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Original Research

SARS-CoV-2 Spike Protein Influences Expression of ICOSL and ICAM-2 in Prostate Cancer

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Abstract

Background: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the novel coronavirus responsible for the COVID-19 pandemic. The viral protein of SARS-CoV-2, spike protein (SP), mediates entry into host cells, contributing to pathogenesis of COVID-19. Prostate cancer is the most common cancer among men in the United States. Inducible T-cell costimulator ligand (ICOSL) and intercellular cell adhesion molecule 2 (ICAM-2) are expressed in cancer cells and their roles in cancer growth remain controversial. It is unknown if SP can affect the expression of ICAM-2 or ICOSL in prostate cancer. This study investigated the effects of SARS-CoV-2 SP on the expression of ICAM-2 and ICOSL and the time-dependent effect of SP on growth and survival of prostate cancer cells. **Methods**: The effect of SARS-CoV-2 SP on the survival of a widely-used prostate cancer cell line, LNCaP, was assessed using clonogenic cell survival assay and quick cell proliferation assay. Reverse transcription-polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) were performed to investigate the expression of ICAM-2 and ICOSL. The survival of an additional prostate cancer cell line, PC-3, was also evaluated by clonogenic survival assay. **Results**: After 3 days, a significant decrease in the percentage of colonies in LNCaP cells treated with SP was found, which was paralleled by a decrease in optical density (OD) value in LNCaP cells in the presence of SP. A significant decrease in the percentage of colonies treated with SP was also found in PC-3 cells evaluated by clonogenic survival assay. In addition, the mRNA expression of ICAM-2 was lower, whereas the mRNA expression of ICOSL was higher in SP-treated LNCaP cells. This was supported by protein expressions for ICAM-2 and ICOSL evaluated with IHC. **Conclusions**: In LNCaP cells, SARS-CoV-2 SP downregulates the expression of ICAM-2 but upregulates the expression of ICOSL. SARS-CoV-2 SP inhibits growth of prostate cancer cells in a time-dependent manner. Further studies are needed to fully address the roles of ICAM-2 and ICOSL in the inhibition prostate cancer growth by SARS-CoV-2 SP.

Keywords: SARS-CoV-2; spike protein; prostate cancer; ICAM-2; ICOSL

1. Introduction

Prostate cancer is very common and a significant public health concern. In fact, prostate cancer is the most commonly diagnosed male cancer as well as the 2nd leading cause of cancer deaths among men in the United States [1]. Several factors are associated with a higher risk of prostate cancer such as older age, family history, ethnicity, smoking, and obesity [2]. Most cases of prostate cancer remain localized and do not pose a significant threat to mortality and can be treated effectively with surgery, radiotherapy, or androgen deprivation therapy [3]. However, if the cancer metastasizes to adjacent lymph nodes, bones, liver, or other tissues, it becomes practically incurable [4]. Thus, most deaths associated with prostate cancer are the result of metastasis.

The immune system plays an important defensive role against cancer in the body. The two divisions of the immune system, the innate and adaptive systems, work to detect and eliminate cancer cells before they become clinically apparent [5]. Unfortunately, there are several factors that disrupt the immune response to cancer cells, such as chemotherapy. Furthermore, cancer cells themselves can evolve and develop mutations such that they evade the immune system, grow into large tumors, and spread throughout the body [6,7]. The tumor microenvironment is one case where the immune response is often significantly inhibited. This can occur due to increased levels of suppressive cytokines, a higher number of immune-suppressing T regulatory cells (Tregs), low expression of major histocompatibility complex (MHC) molecules or antigens, or checkpoint molecules can be expressed to inhibit T cells so that they do not mount a proper immune response [8,9]. Some cancers have a characteristically immunosuppressive tumor microenvironment which can make the cancer more difficult to treat [10].

T Cells are the major directors of the adaptive arm of the immune system. Multiple signals are required for T cells to become activated against potential threats. The first signal is provided to the T cell receptor via antigen presenting cells that display antigens on major-histocompatibility complexes. A second interaction with costimulatory molecules from the antigen presenting cell is required for the T-cell to progress to full activation—a process known as costimulation [11]. Following T cell activation, coinhibitory molecules are produced to suppress and modulate the immune cells to avoid excessive immune responses [12]. These immunity-modulating molecules are primarily expressed on the surface of antigen presenting cells but can also be found on endothelial cells and, importantly, on tumor cells. Our lab is interested in the expression of these molecules in prostate cancer cells as alterations in expression of these proteins could have implications for growth, prognosis, and treatment of cancer.

Inducible T-cell costimulator ligand (ICOSL), part of the B7 family, is the unique ligand for inducible T-cell costimulator (ICOS), which is part of the CD28 family of costimulatory molecules [13,14]. ICOSL has been found to be expressed in dendritic cells, B cells, fibroblasts, endothelial cells, and cancer cells [14–17]. Recent research into the link between the ICOS-ICOSL signaling pathway and cancer illustrates the importance of ICOSL expression in tumor cells. Increased ICOSL expression may assist certain cancers in evading destruction by the immune system, but in other instances it may enhance antitumor immunity [18].

Intercellular cell adhesion molecule 2 (ICAM-2) is a transmembrane glycoprotein of the immunoglobulin superfamily that is primarily expressed on the surface of endothelial cells and can also be found on cancer cells [19]. ICAM-2 is structurally and functionally similar to another important cell adhesion molecule, ICAM-1 [20]. Like ICAM-1, ICAM-2 serves as a receptor to the integrin leukocyte function-associated antigen (LFA)-1 [20]. Cell adhesion molecules like ICAM-2 have important biological roles such as extravasation of leukocytes, T-cell and NK cell costimulation, mediation of intercellular adhesion, and others. Altered expression of these molecules can significantly affect cancer progression.

Coronavirus disease 2019 (COVID-19) has caused the deaths of over 5 million people and afflicted hundreds of millions while causing immense damage and disruptions worldwide [21]. COVID-19 is caused by the highly transmissible severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in Wuhan, Hubei Province, China, in December 2019 [22]. SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus that is structurally composed of 4 major proteins including the nucleocapsid protein, the membrane protein, the envelope protein, and the spike protein (SP) [23]. The SP is present on the surface of the viral particle where it interacts with host proteins leading to infection. The cellular protein, angiotensin-converting enzyme 2 (ACE2) and the transmembrane protease serine 2 (TMPRSS2) have crucial roles in the entry of SARS-CoV-2 virus [24]. ACE2 serves as the main receptor for SP while TMPRSS2 cleaves a specific subunit of the SP leading to fusion of the cell membranes and viral entry [25]. These proteins are not only expressed in respiratory tract epithelium, but they are also found in multiple organs throughout the body, including the prostate and are, therefore, at risk of infection by SARS-CoV-2 [25,26].

Amid the pandemic of COVID-19, and given the prevalence and seriousness of prostate cancer, it is of significant interest to understand if and how prostate cancer is affected in the presence of SARS-CoV-2 SP. A study from our lab recently reported that growth of LNCaP cells is inhibited by SP concentrations of 20 ng/mL, 50 ng/mL, and 200 ng/mL when treated for 72 hours [27]. It was found that 50 ng/mL most significantly inhibited LNCaP cell growth. In addition, all previous cytokine studies in our lab have used the concentration of 50 ng/mL. For this reason, in this *in vitro* study, we focused on investigating if a concentration of 50 ng/mL SARS-CoV-2 SP influences the growth of LNCaP prostate cancer cells in a time-dependent pattern. We also investigated whether this concentration of SP has any effect on the expression of costimulatory/coinhibitory molecules in these cells.

2. Materials and Methods

2.1 Tumor Cell Line

The cell lines, LNCaP and PC-3, are human prostate cancer cell lines, and were obtained from Dr. Lubahn at the University of Missouri, Columbia, MO. The cells were cultured in DMEM (Invitrogen, Carlsbad, CA, USA) for preservation and supplemented with 10% heat-inactivated FBS and 1% penicillin-streptomycin (Invitrogen). Incubation of the cells was conducted at 37 ℃ in a 5% CO_2 incubator. After attaining 70% confluence, the LNCaP and PC-3 cells then underwent the experimental treatments.

2.2 Treatment with SARS-CoV-2 SP

SARS-CoV-2 SP was obtained from BioLegend, San Diego, CA. In our study, SP was dissolved in DMEM, stocked at 50 μg/μL, and we did a 1000-fold dilution with the DMEM to use as our working solution. In preparation for the clonogenic survival assay, the LNCaP and PC-3 cells at 70% confluence were treated with SARS-CoV-2 SP at the concentration of 50 ng/mL for 1, 3, and 5 days. A control group with only medium was also prepared. The other experiments outlined in this study only used LNCaP cells treated with SARS-CoV-2 SP at 50 ng/mL for a period of 3 days, and a control that consisted of LNCaP cells in medium for 3 days.

2.3 Clonogenic Survival Assay (CSA)

The procedure for the CSA was followed as described in prior studies [28–31]. Using TrypLE Express (Invitrogen), the LNCaP and PC-3 cells were retrieved from the culture flasks and then suspended in a phosphate-buffered saline. The cells were subsequently tallied by hemocytometer. A total of 1000 LNCaP and 1000 PC-3 cells were plated in triplicate onto 60-mm Corning petri dishes and then incubated at 37 °C in a humidified 5% CO_2 incubator. Fresh media was added on the 5th day of incubation and on the 9th day, the cells were fixed in 10% formaldehyde and stained with 0.05% crystal violet. Finally, after counting the LNCaP and PC-3 colonies in the treatment groups, the survival was expressed as a percentage of the total colonies found in the control groups.

2.4 Quick Cell Proliferation Assay

LNCaP cell proliferation was studied using the quick cell proliferation assay kit (BioVision). Mitochondrial dehydrogenase activity is directly proportional to the proliferation of viable cells. An increase in activity of these viable cells produced formazan dye which was able to be quantified by a spectrophotometer. Prior studies outline the details of this procedure [28–30].

2.5 Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

In preparation for RT-PCR, the LNCaP cells were washed with a phosphate-buffered saline, homogenized in TRIzol (Invitrogen), and underwent RNA extraction. To confirm the concentration of RNA, Nanodrop was used, and as outlined previously, 1 μg RNA underwent reverse transcription [28–30]. The gene, Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was utilized as an internal control to confirm that the same quantity of RNA was amplified.

2.6 Immunohistochemistry (IHC)

The IHC staining protocol used for Inducible T-cell costimulator ligand (ICOSL), and intercellular cell adhesion molecule 2 (ICAM-2) was followed as described in previous studies [32,33]. A dilution of 1:200 was used for the primary antibody, while a dilution 1:500 was used for the secondary antibody. The images were analyzed using MetaMorph 6.3r6 software (Molecular Devices, Union City, CA) to determine the average staining intensities for the proteins located within the area occupied by LNCaP cells. The same software was used for counting. The results were expressed as the average integrated immunostaining intensity of 3 slides $\pm$ SEM compared to control cell intensity.

2.7 Statistics

Statistical analysis was performed using the Mann-Whitney U test. A value of $p < 0.05$ was considered a significant result. To ensure accuracy, every experiment was done in triplicate.

3. Results

3.1 The Time-dependent Effect of SARS-CoV-2 SP on LNCaP and PC-3 Cell Survival

To evaluate the time-dependent effect of the SARS-CoV-2 SP on the survival of LNCaP and PC-3 cell lines, the cells were treated with SP at the concentration of 50 ng/mL for 1, 3, and 5 days alongside a control that consisted of medium alone. The clonogenic survival assay indicated that there is no statistical difference in clonogenic colony survival after 1 day. After 3 days in the presence of SP, there was a significant decrease in LNCaP cell survival. This was also true at day 5. This suggests that at least 3 days of SP exposure is necessary to reduce colony survival. SP concentrations of 50 ng/mL, added to LNCaP cells showed significant decreases in colony count (Fig. 1A, $p < 0.05$). The colony count percentages suggest that the SP reduces LNCaP cell survival in a time-dependent manner, with values of 88 $\pm$ 8%, 37 $\pm$ 6%, and 24 $\pm$ 3% for the time periods of 1, 3, and 5 days, respectively.

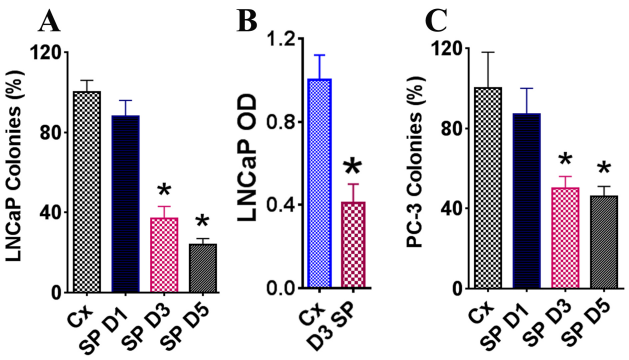

Fig. 1. The time-dependent effect of SARS-CoV-2 SP on LNCaP and PC-3 cell survival. (A) Clonogenic survival assay for LNCaP cells treated with 50 ng/mL of SP for varying periods of time: 1 day (D1), 3 days (D3), 5 days (D5). The control consisted of medium alone with the same concentration of SP and same time periods. Each of the treatment group colonies were quantified and expressed as a percentage of colonies found in the control group. (B) Assessment of the control group and the 3-day (D3) treatment of 50 ng/mL of SP utilizing a cell proliferation kit. Here, two independent experiments are exhibited, and the results are expressed as the average optical density (OD) plus the standard error of the mean (SEM) between the control and SP groups. (C) Clonogenic survival assay for PC-3 cells treated with 50 ng/mL of SP for 1 day (D1), 3 days (D3), 5 days (D5). The control consisted of medium alone with the same concentration of SP and same time periods. Each of the treatment group colonies were quantified and expressed as a percentage of colonies found in the control group. Statistically significant results are indicated by asterisk (*) ($p < 0.05$).

In addition, the Quick Cell Proliferation Assay showed a significant reduction in optical density (OD) values between the SP-treated cells and the control group (Fig. 1B). This is consistent with our previous publication [27]. These results demonstrate that the SP of SARS-CoV-2 negatively affects the proliferation and survival of LNCaP cells. To exclude the possibility that the growth-inhibitory effect of SP is specific to the LNCaP cell line, the effect of SP on the growth of another prostate cancer cell line, PC-3, was also evaluated by clonogenic survival assay. A similar growth-inhibitory effect was found in PC-3 cells (Fig. 1C, $p < 0.05$).

3.2 SARS-CoV-2 SP Upregulates ICOSL in LNCaP Cells

RT-PCR was performed to evaluate the mRNA expression of key costimulatory and coinhibitory molecules in order to elucidate possible mechanisms whereby SARS-CoV-2 SP might influence how prostate cancer interfaces with the immune system. The expression of several costimulatory molecules, coinhibitory molecules, and their respective ligands (PDL1, PDL2, 4-1BBL, ICOSL, OX40) was evaluated. The relative levels of expression were compared between the 3-day treatment with SP group and a control group. ICOSL expression was found to be increased in the LNCaP cells treated with SP for 3 days compared to the control group (Fig. 2, $p < 0.05$). In addition, the staining intensity of the LNCaP cells in the 3-day SP treatment group was evaluated using IHC staining, which revealed a significant increase in staining intensity in the SP group (2.20 ± 0.24) relative to the control group (1.00 ± 0.24) (Fig. 3).

3.3 SARS-CoV-2 SP Downregulates ICAM-2 in LNCaP Cells

RT-PCR was performed to evaluate the mRNA expression of key cell adhesion molecules (ICAM-2, VCAM-1) and the relative levels of expression were compared between the 3-day treatment of SP group and control groups. Expression of ICAM-2 was decreased in the LNCaP cells treatment group as compared to the control group (Fig. 2, $p < 0.05$). Again, IHC was implemented to assess the relative staining intensities of the 3-day SP treatment group and the controls. A decreased staining intensity was noted in the LNCaP cells from the SP treatment group (0.45 ± 0.11) versus the control group (1.00 ± 0.11) (Fig. 4).

4. Discussion

A recent study from our laboratory revealed that SARS-CoV-2 SP not only inhibits LNCaP cell proliferation, but also promotes apoptosis by upregulating the pro-apoptotic molecule, FasL—more fully characterizing the decrease in survival of LNCaP cells when exposed to SP [27]. The current study expanded our understanding by revealing that 1 day of SP exposure is insufficient time to have an effect on LNCaP and PC-3 cell growth—at least 3 days are necessary to begin to see the growth-inhibiting

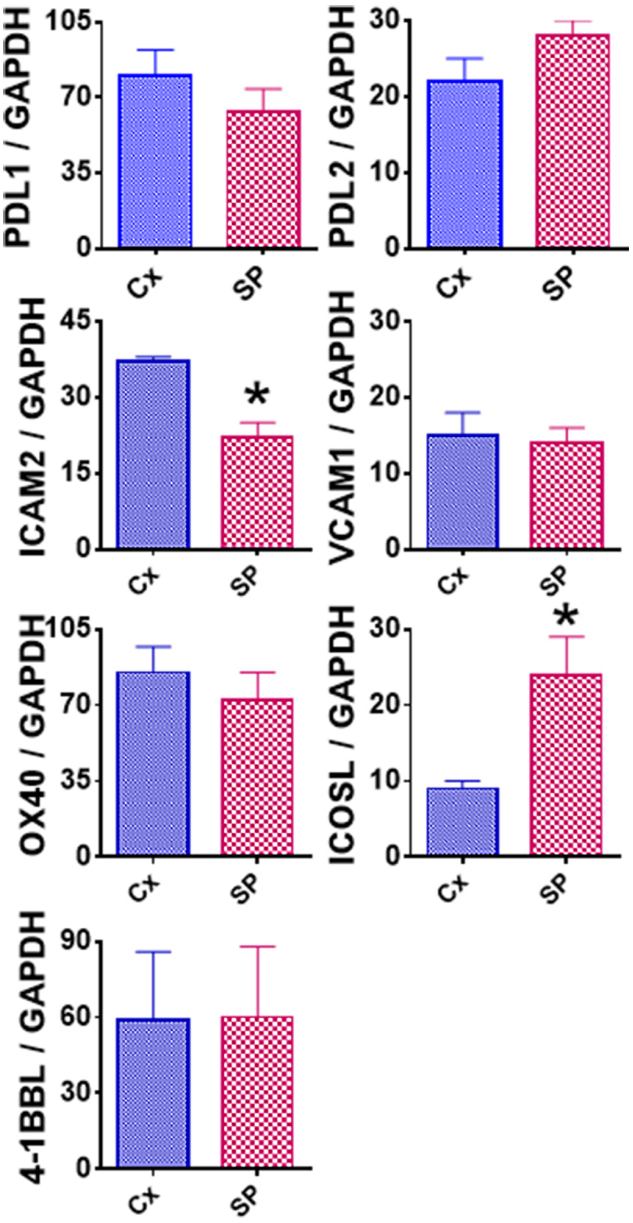

Fig. 2. The effect of SARS-CoV-2 SP on the expression of key costimulatory, coinhibitory, and cell adhesion molecules in LNCaP cells evaluated by RT-PCR. The costimulatory, coinhibitory, and cell adhesion molecules studied include PDL1, PDL2, ICAM-2, VCAM-1, OX40, ICOSL, 4-1BBL. The internal control, GAPDH, was used to compare levels of expression. The experiments were conducted in triplicate with the results depicted on graphs as a mean ratio of molecule densitometric Units/GAPDH + SEM ($\times 100$). Statistically significant results are indicated by asterisk (*) ($p < 0.05$).

effect of SP. This study also elucidated that the SARS-CoV-2 SP modulates the expression of two key costimulatory molecules, ICOSL and ICAM-2. To the best of our knowledge, this is the first study to investigate the direct role of SP on the expression of costimulatory and coinhibitory molecules in prostate cancer.

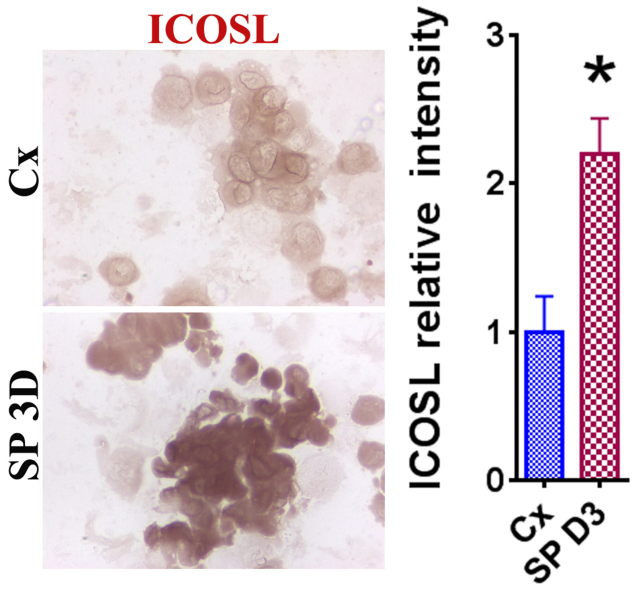

Fig. 3. The effect of SP on the expression of ICOSL in LNCaP cells evaluated by IHC staining. There was an increase in staining intensity observed in the group treated with SP versus the control group, demonstrating an increase in the expression of ICOSL. On 3 slides, 3–5 high power fields were selected randomly, and the relative staining intensities were assessed using MetaMorph image analysis software. Shown are the original 400× magnification IHC images. Statistically significant results are indicated by asterisk (*) ($p < 0.05$).

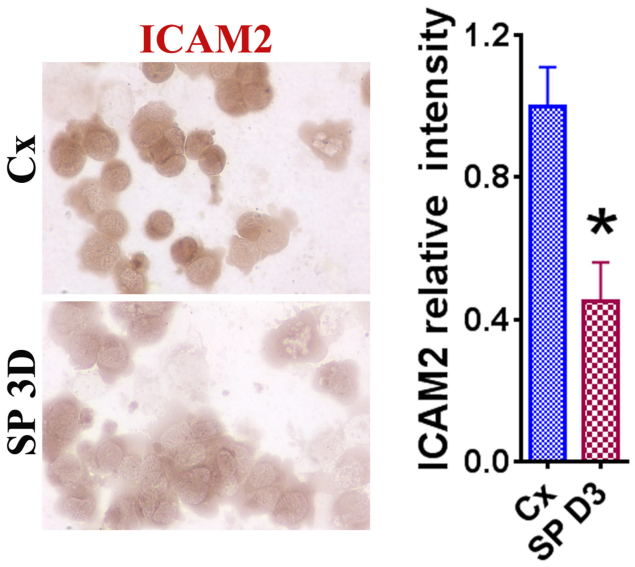

Fig. 4. The effect of SP on the expression of ICAM-2 in LNCaP cells evaluated by IHC staining. There was a decrease in staining intensity observed in the group treated with SP as compared to the control group, demonstrating a decrease in the expression of ICAM-2. On 3 slides, 3–5 high power fields were selected randomly, and the relative staining intensities were assessed using MetaMorph image analysis software. Shown are the original 400× magnification IHC images. Statistically significant results are indicated by asterisk (*) ($p < 0.05$).

When evaluating the growth of prostate cancer, it is important to discuss the androgen receptor signaling pathway as this plays a major role in the growth of prostate cancer. In prostate cancer cells, after binding of a ligand, the androgen receptor can either be translocated to the nucleus where it upregulates cell proliferation genes, or it can exert non-genomic effects that increase proliferation without needing to localize to the nucleus [34]. Thus, the androgen receptor in prostate cancer cells contributes to unregulated cell growth and it does so through a variety of mechanisms. In LNCaP cells, androgens act through the androgen receptor pathway to increase cyclin-dependent kinase activity and promote progression through the cell cycle resulting in overall proliferative effects [35]. One pro-proliferative gene upregulated through this pathway is *CDK4* [35]. This is of particular interest to our lab given that in our recent study CDK4 expression was downregulated in LNCaP cells in the presence of SP, which correlated with suppressed cell proliferation [27]. In this study, we did not test whether the observed inhibitory effects of SP on the growth of LNCaP cells is related to expression levels of the androgen receptor. However, we intend to evaluate the expression levels of the androgen receptor in the presence and absence of SP in future studies.

The upregulation of ICOSL by SARS-CoV-2 SP in prostate cancer cells is interesting because this costimulatory ligand in particular may have an immunosuppressive role in the tumor microenvironment. Melanoma, for example, is a type of cancer that increases expression of ICOSL as a means to more effectively evade detection and destruction by the body's immune system [16]. Upregulation of ICOSL by the melanoma cells serves to promote proliferation and activation of immunosuppressive regulatory T cells (Tregs) in the tumor [16]. In addition, one study determined that ICOSL expression was upregulated in breast cancer cells, which was associated with a worse prognosis [17].

Tregs are a subtype of T cells that act as regulators of the immune response. Tregs suppress immune responses via direct cell-to-cell interactions, or the production of anti-inflammatory cytokines [36]. Tregs can be further subdivided into two groups based on whether or not they express ICOS: ICOS+ and ICOS– [37]. It has been shown that when compared to ICOS– Tregs, ICOS+ Tregs have much more robust immunosuppressive activities and exhibit an increased ability to proliferate [18,38,39]. Studies have shown that the ICOS-ICOSL interaction plays a key role in the proliferation of these ICOS+ Tregs [40]. Moreover, ICOSL expression in tumor cells and tumor-associated plasmacytoid dendritic cells has been shown to increase recruitment and proliferation of ICOS+ Tregs in various types of tumors [16,41–43]. In prostate cancer, blockade of this pathway via anti-ICOS antibodies dimin-

ishes tumor infiltrated Tregs [44]. Together, these studies suggest that an increased number of more efficient Tregs in tumors makes it more difficult for the body to fight the cancer and, in certain situations, increased expression of ICOSL on cancer cells may be responsible for recruitment of these Tregs. Thus, increased ICOSL expression on tumor cells has the potential to worsen clinical situations via immunosuppressive Tregs.

In prostate cancer, a situation could arise in which ICOSL over-expression promotes increased activation of the ICOS-ICOSL pathway leading to an abundance of Tregs that can be associated with poor outcomes. Examination of prostate tumor tissue and the peripheral blood of prostate cancer patients revealed elevated numbers of Tregs, and their abundance was highly correlated with both the severity and poorer prognosis [45]. Exposure of the cancer cells to the SP of SARS-CoV-2 could create such a situation. Although this was only an *in vitro* study, it shows a possible mechanism whereby prostate cancer cells could become better equipped to evade the body's immune system, namely, by increasing ICOSL expression. This could potentially complicate prostate cancer treatment or worsen prognosis. However, further research and direct evidence are needed to definitively make this conclusion.

It is important to note that increased ICOSL expression is not always correlated with poorer outcomes in all cancer types, but it can also be associated with better outcomes and improved survival [46,47]. The ICOS-ICOSL signal can potentiate antitumor activities when acting through effector cells such as T helper cells or cytotoxic T lymphocytes [18,48]. If SP exposure induces ICOSL expression and it favors stimulation of these effector cells in prostate cancer cells, then this may lead to an immune environment that is antagonistic to the cancer. However, at least in prostate cancer, evidence seems to support that the ICOS-ICOSL pathway will give rise to a more favorable environment for cancer cells. But this does demonstrate the dual action of this key costimulatory pathway and the complexity of immunity in the tumor microenvironment. Hence, various approaches aimed at modulating the ICOS-ICOSL pathway are currently being studied as promising avenues for cancer immunotherapy [18].

In our study, ICAM-2 expression was decreased in LNCaP cells when treated with SARS-CoV-2 SP. ICAM-2 is an important costimulatory molecule that has roles in immunity and when downregulated, may affect the immune system's ability to detect and kill cancerous cells. Immune cells rely on cell adhesion molecules to exit vasculature and enter tissues where they can then perform their functions. Various cell adhesion molecules such as ICAM-2, have been reported to be downregulated in tumor-associated vessels in several different cancers [49]. Fewer receptors result in an impaired ability of anti-tumor leukocytes to extravasate to the site of the tumor. ICAM-2 is also directly involved in enhancing immune cell functions. It has been shown that an increased expression of ICAM-2 on cancer cells enhances the cytotoxic activities of immune cells [50]. For example, a higher degree of expression in certain T cells results in a greater capacity to kill pancreatic cancer cells [51]. Moreover, ICAM-2 can enhance antigen presentation and provide costimulatory signals to T cells and natural killer cells [52,53].

ICAM-2 has an additional interesting association with cancer progression. At least in neuroblastoma, ICAM-2 expression is correlated with a favorable tumor stage and indicates a lower metastatic potential [54]. This is interesting because several studies indicate that ICAM-1 expression is highly associated with increased metastatic potential of various cancers [55]. Although ICAM-2 is very similar in structure and function to ICAM-1, it is unclear whether its expression confers malignancy in a similar manner in other cancers.

With the current evidence available, we can only infer what may happen in prostate cancer when ICAM-2 is downregulated. This is because research investigating the role of ICAM-2 in prostate cancer, specifically, is lacking. However, taken together, the above studies indicate that ICAM-2 bolsters the immune system and plays a role in immune-mediated killing of certain cancers. Thus, if prostate cancer cells undergo downregulation of ICAM-2 after exposed to SARS-CoV-2 SP, the cancer cells may be more able to evade destruction by the immune system.

In the context of the current study, the above discussion is only significant if the SARS-CoV-2 SP is ever present in prostate tissue *in vivo*. While evidence is lacking for direct infection of healthy or cancerous prostate cells by SARS-CoV-2, it cannot be ruled out. There are studies that suggest it is likely that prostate tissue can be exposed to the SARS-CoV-2 SP.

Evidence that SARS-CoV-2 could access the prostate was shown in a small study where SARS-CoV-2 was detected in semen samples of six patients [56]. While this does not demonstrate that the prostate was the infected organ in these cases, it does indicate that there can be virus in the male genitourinary tract. SP has also been detected in the urine of SARS-CoV-2 infected patients [57]. It has also been reported that prostate cells could be a target of infection of SARS-CoV-2. A small subset of prostate cells co-express TMPRSS2 and ACE2, making those cells accessible for entry [58]. Moreover, in prostate adenocarcinoma cells, the expression of TMPRSS2 is elevated, which raises susceptibility to infection [59]. A study out of Italy confirmed this increased susceptibility in a study that showed an increased risk of SARS-CoV-2 infection in prostate cancer patients compared to non-cancer patients [60].

A study by Ogata *et al*. [61] showed that the S1 subunit of the SARS-CoV-2, the SP itself, and the nucleocapsid protein could be detected in peripheral plasma of COVID-19 patients at different stages of disease using a highly sensitive assay. The concentrations detected were very mini-

mal, but it confirms that viral particles, including the SP, can circulate in the blood to peripheral sites. Similarly, the S1 subunit of SP and whole SP, were transiently present at detectable levels in the plasma of the mRNA-1273 SARS-CoV-2 vaccine recipients [62]. The concentration of the levels of SP were again very low, but considering the results of our study, this does suggest possible implications that mRNA vaccines might have for prostate cancer patients. It is unknown if the SP produced after administration of SARS-CoV-2 vaccines could produce the effects that were shown in our *in vitro* study with viral SP, nor is it known if the vaccine-produced SP reaches sufficient concentrations in the body to influence prostate cells. However, at least *in vitro*, the presence of SP influences prostate cancer cell expression of costimulatory molecules and affects survival after at least 3 days of exposure. Whether it be by vaccination or infection, if prostate cancer cells are exposed to SP in a manner that produces the effects found in this study, there could be a variety of outcomes as discussed.

As an *in vitro* study, there are several limitations, and we cannot make any definitive conclusions about *in vivo* processes. Discussions about possible implications that our study may have for prostate cancer growth are logical extensions based on current research. To make conclusions of clinical import regarding the effects of SP on prostate cancer, research in the context of the body's immune system is needed. The practicality of our data also depends on more knowledge of how the SARS-CoV-2 SP is distributed throughout the body during COVID-19 or following mRNA vaccination. Furthermore, our laboratory continues investigating the effects of the SARS-CoV-2 SP in various cancer cell lines.

5. Conclusions

In summary, our study demonstrated that in the presence of the SP of SARS-CoV-2, LNCaP prostate cancer cells upregulated the expression of ICOSL and downregulated the expression of ICAM-2. We also determined that at least 3 days of SP exposure is necessary to inhibit growth of LNCaP and PC-3 cells *in vitro*. Further studies are needed to fully address the roles of ICAM-2 and ICOSL in the inhibition of prostate cancer growth by SARS-CoV-2 SP. Further conclusions are unable to be made, but we feel that the results of this study have illuminated interactions between the SP of SARS-CoV-2 and prostate cancer cells.

Author Contributions

YF designed the study. YF, ME, CP, HX, ML, SL, QB performed the experiment, YF, ZD, ZZ, ME and MRW analyzed the data. YF, ZD, ZZ, MRW interpreted the data. ME wrote the draft and YF and ZD revised the manuscript critically. ME and ZD may work differently in each part, however, overall, they contributed equally to this study.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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