ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-8 | Issue-1 | Jan-Feb -2026 | **DOI: https://doi.org/10.36346/sarjbab.2026.v08i01.006**

Original Research Article

Resveratrol Suppresses Prostate Cancer Cell Growth by Modulating STAT3 Signaling Pathways

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Article History
Received: 04.12.2025
Accepted: 31.01.2026
Published: 06.02.2026

Abstract: Prostate cancer is part of male reproductive system cancers that causes of cancer related death. Although, multiple strategies have been developed, prostate cancer continue to represent a significant disease in globally. To overcome this problem, a deeper understanding of molecular mechanism that drive prostate cancer proliferation and metastasis to develop effective cure. In the current study, we investigate the signal transducer and activator of transcription 3 (STAT3) as oncogene that implicated in prostate cancer progression. Our results show a substantial level of *STAT3* in DU145 and LNCaP cells. Furthermore, we show that chemo-preventing of STAT3 by resveratrol treatment or knockout of *STAT3* expression in prostate cancer cells (DU145 and LNCaP) decrease cell viability. Resveratrol at different concentration show an antiproliferative activity against prostate cancer cells (DU145 and LNCaP), with most activity accompanied with 50 µM. For the first time in our lab, using CRISPR Cas9 technology to delete STAT3 gene to showed a significant reduces cell viability of DU145 and LNCaP, furthermore, the colony formation of both cells was inhibited. Interestingly, resveratrol follows the same trend as knockout of STAT3 by ameliorating DU145 and LNCaP viability and colony formation. Furthermore, Western blotting analysis showed that resveratrol inhibits STAT3 expression in both DU145 and LNCaP. These data indicate that STAT3 mediates prostate cancer progression. Inhibition of STAT3 by resveratrol mitigates prostate cancer activity progression.

Keywords: Prostate Cancer, STAT3, Resveratrol, Oncogene, Cell Viability.

INTRODUCTION

Prostate cancer (PCa) is one of the most cancers that lead to death in men. Globally, prostate cancer emerges in 1 in 8 men in their lifetime, with highest incidence frequency in aged people, especially those over 65 years [1, 2]. Several risk factors have been proposed in the development of prostate cancer. Non-modifiable factors include genetic alteration such as mutation in the signal transducer and activator of transcription 3 (*STAT3*) gene, loss or mutation in tumor suppresser genes like phosphatase and tensin homolog (PTEN), modification in androgen receptors, race, and family history [1-4]. In contrast, modifiable factors include obesity, alcohol consumption, smoking, and dietary habits [5, 6]. Epigenetic modification like DNA methylation or modification in histone modifying enzymes have been proposed to play important a critical role in the initiation and progression of prostate cancer [7, 8]. Although significant advances have been made in the available treatment strategies, prostate cancer remaining one of the major public health challenges worldwide [9]. Recent studies showed that targeting the molecular mechanisms associated with tumor progression is promising approach or strategy for preventing disease development [10-12]. One emerging strategy involves augmentation the immune system to combat tumor growth, which can be achieved using natural compounds that synergize with conventional treatments [13,14].

Many plant-derived chemotherapeutic compounds like paclitaxel and vincristine have been widely utilized to treat different cancers, this highlighted the importance of natural compounds including phytochemical and bioactive molecules [15]. Natural products like isoflavones, resveratrol and epigallactocatechin-3-gallate (EGCG) have demonstrated efficacy across different cancers like prostate cancer, breast cancer, and head and nick cancer [1-18]. Notably, some of these

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compounds have been advanced to phase III clinical studies for cancer prevention [1]. Despite these advances, intensive preclinical studies and investigation are still needed to fully characterize the therapeutic potential and mechanism of action of natural compounds in cancer treatment. Resveratrol (trans-3, 4', 5-trihydroxystilbene), a polyphenolic antioxidant found in grapes and peanuts, exhibited anti-inflammatory [19], anti-oxidative [20], and anti-cancer properties [1]. It exerts therapeutic effects against multiple diseases via reducing oxidative stress, inhibiting cell proliferation, inducing cancer cells apoptosis in cancer cells. It has demonstrated antiproliferative activity against several human cancers like prostate cancer cell lines such as DU145[1] and hematologic malignancy like acute lymphoblastic leukemia [21]. Resveratrol halts cell cycle in prostate cancer cells, including PC3 and DU145 [22]. Furthermore, epidemiologic evidences showed that individuals who consume resveratrol-primarily through dietary intakes of grapes, have up to 50% lower risk developing prostate cancer [23]. Its activity has been partially attributed to similarity to specific kind of hormones such as androgen and estrogen, this propose that resveratrol may exert its e beneficial effects through hormone receptors-mediated pathways [24]. Previous study showed the ability of resveratrol to suppress prostate cancer progression by blocking the *AKT/miR-21* pathway [25], our finding also demonstrated that resveratrol modulates *miR-21* oncogene and increase *prostate cancer–associated transcript 29* (*PCAT29*) gene, therefore, reduces proliferation of prostate cancer cell line [1]. This poly-mechanism profile underscores its strong potential as promising therapeutic agent for prostate cancer.

Inflammation has been widely recognizing as critical components of tumor progression tumor progression. Pro-inflammatory cytokines initiate or activate inflammatory signaling pathways, leading to activation mediators such as interleukin 6 (IL-6), tumor necrosis factor-α (TNF- α), and related mediators [26]. These mediators orchestrate neutrophil migration to the sites of tumor growth and enhance establish a tumor-supportive microenvironment that could contribute tumor progression. chemokines such as CXCL1, CXCL2, and CXCL12 play positive role in in driving prostate cancer development by enhancing this supportive microenvironment [27]. The precise role of these mediators is represented by recruitment of neutrophils and myeloid-derived suppresser cells into the tumor milieu, creating an immunosuppressive that aid tumor growth [28]. Furthermore, upregulation of STAT3 has been implicated in prostate cancer metastasis to lymph node and modulates the chemoresistance behavior of prostate cancer cells [29, 30].

A preliminary cytokine array in our laboratory showed that prostate tumor tissue exhibits substantial expression of pro-inflammatory cytokines, including STAT3. Furthermore, our previous study demonstrated that IL-6 upregulation positively enhances prostate cancer cell proliferation via activation of STAT3, while siRNA-mediated STAT3 knockdown reverse this action. Interestingly, the chemo-preventative agent, resveratrol, ameliorated the viability of prostate cancer cell by blocking STAT3 and miR-21 signaling pathway [1]. In current study, we generated knockout a stable STAT3 using clustered regularly interspaced short palindromic repeats (CRISPR) technology to investigate the molecular mechanism of resveratrol inhibits prostate cancer progression. We show that STAT3 is highly upregulated in DU145 and LNCaP cells, Importantly, either deletion of STAT3 or chemo-preventative agent, resveratrol, significantly reduced prostate cancer proliferation, in part through interfering with STAT3.

MATERIALS AND METHODS

Materials

Resveratrol was purchased from Sigma-Aldrich (R5010), pSTAT3, STAT3, and β-actin antibodies was purchased from Cell Signaling Technology®. DU145 and LNCaP media, RPMI 1640, was obtained from Gibco, while the antibiotic like penicillin and streptomycin were purchased from ThermoFisher Scientific. Fetal bovine serum (FBS) was obtained from Atlanta Biologicals. RNA extraction reagent (TRIzol) reagent was obtained from Sigma-Aldrich. Kit for cDNA Synthesis was bought from Bio-Rad, whereas Fast SYBR™ Green Master Mix was obtained from Applied Biosystems. Cell Proliferation AQueous One Solution Kit was purchased from Promega. Synthetic pSpCas9 BB-2A-GFP plasmid for STAT3 knockout (cat. No. 210450) and their negative controls (scramble) were purchased from GenScript, while the Lipofactamine 3000 (Cat. No. L3000001) transfection reagent was purchased from ThermoFisherScintific.

Cell Culture

RPMI 1640 media (Gibco) were used to culture DU145 and LNCaP cells, the media was supplemented with 10% fetal bovine serum (Atlanta Biologicals), penicillin (50 units/ml) and streptomycin (50 µg/ml). Both cell lines were grown at 37°C with 5%CO_2 and 95% ambient air. Sub-confluent monolayers of both cell lines were used to conduct all experiments.

Cell Viability Assay (MTS Assay)

DU145 and LNCaP cells viability was measured utlizing CellTiter 96® AQueous One Solution. A 96-well plate that contain 100 µl of culture media in each well was used to seed 3000 cells per well. After 24 h, cells were treated with different concentrations of resveratrol and incubated for an additional 24 then treated with 20 µl of CellTiter 96® AQueous One Solution reagent for 2 to 3 h. An ELISA plate reader and absorbance at 490 nm was used to measure the development the of the brown color resulted from colored formazan production which is reflect the viable cells. The cell viability depicted as the percentage comparing to control group.

CRISPR Cas9 Plasmid Transfection

Synthetic pSpCas9 BB-2A-GFP plasmid (10 nM) and scramble (as controls) were transferred into prostate cancer cells (DU145 and LNCaP) utilizing Lipofactamine 3000 transfection solution. DU145 and LNCaP cells were seeded in a 6-well plate and incubated until they reach 60-70% confluence. PSpCas9 BB-2A-GFP plasmid (10 nM) and negative controls were mixed with transfection media (150 µl) and 9 µl of transfection reagent (Lipofactamine 3000) for 5 min, allowing formation the transfection mix. Then, 150 µl of transfection mix was added each well in the 6-well plate. 24 h post transfection, cells were collected and sorted for single cell in 96-well plate using fluorescence-activated cell sorting (FACS), through excited 488 nm laser. Only GFP-positive cells were used in Western blotting and qPCR experiments.

Western Blot

Western blotting technique was utlized to estimate protein expression [1]. Briefly, cells were seeded in 6-well plates and incubated until 70-80% confluence. After treatment, ice cooled PBS was used to wash cells, then cells were subjected to whole-cell lysates by homogenizing in pre-cold lysis buffer (50 mM Tris-HCl, 1.0% NP-40, 0.5% sodium deoxycholate, 150 mM NaCl, and 0.1% sodium dodecyl sulfate), the lysis buffer was supplemented with inhibitor 2 and 3 for phosphatase enzyme. Then, protein concentration was estimated by Bradford method, and an equal amount of cell lysate was resolved by gel electrophoresis using SDS polyacrylamide. The resolved proteins were then transported to 0.4 µm of nitrocellulose membrane and blocked with blocking buffer (sigma). The plots were then incubated with primary antibody overnight and washed thrice with TBST solution. Mouse or rabbit specific IgG secondary antibody with fluorescent-tagged dye was used to probe with primary antibody and LI-COR Odyssey® imaging system was used to visualize the protein of interest. ImageJ software was used to analysis the intensity of proteins band and results were depicted as percent relative to control which was normalized to of control 100%.

RNA Isolation

Total RNA was extracted from the cells seeded in 6-well plate by adding 500 µl of TRI reagent (Sigma) for each well. TRI reagent and cells were collected in Eppendorf tube, then 100 µl of Chloroform was added to the tubes and shacked for 15 sec. Tubes were then centrifuged for 15 min at 12,000 rpm. Layer that contains RNA, top layer, was transferred to new pre-cooled Eppendorf tube, then pre-cold isopropanol (0.5 ml) was added to each tube and centrifuged for 15 min at 12,000 rpm. The Isopropanol containing RNA like-gel pellet was removed and ethanol (75%) was used to wash the pellet, then tubes were centrifuged for 15 min at 12,000 rpm again. Ethanol was discarded and RNA like-gel pellet was dried for 5 min by inverting the tube on delicate tissue that absorbed the remaining of ethanol. A 50 µl of nuclease free water was added to each tube to dissolve RNA, and the purity and concentration of RNA was measured utilizing a Nanodrop® ND-1000 [26].

RT-PCR

A 1 µg of RNA was used to synthesis cDNA utilizing iScript cDNA Synthesis Kit. The components used to synthesis cDNA were: RNA (1 µg), reaction mix (4 µl), reverse transcriptase enzyme (1 µl), and nuclease-free water to adjust the final volume at 20 µl. Each reaction tube was incubated at PCR machine which was set it up at 25°C for 5 min (priming), then 42°C for 30 min (revers transcription reaction) followed by 85°C for 5 min (reverse transcription reaction inactivation). The synthesized cDNA was used to measure the gene expression using qPCR quant studio 3 systems machine. The qPCR reaction mixture components were mixed as follow: cDNA (1 µl), SYBR™ Green Master Mix (10 µl), 1.2 µl of forward and reverse primers (0.6 µl for each), and nuclease-free water (7.8 µl) of nuclease-free water adjust the final volume at 20 µl. The qPCR plated was sealed with Sealing Film and then subjected to the following reaction conditions: 20 sec at 95°C (hold), followed by 40 cycles of 95°C for 3 sec (denature), and 60°C for 30 sec (anneal/extend) [26]. The primers for STAT3 and GAPDH (*H. sapiens*) were obtained from integrated DNA technology:

STAT3 (sense): 5'-ACCGTAAGTGGCTTCCTTTC -3'
(antisense): 5'-CTTCCAACCTTTGGCAGATTAAC-3'
GAPDH (sense): 5'-AATCCCATCACCATCTTCCA-3'
(antisense): 5-TGGACTCCACGACGTACTCA-3'

Statistical Analysis

Analysis of variation (ANOVA) was used to perform statistical analysis followed by Bonferroni *post hoc* correction for multiple comparisons. $p<0.05$ was considered as statistically significant. The data were presented the mean ± SEM [26].

RESULTS

Resveratrol Inhibits Viability and Invasiveness of DU145 and LNCaP Cells

Previously, we used 50 µM resveratrol to assess its antiproliferative effects on DU145 and LNCaP prostate cancer cells [1]. To further investigate the effect of antiproliferative of different resveratrol doses on DU145 and LNCaP cancer cells, cells were cultured (2500-300 cells per well) in 96-well plate then incubated for 3 days with control (vehicle) or resveratrol at dose-dependently of 10, 25, 50 and 100 µM. The cell viability was determine using CellTiter 96® AQueous

One Solution. The percentage of viability in DU145 cells following resveratrol treatment at 10, 25, 50 and 100 µM was reduced to 83.0±4.4, 62±5.7, 33.1±7.9, and 33±5.8, respectively, compared to control-treated group which was normalized to 100 % **(Fig. 1 A)**. Similarly, LNCaP cell viability was decreased to 71.7±1.7, 60.4±1.9,28±9.1 and 24.4±9.5 at same concentrations relative to control-treated group that normalized to 100 % **(Fig. 1 B)**. The inhibition effect of lovastatin exerted on prostate cancer cells viability could be explained due to increased apoptosis of these cells as determined by Ashraful *et al*., The most significant inhibition of DU145 and LNCaP cells viability was observed at 25 and 50 µM **(Fig. 1 A and B),** based on these results we selected 50 µM of resveratrol in the subsequent experiments.

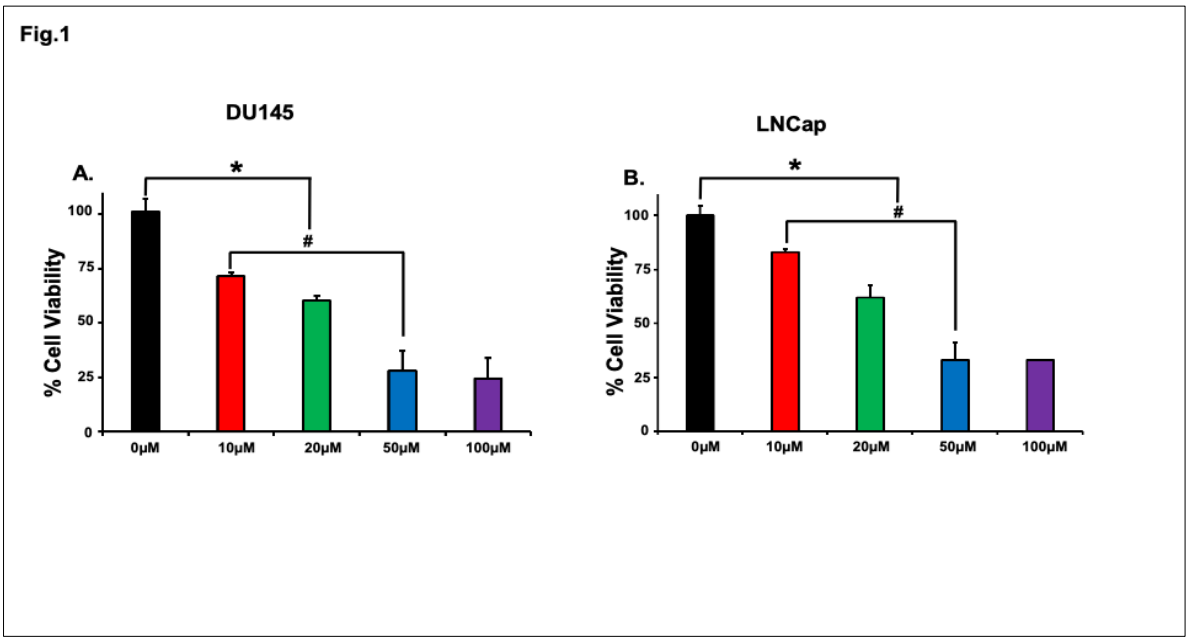

Figure 1: Resveratrol inhibits DU145 and LNCaP cell viability in dose-dependent manner

DU145 and LNCaP cells were treated with either vehicle or resveratrol at to various concentrations (10-100 µM) for 72 h. The cell viability was measured by MTS assay. Resveratrol treatment produced a progressive inhibition in cell viability in **(A)** DU145 and **(B)** LNCaP, with most significant effect at 50 µM. Data presented as the mean fold change ± SEM (n=4). (*) indicates significant difference ($p<0.05$) from vehicle group, while (#) indicate statistically significant difference among resveratrol-treated groups. Statistical analyses among groups were tested using one-way analysis of variance (ANOVA).

STAT3 Knockout Using CRISPR Cas9 Plasmid Transfection

Given the fact that DU145 and LNCaP cells harbor constitutively active STAT3. We furthered used CRISPR Cas9 technology to delete the role of STAT3 gene in the pathology of androgen-sensitive (AR-sensitive LNCaP cells and androgen-independent DU145 cell [31]. First, we established the permanent deletion of STAT3 by transfection both DU145 and LNCaP cells with synthetic pSpCas9 BB-2A-GFP plasmid that harboring guide RNA targeting STAT3. The plasmid system constitutively express GFP along with the guide RNA and Cas9 enzyme. When functionally active Cas9/gRNA complexes are delivered into DU145 and LNCaP cells, the guided RNA drives Cas9 nuclease to creates double-stranded breaks at the target S*TAT3* sequence, this induces the cellular repair machinery to restore and repair the DNA breakage via homologous end-Joining (NHEJ) pathway [32-34]. This repair mechanism often introduces insertion or deletion that generate deleterious premature stop codon, thereby disturbing the target gene [35], successful plasmid transfection is indicated by GFP expression **(Fig 2 A).** Next, we sorted *STAT3* knockout (GFP^+) cells using fluorescence-activated cell sorting (FACS) to isolate single STAT3 deleted cell from the mixed population. GFP-positive cells that has STAT3 knockout were sorted into single cell using FACS in 96-well plate and incubated until colony formation. qPCR and Western blot analysis of wild type and knockout DU145 and LNCaP cells were conducted to confirm the complete loss of *STAT3* gene and protein**.** qPCR results showed a complete loss of *STAT3* gene expression in DU145 and LNCaP cells. *GAPDH* was used as internal control **(Fig. 2 B)**. Consistence with these results, Western blot analysis demonstrated a complete absence of STAT3 protein in the knockout cells, confirming successful disruption of *STAT3* gene at both cell transcription and translation level **(Fig 2 C)**. The Western blot intensity was depicted in **Fig. 2 D,** the intensity value showed a slight expression which resulted from the secondary antibody fluorescence which was subtracted from background of the blot but we have shown this intensity for consistency, but we still have a complete knockout as the qPCR results shown **(Fig. 2 B)** These finding validate the efficiency of our CRISPR0Cas9 mediated gene deletion and established reliable model to investigate the implication of STAT3 in prostate cancer progression.

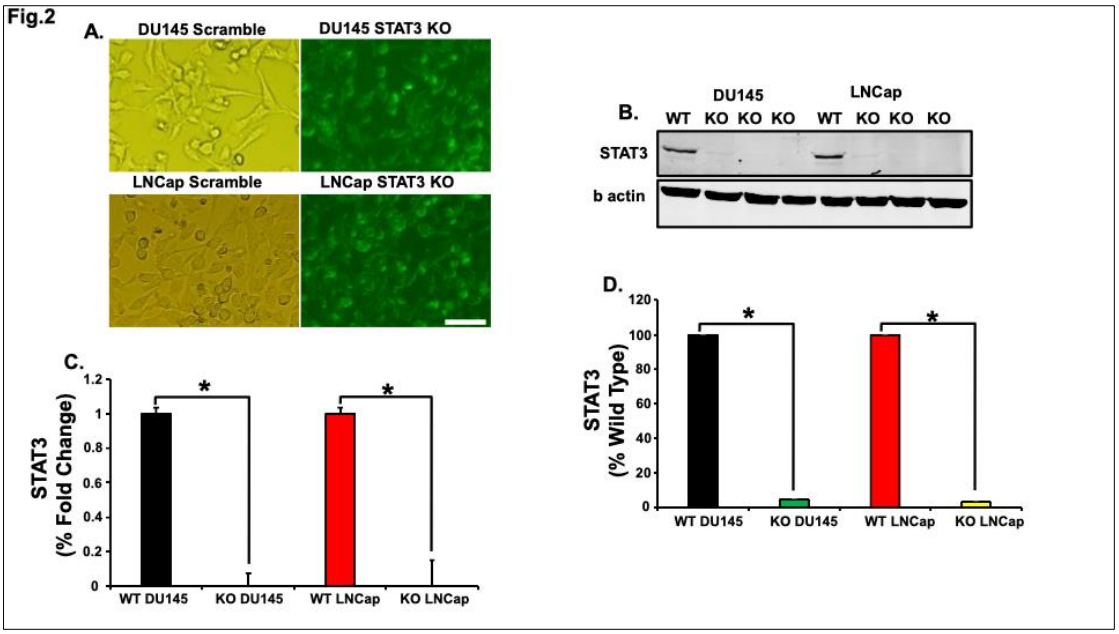

Figure 2: Knockout of *STAT3* in DU145 and LNCaP

(A) DU145 and LNCaP cells were transfected with Synthetic pSpCas9 BB-2A-GFP plasmid (10 nM) and their negative controls (scramble) for 24 h. Cells were then imaged for fluorescence expression and sorted for single cell in 96-well plate using fluorescence-activated cell sorting (FACS), scale bar is 20 µ. **(B)** GFP^+ DU145 and LNCaP and the wild type DU145 and LNCaP cells were cultured in 6-well plate for 24 h then used to evaluated STAT3 expression by RT-PCR. Wild type cells showed significant expression of STAT3 while the knockout cells showed no expression of STAT3 in both cells. **(C, D)** GFP^+ DU145 and LNCaP and the wild type DU145 and LNCaP cells were cultured in 6-well plate for 24 h. Western blotting analysis showed significant level of STAT3, while the knockout cells showed no expression of STAT3 in both cells. Data are presented as the mean ± SEM of at least 3 independent experiments. Asterisks (*) indicate statistically significantly difference ($p < 0.05$) from wild type.

Resveratrol Abolish STAT3 Expression and Mitigate Prostate Cancer Progression

Next, we tested the effect of resveratrol on STAT3 activity. Western blotting analysis revealed that 24-h treatment with resveratrol abolished STAT3 phosphorylation in DU145. The pSTAT3 level was in significantly reduced to 13±1.68%, compared to vehicle-treated group which was normalized to 100% **(Fig. 3A)**. Notably, the extent of STAT3 inhibition by resveratrol was close to the effect of STAT3 knockout which showed that resveratrol could be a good pharmacological inhibitor of STAT3 **(Fig. 3B)**. Similarly, resveratrol had comparable effect on LNCaP cells. A 24-h treatment reduced STAT3 phosphorylation to 6.6±4.2 relative to vehicle-treated group which was normalized to 100% **(Fig. 3C)**. The inhibitory effect of resveratrol mirrored the results observed in STAT3 knockout in LNCaP cells, **(Fig. 3D)**, altogether, these results support that resveratrol as an effective STAT3.

We next test the antiproliferative activity of resveratrol against DU145. As previously described, we compared the inhibitory effects of resveratrol with that of STAT3 knockout. Consistent with the inhibitory activity, 50µM of resveratrol reduced DU145 viability to 29±9.2 % compared to control group **(Fig. 4A)**. As anticipated, this inhibitory effect was close to that observed in DU145-STAT3 knockout, where cell viability was 24.7±9.9 %. While both resveratrol and STAT3 knockout significantly decreased the viability relative to control group, there was no significant difference between resveratrol-treated and STAT3 knockout groups **(Fig. 4A)**. A similar trend was observed in LNCaP cells where treatment with 50 µM resveratrol significantly reduced cell viability to 30.8±7.2 % compared to control group, which was normalized to 100%. Both resveratrol and STAT3 knockout significantly decreased LNCaP cell viability relative to control group. Importantly, there was no significant difference in viability between the resveratrol-treated and STAT3 **(Fig. 4D)**. These results indicate that DU145 cells viability is largely driven by STAT3, while it reverses by resveratrol.

Furthermore, we tested the colony forming inhibitory exerted by resveratrol against DU145 DU145 and LNCaP cells. The level of colony formation in control treated group was highly aggressive in both cell lines, however the colony forming for both cell lines was significantly decreased following 50 µM of resveratrol treatment. The colony forming percentage in DU145 cell was decreased to 36±1.6 % following resveratrol treatment compared to control group, similarly, the treatment of LNCaP with same dose of resveratrol reduced their colony formation to36.2±1 %. Moreover, knockout STAT3 in DU145 and LNCaP significantly the cell line **(Fig. 4 B, C, E and F)**.

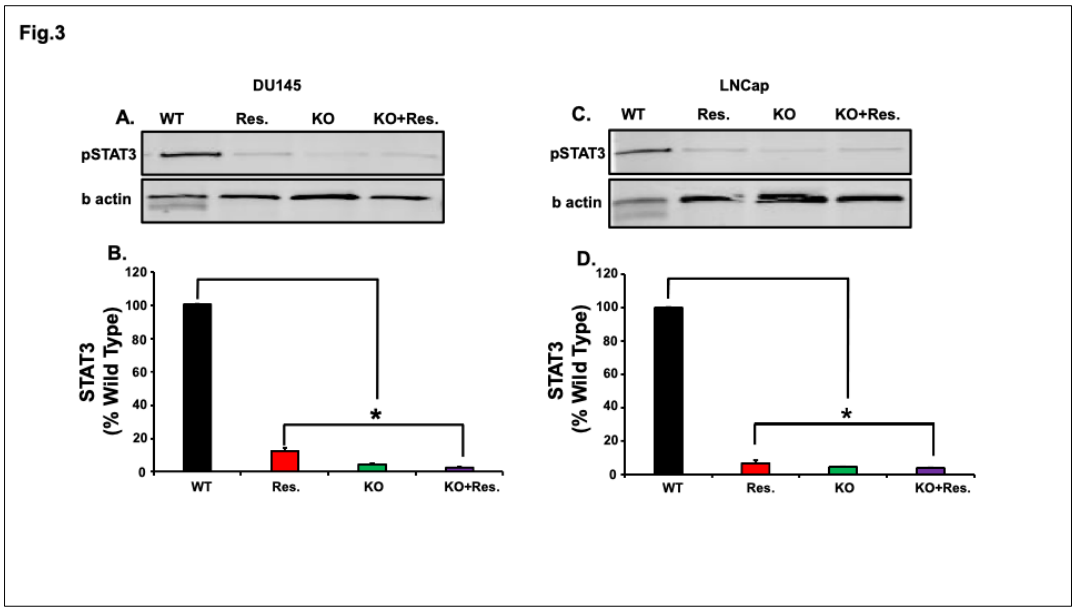

Figure 3: Resveratrol ameliorate STAT3 in DU145 and LNCaP

Wild type and knockout DU145 and LNCaP cells were cultured in 6-well plated and incubated for until reach 70 percentage of confluency, then treated with resveratrol (50 µM) and vehicle for 24 h. Cell lysates were prepared and used for Western blotting analysis. **(A, B)** Resveratrol significantly decreased STAT3 level in DU145 cells treated with resveratrol. Similarly, **(C, D)** resveratrol significantly decreased STAT3 level in LNCaP cells treated with. Data are presented as the mean ± SEM. Asterisks (*) showed significance difference ($p < 0.05$) from wild type.

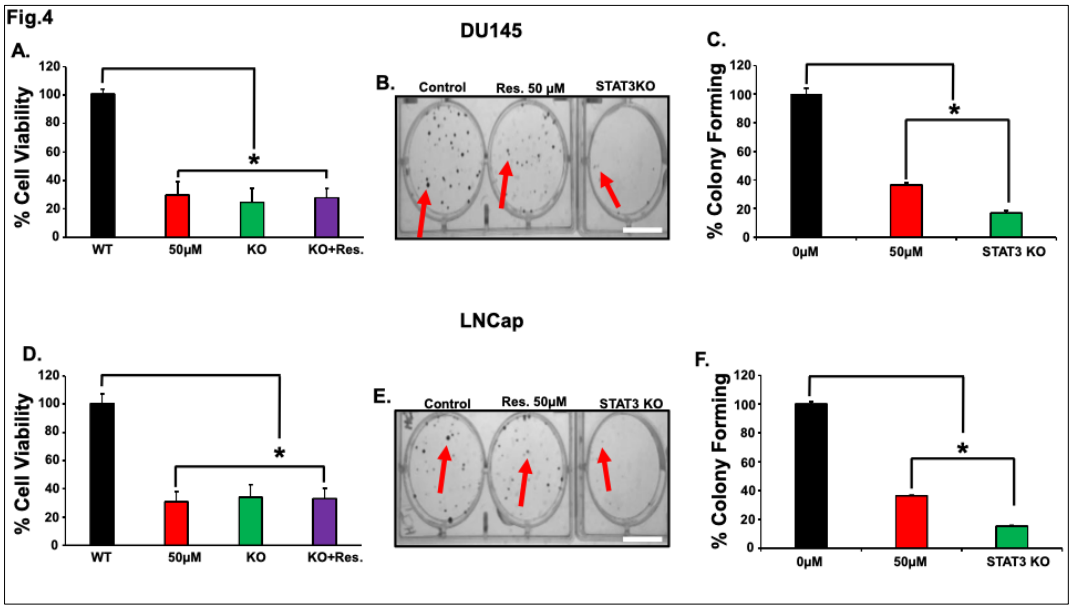

Figure 4: Resveratrol inhibits DU145 and LNCaP cell viability

(A, D) Wild type and knockout DU145 and LNCaP cells were cultured in 96-well plated (2500-300 cell per well) and incubated overnight, then treated with resveratrol (50 µM) or vehicle for 72 h. MTS assay was assigned to measure cells viability. Resveratrol treatment and STAT3 knockout significantly decreases DU145 and LNCaP cell viability. **(B, C, E, and F)** Wild type and knockout DU145 and LNCaP cells were cultured in 6-well plate (15-200 cells per well). 3 days later, cells were treated with either vehicle of resveratrol (50µM) until 21 days. Then the colonies were stained in 1% methylene blue for 30 min. and washed with water. Resveratrol treatment and STAT3 knockout significantly decreased colony formation in DU145 and LNCaP cells comparing to control. Asterisks (*) Data are presented as the mean ± SEM. Asterisks (*) showed significance difference ($p < 0.05$) from wild type.

DISCUSSION

The current study demonstrated that STAT3 is upregulated in prostate cancer cell (DU145 and LNCaP). It appears to play crucial role in prostate cancer progressing via enhancing cell viability and promoting colony formation. Our finding indicates that STAT3 linked to prostate cancer progression, as either deletion of STAT3 or pharmacological inhibition of STAT3 mitigates STAT3-mediated oncogenic activity. The pathology of STAT3-mediated prostate cancer progression.

High level of STAT3 has been found in many cancers, this level is linked to prostate cancer progression [36]. As pro-inflammatory transcription factor, STAT3 contributes to prostate cancer development and implicate in transfer prostate cancer into more aggressive via stimulation of cyclin D1-mediated cell proliferation and induce the expression of Bcl2 protein (anti-apoptotic) [1]. Previously, finding also demonstrated that IL-6/STAT3 signaling pathway play an important role in prostate cancer progression. IL-6-mediated activation of STAT3 significantly increased the viability of DU145 and LNCaP, whereas STAT3 knockdown using siRNA abolished cell viability and progression. Interestingly, elevated level of both STAT3 and IL-6 were detected in prostate cancer tissue from patients [1].

Resveratrol used to protect against many diseases and consider as a chemo-preventive agent [37]. This activity resulted from its ability to interact with many targets or block pathways that involve in cancer cell progression [38]. In fact, resveratrol showed ability to block androgen and estrogen receptors, this activity was ascribed to its similarity these hormones [39].

Multiple evidences have demonstrated the ability of resveratrol to target STAT3 and inhibit prostate cancer cells [38]. Al Aameri *et al*., showed that, resveratrol inhibits STAT3 oncogenes expression in prostate cancer cells, whereas enhances the activity of tumor suppressor genes such as PDCD4 and PCAT29 [1]. Moreover, Knockdown of PDCD4 mitigate the activity of resveratrol as antiproliferative agent in prostate cancer cells [40]. Overall, these data provide evidence that STAT3 is a promising target of resveratrol to ameliorate prostate cancer progression. STAT3 can upregulate AKT via as a compensatory survival mechanism in cancer by directly binding to the AKT promoter [41], or through pathways like IL-6/JAK, leading to increased AKT expression and activation, which promotes cell proliferation, survival, and resistance to therapies targeting either pathway alone [42]. In PC-3M-MM2, the expression level of phosphorylated Akt is high and resveratrol showed the ability to inhibit AKT [43]. In fact, blocking the STAT3 by siRNA increased PDCD4 levels [44], thus, inhibition of STAT3 by resveratrol represents an important mechanism to reduce prostate cancer progression and invasiveness. Recently, studies showed that STAT3 may activated Akt, thus increase the phosphorylation of Ser [67], and Ser [4-7], residues of PDCD4 leading to decrease the activity of activator protein-1 (AP-1) promoter [45]. Moreover, STAT3 positively implicated in Akt-mediated proteasomal degradation of PDCD4 multiple cancer like prostate and overlain cancers [46]. Since resveratrol was able to inhibit *STAT3* expression. Therefore, resveratrol can exert anti-tumor activity by different mechanism which is regulated by STAT3 as transcription factor.

Collectively, our study demonstrated that STAT3 mediated prostate cancer progression while STAT3 deletion using CRISPR Cas9 technology or inhibition by resveratrol mitigates prostate cancerprogression.

ACKNOWLEDGMENTS

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad, Iraq for its providing support in the current work.

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