cas-8 and Cas-9 protein expressions. It has contributed to tumor development delay as well as a lower level of tumor tissue weight. In addition, FUC treatment alone caused a reduction in tumor size through apoptosis by increasing active Caspase-3, Caspase-8, and Caspase-9 protein expressions in tumor tissues. In this study, both the preventive and therapeutic efficacies of fucoidan in a prostate cancer xenograft model have been shown for the first time with a delay in tumor development/lower tumor tissue volume or a decrease in tumor tissue weight through apoptosis These results suggested that FUC alone can be an important therapeutic molecule in terms of both the prevention and the treatment of prostate cancer.

A limited number of studies that fucoidan triggers autophagic cell death in cancer cells have been evaluated.[36] It has been shown that the administration of fucoidan to human gastric and oral squamous cancer cells causes autophagic cell death mediated by LC3A/B and Beclin-1 protein accumulation.[36] Meanwhile, it has been shown that fucoidan has apoptotic effects in these cancer cells, through a decrease in anti-apoptotic Bcl-2 and Bcl-xl protein expressions as well as caspase activations.[36] In another xenograft study, apoptotic cell death was not induced in the tumor tissue of a hepatocellular carcinoma model.[37] Moreover, this study showed that fucoidan administration induced autophagic cell death due to an increase in LC3A/B

protein expression. At the same time, in the Pt-D group, tumor growth was inhibited through the induction of autophagic cell death in the tumor tissue by the increase of both anti-apoptotic Bcl-2, autophagic LC3A/B, and Beclin-1 protein expressions.

In this study, DOC treatment alone decreased the tumor development by triggering both apoptotic cell death and autophagic cell death by increasing LC3A/B, Beclin-1, and decreasing the anti-apoptotic Bcl-2 protein expressions. Studies have shown that autophagy has a role in the development of resistance to DOC treatment in castration-resistant prostate cancer.[38] In this sense, targeting autophagy to reduce DOC resistance also emerges as an important issue.[39] In this study, it was shown for the first time that fucoidan was effective in combination with DOC by inhibiting tumor growth with an increase in the expression of autophagic LC3A/B and Beclin-1 proteins in tumor tissue. In this regard, the addition of different agents, such as FUC, to DOC treatment in castration-resistant prostate cancer may be a useful approach for the prevention of resistance development and the continuation of the efficiency of DOC treatment.

Apart from the anti-apoptotic mechanism, Bcl-2 may bind to Beclin-1, suggesting that it may inhibit autophagy by preventing the formation of the pre-autophagosomal structure.[40] In the Pt-D group, Beclin-1 expression increased with raised anti-apoptotic Bcl-2 protein expression and remained at a similar level to that of the control group, indicating that cell death in the Pt-D group may be mediated by both apoptotic and autophagic mechanisms. It was suggested that preventive fucoidan administration may be effective in preventing tumor development caused by a decrease in Bcl-2 and an increase of Caspase-8 and Caspase-9 pro-apoptotic protein expressions in the tumor tissue.

The potential effects of fucoidan on autophagic or necroptotic cell death in prostate cancer have not been studied to date. In this study, fucoidan inhibited the tumor growth by increasing necroptotic MLKL protein expression. It is predicted that the necroptotic cell death through the increase in RIP1, and RIP3 protein expressions in the Pt-D group, may be one of the potentially effective mechanisms mediating the prevention of tumor development.

In this study, the effects of FUC together with DOC on apoptotic, necroptotic, and autophagic molecules in prostate cancer were investigated for the first time. Besides, it was shown for the first time that the combined treatment of FUC+DOC contributes to the reduction in prostate cancer tumor volume by increasing the necroptotic protein expressions similar to other cell death-related protein expressions. In addition, it has been evaluated for the first time using a xenograft prostate cancer model that both the preventive and the anti-tumorigenic effects of fucoidan in both the presence and absence of DOC. FUC would induce different cell death mechanisms with or without DOC, through the modulation of either apoptotic, autophagic, or necroptotic molecules. It can be suggested in light of this study's results that fucoidan should be evaluated for its efficiency-increasing effects on DOC therapy response in metastatic prostate cancer.

The formation and regulation of oxidative stress are important for triggering cell death in conventional chemotherapy. Oxidative stress-related MDA, GSH, and GPx markers were also examined within the scope of this study and all markers were changed in all groups compared to the control group. In particular, the induction of lipid peroxidation in the control group might indicate the role of oxidative stress in the development of cancer. This study's results were consistent with some clinical studies, indicating that MDA level was increased in prostate cancer. It could be correlated with the disease stage.[41] In this study, levels of MDA were decreased in FUC alone and FUC+DOC combination groups compared to the control group. However, the Pt group did not show a significant decrease in MDA levels. Freitas *et al*.[42] evaluated GSH and GSH reductase activity to assess the adaptation of metastatic prostate cancer cells to increased ROS levels. They found a decrease in GSH reductase activity and GSH content, besides a decrease in cell proliferation due to apoptotic or necrotic cell death. The fact that the antioxidant GPx levels were significantly increased in the Pt, Pt-D, and FUC groups, except the DOC group, in comparison with the control, suggested that fucoidan supports the antioxidant capacity alone against oxidative stress in prostate cancer. In this study, compatible with the study of Freitas, it is suggested that the FUC+DOC combination contributes to the development of anti-tumoral effects mediated by oxidative stress-related GPx increase and antioxidant GSH decrease.

In light of the results of this study, it was stated for the first time DOC, FUC, and FUC+DOC combination treatments in prostate cancer can trigger autophagic and necroptotic cell deaths in addition to apoptotic cell death. They showed antitumoral effects mediated by increasing the expression of the proteins associated with these cell death mechanisms. In this study, it was shown for the first time that FUC alone and in combination with DOC are important for increasing the efficacy of DOC therapy response in metastatic prostate cancer. It was

thought that fucoidan could balance docetaxel resistance and increase the antitumor efficacy of docetaxel. Not using docetaxel-resistant cells is a limiting factor in this study. The effect of fucoidan on the autophagic death mechanism can be investigated for further studies, which are needed for the potential effect of FUC for advanced prostate cancer.

Conclusion

This study showed that fucoidan alone and in combination with DOC are potential anticancer agents through the modulation of apoptotic, necroptotic, and autophagic cell death molecules in prostate cancer. In the pre-treatment fucoidan group, prostate cancer development emerges through temporal and dimensional delay which suggests that it could be considered as a potential preventive agent.

The results of this study showed that fucoidan is an effective agent for the prevention and treatment of prostate cancer, alone or in combination with DOC. Further experimental and clinical studies are needed to use fucoidan in the clinic as a potential anticancer agent, both preventive and curative in prostate cancer.

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Conflicts of interest

There are no conflicts of interest

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