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RESEARCH ARTICLE

Curcumin inhibits IFN-γ induced PD-L1 expression via reduction of STAT1 Phosphorylation in A549 non-small cell lung cancer cells

Dian Jamel Salih[1,2,3] · Saraa Hanna Barsoom[4] · Ghazwan Fawzi Ahmed[2] · Shler Qasim Hussien[5] · Qais Al Ismaeel[2] · Asaad A. B. Alasady[2] · Tahseen A. Alsalim[6] · Ahmed Mohammed Salih[7]

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Abstract

Background Immune evasion in non-small cell lung cancer (NSCLC) is largely mediated by programmed death-ligand 1 (PD-L1), which is upregulated by interferon-gamma (IFN-γ)-induced STAT1 activation. Targeting this pathway may improve immunotherapy outcomes. Curcumin, a natural polyphenol, has been reported to modulate various oncogenic signaling pathways, but its role in inhibiting IFN-γ-driven PD-L1 expression in NSCLC remains unclear.

Methodology The NSCLC cell line A549 were treated with curcumin (50 μM) for 2 h before stimulation with IFN-γ (500 U/ml). Western blot, qRT-PCR, and immunofluorescence microscopy were used to evaluate STAT1 phosphorylation, PD-L1 expression, and the localization of phosphorylated STAT1 (p-STAT1). The expression of interferon-stimulated genes (ISGs), including SOCS1 and ISG15, was also examined. Additionally, the Resazurin assay was performed to assess cell viability.

Results IFN-γ significantly induced STAT1 phosphorylation, leading to a time-dependent upregulation of PD-L1 expression. Immunofluorescence confirmed that p-STAT1 is translocated to nucleus. Curcumin treatment inhibited STAT1 phosphorylation by 68% ($p < 0.001$), leading to a marked reduction in PD-L1 expression. Moreover, curcumin suppressed IFN-γ-induced SOCS1 (63%) and ISG15 (54%) expressions, indicating a broader effect on STAT1-mediated immune evasion. Finally, curcumin enhanced IFN-γ-mediated growth inhibition, reducing cell viability by 47% at 48 h ($p < 0.01$).

Conclusion Curcumin effectively inhibits IFN-γ-induced STAT1 phosphorylation and PD-L1 expression, downregulates ISGs, and enhances IFN-γ-mediated tumor suppression. These findings suggest that curcumin may serve as a therapeutic adjuvant in NSCLC, potentially improving immune checkpoint inhibitor (ICI) efficacy.

Keywords Lung cancer · Curcumin · STAT1 · PD-L1 · IFN-γ

1 Introduction

Lung cancer continues to be the most prevalent cancer-related mortality globally, responsible for more than 1.8 million deaths per year (Siegel et al. 2024). Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancer cases, an issue that is of significant public health importance and also a target for oncological research (Salih et al. 2025). Over the last decade, the development of immune checkpoint inhibitors (ICIs) targeting the programmed death- 1/programmed death-ligand 1 (PD- 1/PD-L1) axis has shifted the treatment landscape for NSCLC, significantly improving survival rates in a particular group of patients (Rizvi et al. 2015). Nevertheless, with these advancements, a large proportion of NSCLC patients either show a lack of responsiveness to ICIs (primary resistance) or develop resistance

✉ Dian Jamel Salih
dian.salih@unifg.it

1 Department of Medical and Surgical Sciences, University of Foggia, Via Dattoli, 42, 71121 Foggia, FG, Italy

2 Department of Anatomy, Biology and Histology, College of Medicine, University of Duhok, Duhok, Iraq

3 College of Pharmacy, University of Nawroz, Duhok, Iraq

4 Department of Clinical Biochemistry, College of Health Science, Hawler Medical University, Erbil, Iraq

5 Department of Medical Laboratory, Erbil Technical Health and Medical College, Erbil Polytechnic University, Erbil, Kurdistan Region, Iraq

6 Department of Chemistry, College of Education, University of Basrah, Basrah, Iraq

7 College of Nursing, University of Duhok, Duhok, Iraq

("acquired resistance") over time that significantly restricts their clinical efficacy (Sharma et al. 2017; Rotte 2019).

Immune evasion of NSCLC is significantly attributed to the expression of PD-L1, a transmembrane molecule that binds to PD- 1 receptors located on cytotoxic T cells and induces immune suppression and tumor tolerance. PD-L1 overexpression is often linked to poor prognosis, tumor aggression, and poor response to ICIs (Rizvi et al. 2015; Kim and Chen 2016). One of the major regulators of PD-L1 expression in the tumor microenvironment is IFN-γ, a key cytokine modulating the immune system. Although IFN-γ is classically described to have tumor-suppressive effects, paradoxically, it also plays a pro-tumorigenic role by inducing PD-L1 expression, enabling tumor cells to escape immune surveillance (Garcia-Diaz et al. 2017).

IFN-γ signaling is largely achieved via Janus Kinase—Signal Transducer and Activator of Transcription (JAK-STAT) pathway and, in particular, via STAT1. Binding of IFN-γ to its receptors, JAK1 and JAK2, phosphorylates STAT1 on Tyr701. Upon phosphorylation, STAT1 dimerizes, translocate to the nucleus, and binds to gamma-activated sequence (GAS) motifs of the PD-L1 promoter, in turn increasing the level of PD-L1 transcription (Sumitomo et al. 2022).

As confirmed by several studies, IFN-γ-induced activation of STAT1 is one of the most potent inducers of PD-L1 expression in NSCLC, which in turn leads to adaptive immune resistance that prevents T-cell-mediated tumor destruction (Drake et al. 2006; Shin et al. 2017). Since IFN-γ levels are typically increased in the tumor microenvironment of NSCLC, strategies that can inhibit IFN-γ-driven upregulation of PD-L1 promise to be valuable for improving the efficacy of ICIs (Spranger et al. 2013).

Despite extensive research regarding PD-L1 regulation in NSCLC, therapeutic approaches that specifically aim to silence IFN-γ-induced PD-L1 expression are still limited. Strategies presently are based on ICIs in synergy with kinase inhibitors, epigenetic modulators, or chemotherapy, but such pharmacologic efforts are typically associated with toxicity and short-term failure (Zhou and Yang 2023). Accordingly, the discovery of non-toxic, naturally derived compounds that effectively and specifically suppress IFN-γ-mediated PD-L1 expression but do not negatively affect the host immune function is of significant interest.

Curcumin, a polyphenolic compound isolated from the extract of *Curcuma longa* (turmeric), has attracted attention as a multi-targeted therapeutic agent due to its anti-inflammatory, antioxidant, and anti-cancer functions (Allegra et al. 2017). It has been shown to suppress a variety of oncogenic signaling pathways such as nuclear factor-kappa B (NF-κB), cyclooxygenase- 2 (COX- 2), STAT3, and AKT, leading to growth inhibition and apoptosis in a variety of cancers (Kumar et al. 2021).

Several preclinical studies have suggested that curcumin can downregulate PD-L1 expression in different cancer models, including breast and colon cancer, by inhibiting STAT1 phosphorylation and preventing its nuclear translocation (Midura-Kiela et al. 2012). Nevertheless, its specific involvement in downregulating IFN-γ-induced PD-L1 expression in NSCLC, especially in A549 cells, is unknown. As high PD-L1 expression is associated with poor response to ICIs, understanding natural products (like curcumin) that could regulate IFN-γ-induced PD-L1 expression might provide new strategies for upgrading the outcome of NSCLC treatment.

Although these encouraging results are promising, there are some deficiencies in our knowledge about how curcumin functions in regulating IFN-γ/STAT1 signaling in lung cancer. Firstly, while previous reports have shown that curcumin can block the phosphorylation of STAT1, they have not specifically evaluated the effect of curcumin on the expression of PD-L1 in NSCLC. Second, the underlying mechanism of curcumin's inhibitory action on STAT1 is still unknown, especially whether curcumin directly inhibits STAT1 phosphorylation or inhibits its nuclear translocation. Third, the ability of curcumin to enhance the efficacy of ICIs by decreasing PD-L1 expression has not been extensively studied, and this should be explored.

Given these gaps in knowledge, the present study aims to investigate the effect of IFN-γ on STAT1 phosphorylation and PD-L1 expression in A549 cells and determine whether curcumin can inhibit IFN-γ-induced STAT1 activation and subsequent PD-L1 upregulation. In addition, explore the impact of curcumin on IFN-γ-induced expression of interferon-stimulated genes (ISGs), such as SOCS1 and ISG15, which are involved in immune signaling and tumor immune escape, and evaluate whether curcumin enhances the antiproliferative effects of IFN-γ, suggesting a potential therapeutic benefit beyond immune modulation.

2 Material and methods

2.1 Cell culture and treatments

Human non-small cell lung cancer (NSCLC) cell, A549, was obtained from American Type Culture Collection (ATCC) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS) (Life Technologies), 1% penicillin–streptomycin, and 2 mM L-glutamine. Cells were cultured in a humidified incubator at 37 °C and 5% CO_2 with regular 2–3-day passaging in a 0.25% trypsin–EDTA suspension. All experiments were performed on cells between passage 5 and passage 15, thereby permitting reproducibility. Recombinant human IFN-γ was purchased from PeproTech (EC Ltd PeproTech, London,

UK) and stored in sterile phosphate-buffered saline (PBS) at 10^5 U/ml stock concentration. Curcumin ≥ 95% was purchased from Santa Cruz (Heidelberg, Germany) and dissolved in dimethyl sulfoxide (DMSO) (≥ 99.9% purity) to make a 50-mM solution. The working dilutions of curcumin were freshly prepared in the complete DMEM prior to each experiment. Cells were pretreated with curcumin (50 µM) for 2 h prior to the IFN-γ (500 U/ml) stimulation for studied time points. Control groups were treated with vehicle (DMSO < 0.1%).

2.2 Cell viability assay

Cell viability was assessed using the Resazurin assay (Sigma-Aldrich, #R7017 - 1G). A549 cells were seeded in 96-well plates at a density of 3×10^4 cells per well (final volume of 100 µL/well) and allowed to adhere overnight. Following 24 h treatment with IFN-γ, curcumin, or a combination of both, 20 µl of Resazurin reagent (0.15 mg/ml) was then added to each well, and incubation was performed for 2–4 h at 37 °C. The conversion of Resazurin to resorufin was measured by fluorescence using a microplate reader (excitation 560 nm, emission 590 nm) or by absorbance at 570 nm with 600 nm as a reference wavelength. Cell viability was determined as a percentage compared to untreated control cells.

Cell Viability (%) = (Absorbance of control cells\Absorbance of treated cells) × 100.

2.3 Western blot analysis

Total protein lysates were extracted from treated A549 cells using ice-cold radioimmunoprecipitation assay (RIPA) buffer supplemented with protease and phosphatase inhibitors and centrifuged at 10,000 × g for 10 min at 4◦C. The total protein concentrations were measured with the bicinchoninic acid (BCA) protein assay kit (Pierce, Thermo Fisher Scientific, USA). Equal amounts of protein (20 µg per sample) were separated on 10% sodium dodecyl sulfate–polyacrylamide gels (SDS-PAGE) and transferred onto 0.45 µM nitrocellulose membranes (Merck). Membranes were blocked with 5% BSA in Tris-buffered saline with 0.1% Tween- 20 (TBST) for 1 h at room temperature to prevent non-specific binding and incubated overnight at 4 °C with primary antibodies against phospho-STAT1 (Tyr701), total STAT1, PD-L1, and β-actin (Santa Cruz Biotechnology, Dallas, TX, USA). After washing with TBST, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary anti-mouse IgG antibody or anti-rabbit IgG, HRP-linked antibody, for 1 h at room temperature. Protein bands were detected using enhanced chemiluminescence (ECL) reagent (GE Healthcare, USA) and visualized using a ChemiDoc imaging system (Bio-Rad, USA). Analysis of protein bands was performed using ImageJ software (NIH, Bethesda, MD, USA). The primary and secondary antibodies are listed in Table 1.

2.4 Quantitative real-time PCR (qRT-PCR) analysis

Total RNA was extracted from A549 cells using the RNeasy Mini-Kit (Qiagen, #74,104) according to the manufacturer's instructions, and the eluted RNA purity and concentration were assessed using a NanoDrop One spectrophotometer (Thermo Scientific, USA). For cDNA synthesis, 500 ng of RNA was reverse transcribed using the RevertAid First Strand cDNA Synthesis Kit to cDNA as per the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was conducted using the SYBR Green PCR Master Mix (Applied Biosystems™) on a QuantStudio 5 Machine (Thermo Fisher Scientific, Inc.). The relative expression levels of PD-L1, STAT1, and ISGs (ISG15 and SOCS1) were normalized to the housekeeping gene GAPDH and analyzed using the 2^ − ΔΔCt method. The PCR reactions were carried out in duplicate with 40 cycles of denaturation (15 s at 95 °C), annealing (20 s at 65 °C), and elongation (20 s at 72 °C) after an initial enzyme activation (15 min at 95 °C). The primer sequences used are presented in Table 2.

2.5 Immunofluorescence microscopy

A549 cells were seeded onto sterile coverslips in 12-well plates and treated as described. After incubation, cells were fixed with 4% paraformaldehyde for 15 min and permeabilized with 0.1% Triton X- 100 for 10 min. After blocking with 1% bovine serum albumin (BSA) for 30 min, cells were incubated overnight at 4 °C with primary antibodies against

Table 1 The description of primary and secondary antibodies

Antibody	Species	Clone	Dilution	Ca#	Source
PD-L1	Rabbit	Monoclonal	1:1000	ab213524	Abcam
STAT1	Mouse	Monoclonal	1:1000	9176	Cell Signaling Technology
pSTAT1	Rabbit	Monoclonal	1:1000	9177S	Cell Signaling Technology
β-actin	Mouse	Monoclonal	1:5000	47,778	Santa Cruz Biotechnology, Dallas, TX, USA
Anti-mouse	Horse	-	1:10,000	7076	Cell Signaling, Danvers, MA, USA
Anti-Rabbit	Goat	-	1:10,000	7074	Cell Signaling, Danvers, MA, USA

Table 2 List of PCR primers designed using NCBI/Primer-BLAST program

Primer	Primer sequences	
	Forward	Reverse
CD274 ($^{PD-L1}$)	5′-TGGCATTTGCTGAACGCATTT- 3′	5′-AGTGCAGCCAGGTCTAATTGT- 3′
ISG15	5′-ATCACCCAGAAGATCGGCGT- 3′	5′-TCGCATTTGTCCACCACCAG- 3′
SOCS1	5’- TTCGCCCTTAGCGTGAAGATGG- 3′	5’- TAGTGCTCCAGCAGCTCGAAGA- 3′
GAPDH	5′-GGAAGGTGAAGGTCGGAGTC- 3′	5′-TGAAGGGGTCATTGATGGCA- 3′

PD-L1 and phospho-STAT1. Following PBS washes, cells were incubated with Alexa Fluor 488- or 594-conjugated secondary antibodies for 1 h at room temperature. Nuclei were counterstained with DAPI, and images were captured using a fluorescence microscope (Zeiss Axio Observer, Germany).

2.6 Statistical analysis

All experiments were performed in triplicate, and data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism 10 (GraphPad Software, USA). One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare multiple groups, and an unpaired Student's t-test was used for pairwise comparisons. Differences were considered statistically significant at $p < 0.05$.

3 Results

3.1 IFN-γ-induced STAT1 phosphorylation in A549 cells at Tyr701

STAT1 phosphorylation at tyrosine 701 (Tyr701) is a key regulatory event in IFN-γ-mediated signaling, leading to STAT1 dimerization, nuclear translocation, and transcriptional activation of ISGs, including PD-L1. To confirm the activation of the JAK-STAT1 pathway in A549 cells, we analyzed phospho-STAT1 (Tyr701) levels by Western blotting after stimulation with 500 U/ml of IFN-γ for different time points (0, 1, 2, 6, 12, and 24 h). Our results demonstrated a time-dependent increase in STAT1 phosphorylation. A significant induction observed as early as 30 min post-treatment ($p < 0.01$), peaking at 2 h ($p < 0.001$), and remaining elevated up to 24 h. Total STAT1 protein levels remained unchanged across all time points, indicating that the increase in phospho-STAT1 was due to phosphorylation rather than upregulation of STAT1 expression, as shown in Fig. 1.

To further investigate the effect of IFN-γ on STAT1 activation, we performed immunofluorescence microscopy to visualize the cellular localization of phosphorylated STAT1 (p-STAT1) in A549 cells following a 2-h treatment with

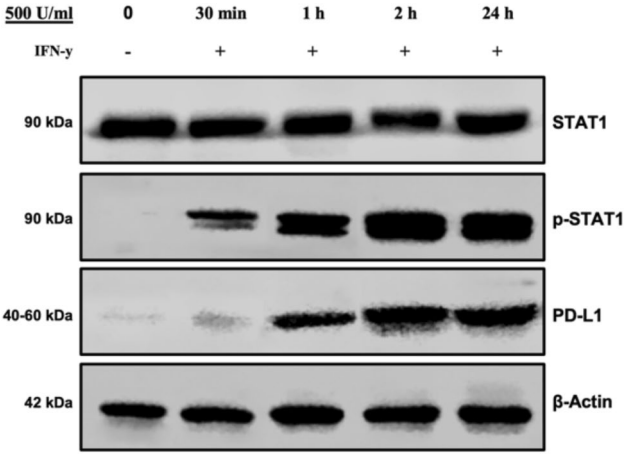

Fig. 1 Western blot analysis showing STAT1 phosphorylation and PD-L1 expression in A549 NSCLC cells following IFN-γ treatment. A549 cells were treated with 500 U/mL of IFN-γ for various time points (0, 30 min, 1 h, 2 h, and 24 h). Protein lysates were collected and analyzed by Western blot using antibodies against phosphorylated STAT1 (Tyr701), total STAT1, and PD-L1. β-Actin was used as a loading control

IFN-γ (500 U/ml). In untreated control cells, STAT1 was primarily localized in the cytoplasm, with minimal nuclear fluorescence detected. However, upon IFN-γ stimulation, a marked increase in nuclear accumulation of p-STAT1 was observed, as shown in Fig. 2, indicating its activation and translocation to the nucleus, where it functions as a transcription factor. This translocation pattern was confirmed through co-staining with DAPI, a nuclear marker, which showed strong co-localization of p-STAT1 within the nucleus.

3.2 IFN-γ-induced STAT1 phosphorylation led to PD-L1 upregulation

Studies reveal that IFN-γ dramatically increases the expression of PD-L1 on cancer cells, mainly by activating the STAT1 signaling pathway. To validate this association, we examined PD-L1 upregulation upon IFN-γ treatment in A549 cells.

Western blot analysis revealed a time-dependent increase in PD-L1 protein levels, with minimal expression in untreated control cells. Upon IFN-γ stimulation, PD-L1

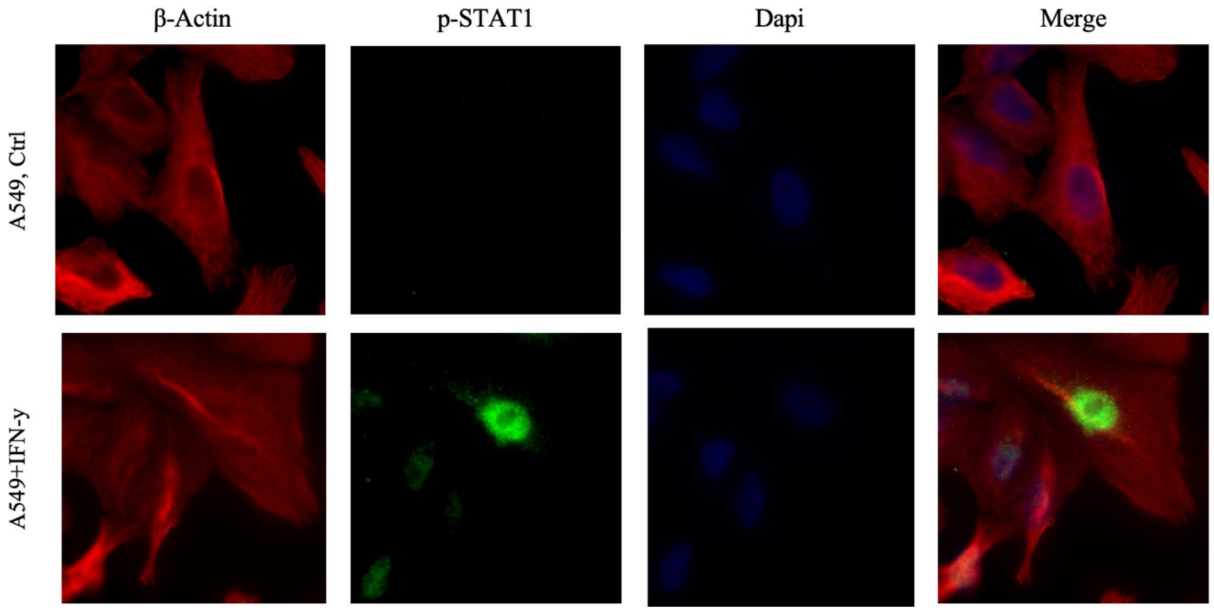

Fig. 2 Immunofluorescence microscopy showing p-STAT1 localization in A549 cells treated with IFN-γ. A549 cells were treated with 500 U/mL of IFN-γ for 2 h, then analyzed by immunofluorescence microscopy to assess p-STAT1 localization. In untreated cells, p-STAT1 was primarily detected in the cytoplasm. Following IFN-γ treatment, increased nuclear localization of p-STAT1 was observed. DAPI was used for nuclear staining, and β-actin staining was used to visualize the cytoskeleton

levels began to increase at 30 min and 1 h, corresponding with the early activation of STAT1 phosphorylation (p-STAT1, Tyr701). By 2 h, PD-L1 expression was noticeably upregulated, and by 24 h, it reached its maximum induction ($p < 0.01$). This trend correlated with sustained STAT1 phosphorylation, indicating that IFN-γ-driven STAT1 activation plays a key role in PD-L1 upregulation in A549 cells (Fig. 1).

To further assess the effect of IFN-γ on PD-L1 regulation, we performed quantitative real-time PCR (qRT-PCR) to measure PD-L1 mRNA expression levels in A549 cells treated with IFN-γ (500 U/ml) for different time points. The results demonstrated a time-dependent increase in PD-L1 mRNA expression. At 6 h, PD-L1 mRNA levels were significantly upregulated by approximately 1.8-fold compared to untreated control cells ($p < 0.05$). By 12 h, PD-L1 expression increased further to 2.9-fold ($p < 0.01$), and by 24 h, the induction reached a peak with a 3.8-fold increase ($p < 0.001$). These findings confirm that IFN-γ robustly upregulates PD-L1 expression at the transcriptional level over time, supporting its role in immune evasion mechanisms (Fig. 3).

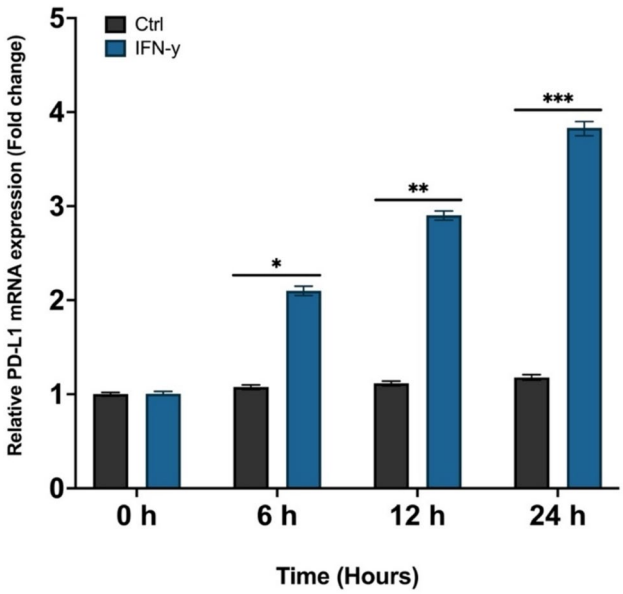

Fig. 3 qRT-PCR analysis showing time-dependent upregulation of PD-L1 mRNA expression in A549 cells following IFN-γ treatment. A549 cells were treated with 500 U/mL of IFN-γ for 6, 12, and 24 h. Total RNA was extracted and analyzed by qRT-PCR to measure PD-L1 mRNA expression levels. Data are presented as mean ± SD relative to untreated controls. Statistical significance is indicated (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

3.3 Curcumin-mediated inhibition of IFN-γ-induced STAT1 phosphorylation leads to PD-L1 downregulation

To investigate whether curcumin inhibits IFN-γ-induced STAT1 phosphorylation, A549 cells were pretreated with curcumin (50 μM) for 2 h before stimulation with IFN-γ (500 U/ml) for 24 h. Western blot analysis revealed a significant reduction in phosphorylated STAT1 (p-STAT1,

Tyr701) levels in curcumin-pretreated cells, with a 68% decrease compared to IFN-γ-treated cells alone ($p < 0.001$). Notably, total STAT1 protein levels remained unchanged, confirming that the decrease in p-STAT1 was due to inhibition of phosphorylation rather than reduced STAT1 expression. Additionally, PD-L1 expression, which is regulated by STAT1 activation, was also markedly reduced in curcumin-treated cells, with 50 μM curcumin reducing PD-L1 levels by 72% compared to IFN-γ-treated cells ($p < 0.01$). These findings indicate that curcumin effectively suppresses IFN-γ-induced STAT1 activation and its downstream signaling, including PD-L1 upregulation, in A549 cells (Fig. 4).

3.4 Curcumin reduces the expression of ISGs in IFN-γ-treated cells

Since STAT1 regulates the expression of multiple ISGs involved in immune evasion, we next evaluated the effect of curcumin on the expression of ISG15 and SOCS1. qRT-PCR analysis showed that IFN-γ significantly upregulated ISG15 and SOCS1 mRNA expression by 3.2-fold and 4.5-fold, respectively, compared to untreated controls ($p < 0.001$). However, pretreatment with curcumin (50 μM) led to a suppression of ISG15 (54% reduction) and SOCS1 (63% reduction) expression, further supporting the role of curcumin in inhibiting IFN-γ-driven STAT1 signaling (Fig. 5).

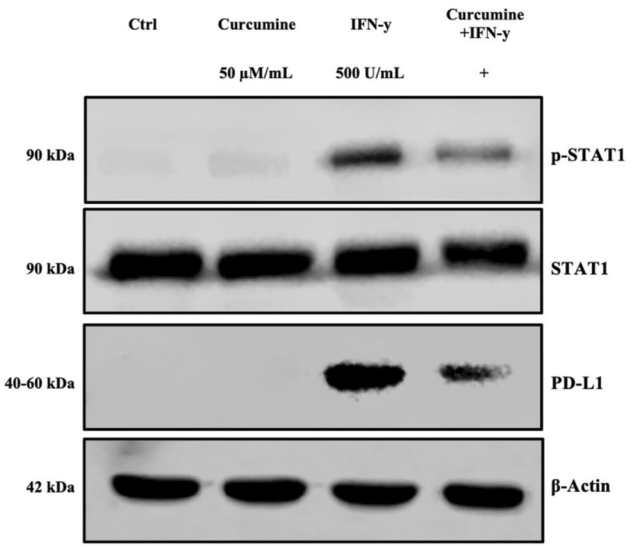

Fig. 4 Western blot analysis showing the effect of curcumin on IFN-γ-induced STAT1 phosphorylation and PD-L1 expression in A549 cells. A549 cells were pretreated with curcumin (50 μM) for 2 h, followed by stimulation with IFN-γ (500 U/mL). Protein lysates were collected and analyzed by Western blot for phosphorylated STAT1 (Tyr701), total STAT1, and PD-L1 expression. β-Actin was used as a loading control

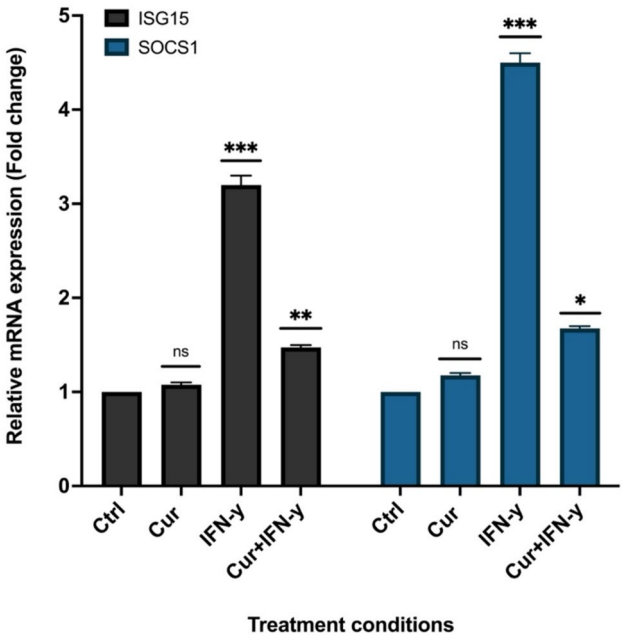

Fig. 5 qRT-PCR analysis showing the effect of curcumin on IFN-γ-induced expression of interferon-stimulated genes (ISGs) in A549 cells. A549 cells were treated with IFN-γ (500 U/mL) for 24 h, with or without curcumin pretreatment (50 μM for 2 h). Total RNA was extracted and analyzed by qRT-PCR for SOCS1 and ISG15 mRNA expression. Data are presented as mean ± SD. Statistical significance is indicated (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

3.5 Curcumin enhances IFN-γ-mediated growth inhibition

To evaluate the time-dependent effects of curcumin on IFN-γ-induced growth suppression, cell viability was assessed using the Resazurin assay at 0, 12, 24, and 48 h following treatment. At 12 h, a modest reduction in cell viability (10%) was observed in response to IFN-γ and curcumin co-treatment. By 24 h, cell viability had decreased further (21% reduction), consistent with previous findings on IFN-γ-mediated cytotoxicity in A549 cells. Notably, at 48 h, the combination of curcumin and IFN-γ resulted in a 47% reduction in cell viability, demonstrating a significant time-dependent enhancement of IFN-γ's anti-proliferative effects ($p < 0.01$). These findings suggest that curcumin sensitizes NSCLC cells to IFN-γ-induced growth suppression in a time-dependent manner, highlighting its potential as a therapeutic adjuvant (Fig. 6).

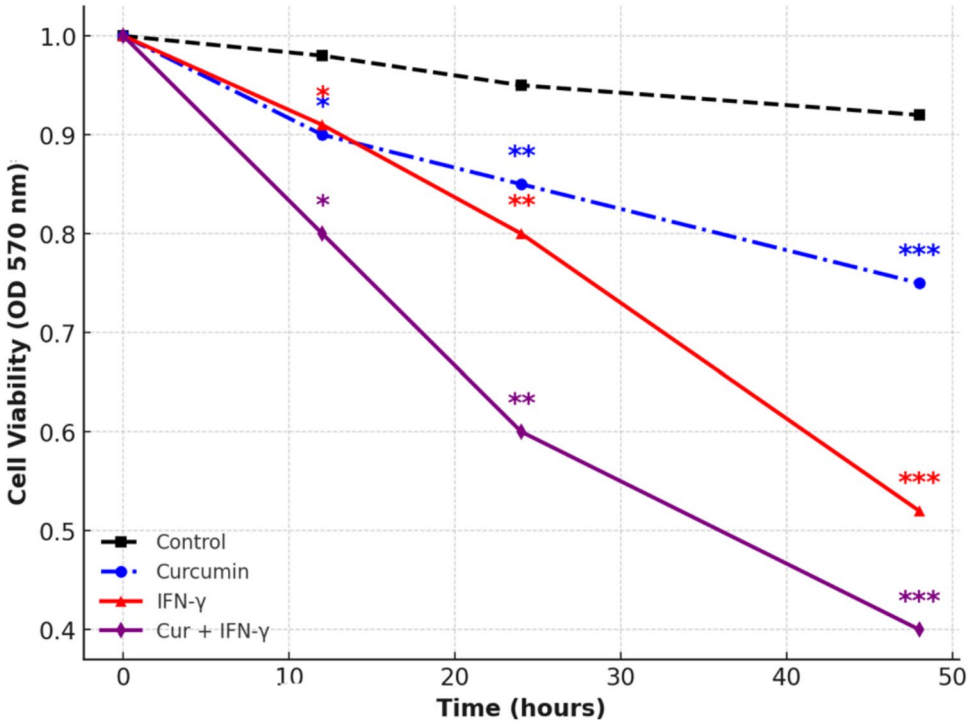

Fig. 6 Resazurin assay showing the effect of curcumin and IFN-γ co-treatment on cell viability in A549 NSCLC cells. **A549** cells were treated with curcumin (50 μM) and IFN-γ (500 U/mL) for 12, 24, and 48 h. Cell viability was assessed using the Resazurin assay. Data are presented as mean ± SD, showing a time-dependent decrease in viability with combination treatment. Statistical significance is indicated (*$p <$ 0.05, **$p < 0.01$, ***$p < 0.001$)

4 Discussion

Immune evasion remains a significant challenge in the treatment of NSCLC, with PD-L1 upregulation being one of the primary mechanisms by which tumors escape immune surveillance (Cui et al. 2024). The IFN-γ/STAT1 signaling pathway plays a crucial role in PD-L1 regulation, enabling tumor cells to suppress T-cell-mediated immune responses and resist immune checkpoint blockade therapy (Padmanabhan et al. 2022). In this study, we investigated how IFN-γ-induced STAT1 phosphorylation leads to PD-L1 upregulation in A549 cells and explored the potential of curcumin, a bioactive polyphenol with known anti-inflammatory and anti-cancer properties, as a therapeutic agent capable of modulating this pathway. Our findings provide strong evidence that curcumin inhibits IFN-γ-induced STAT1 activation, thereby reducing PD-L1 expression and enhancing the anti-proliferative effects of IFN-γ in NSCLC.

Consistent with previous studies, we observed that IFN-γ induces a robust phosphorylation of STAT1 at Tyr701 in A549 cells, leading to its nuclear translocation and subsequent activation of target genes, including PD-L1. The kinetics of STAT1 phosphorylation followed a time-dependent pattern, with phosphorylation detected as early as 2 h, peaking at 6 h, and remaining elevated up to 24 h.

These results are in agreement with earlier reports that demonstrated a similar pattern of IFN-γ-induced STAT1 activation in various cancer models, including melanoma (Schmitt et al. 2012), colorectal cancer (Zhao et al. 2020), and lung adenocarcinoma (Gao et al. 2018). STAT1 phosphorylation is a prerequisite for its dimerization and nuclear translocation, which is required for the transcriptional activation of ISGs (Wang et al. 2017). Immunofluorescence analysis confirmed that IFN-γ treatment led to a marked accumulation of p-STAT1 in the nucleus, reinforcing the notion that STAT1 plays a crucial role in IFN-γ-mediated transcriptional regulation. These findings align with studies showing that sustained STAT1 activation promotes an immunosuppressive tumor microenvironment by inducing PD-L1 expression and other immune-regulatory genes.

The upregulation of PD-L1 in response to IFN-γ was confirmed at both the mRNA and protein levels, as demonstrated by qRT-PCR and Western blotting. The increased surface expression of PD-L1 following IFN-γ treatment highlights the functional significance of this regulation, as surface PD-L1 interacts with PD- 1 on T cells to inhibit anti-tumor immune responses (Arak et al. 2021). These results are in accordance with previous reports showing that IFN-γ is one of the most potent inducers of PD-L1 in NSCLC, facilitating immune escape and tumor progression (Pawelczyk et al. 2019). Additionally, the use of fludarabine, a STAT1 inhibitor, significantly attenuated IFN-γ-induced PD-L1 expression, confirming that STAT1 is the primary mediator of this regulatory axis. This finding corroborates prior studies demonstrating that STAT1-deficient cells fail to upregulate PD-L1 in response to IFN-γ, emphasizing the centrality of STAT1 in this pathway.

One of the most significant findings of this study is the ability of curcumin to inhibit IFN-γ-induced STAT1 activation and PD-L1 expression in A549 cells. Western blot analysis revealed that curcumin suppressed STAT1 phosphorylation in a dose-dependent manner, with the highest concentration (50 μM) reducing phosphorylation by 68%. This effect was not due to a decrease in total STAT1 protein levels, indicating that curcumin selectively inhibits STAT1 activation rather than its expression. Previous studies have reported that curcumin can interfere with JAK-STAT signaling in other cancer types. Curcumin directly inhibits the phosphorylation of STAT3, a key component of the JAK-STAT signaling pathway in breast cancer (Golmohammadi et al. 2024) and prostate cancer (Li et al. 2024), as well as the downregulation of the STAT1 in melanoma (Xu et al. 2018), but its specific effect on STAT1 in IFN-γ-stimulated NSCLC cells had not been previously explored. Our findings extend these observations by demonstrating that curcumin effectively blocks STAT1 activation in lung cancer cells, preventing the downstream induction of PD-L1.

The suppression of PD-L1 expression by curcumin was observed at both the transcriptional and translational levels, as evidenced by qRT-PCR and Western blotting. This finding is particularly relevant in the context of NSCLC, where high PD-L1 expression correlates with poor prognosis and resistance to immunotherapy. Previous studies have reported that curcumin downregulates PD-L1 in other cancer models, such as melanoma (Xu et al. 2018) and hepatocellular carcinoma (Guo et al. 2021), but the specific inhibition of IFN-γ-induced PD-L1 expression in NSCLC had not been thoroughly investigated. Our study provides the first evidence that curcumin can effectively suppress IFN-γ-mediated PD-L1 upregulation in lung cancer cells via STAT1 pathway, highlighting its potential as an immune-modulatory agent.

In addition to PD-L1, STAT1 regulates the expression of multiple ISGs involved in immune evasion, including SOCS1 (Ilangumaran et al. 2024) and ISG15 (Desai 2015). Our results demonstrated that IFN-γ significantly upregulated both SOCS1 and ISG15, reinforcing the notion that IFN-γ signaling contributes to an immunosuppressive tumor microenvironment. Curcumin pretreatment, however, led to a significant reduction in both SOCS1 and ISG15 expression, further supporting its ability to interfere with IFN-γ-driven STAT1 signaling. SOCS1 is known to act as a feedback inhibitor of JAK-STAT signaling (Liau et al. 2018), but paradoxically, its overexpression in tumors has been associated with immune escape mechanisms. By suppressing SOCS1 expression, curcumin may enhance the responsiveness of tumor cells to immune-mediated clearance. Similarly, ISG15 has been implicated in tumor progression and resistance to therapy (Meng et al. 2024), suggesting that its downregulation by curcumin may have additional therapeutic benefits.

Finally, we observed that curcumin enhances the antiproliferative effect of IFN-γ in A549 cells. While IFN-γ alone resulted in a modest reduction in cell viability (21%), the combination of IFN-γ and curcumin led to a significantly greater reduction (47%), suggesting a synergistic effect. These findings align with previous reports that curcumin enhances the anti-tumor activity of cytokines by modulating cell cycle regulators and apoptotic pathways (Hu et al. 2018). The precise mechanism by which curcumin sensitizes NSCLC cells to IFN-γ-induced growth suppression remains to be elucidated, but it may involve inhibition of survival pathways downstream of STAT1 activation. Given that STAT1 has been implicated in both pro-apoptotic and pro-survival signaling, the net effect of its inhibition may depend on the cellular context and additional regulatory factors.

In conclusion, our study provides novel evidence that IFN-γ induces PD-L1 expression in NSCLC cells via STAT1 activation and that curcumin effectively inhibits this process by suppressing STAT1 phosphorylation and nuclear translocation. Furthermore, curcumin downregulates IFN-γ-induced ISGs and enhances IFN-γ-mediated tumor cell growth suppression, highlighting its potential as a therapeutic adjuvant in NSCLC. These findings suggest that curcumin could be used to improve the efficacy of immune checkpoint inhibitors by reducing tumor immune evasion. Future studies should focus on elucidating the precise molecular mechanisms underlying curcumin's effects on STAT1 signaling and investigating its potential synergistic effects with existing immunotherapies in preclinical and clinical settings.

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Author contributions DJS and AHS, conceived and designed the study. DJS and SHB performed the experiments. DJS wrote the paper. GFA, SQH, QAI, AAA, TAA and AMS reviewed and edited the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was conducted in accordance with the principles of the Declaration of Helsinki and the ethical guidelines set forth by the Institutional Ethics Committee of the University of Foggia. Ethical approval was obtained from the Institutional Review Board (approval number DDG N. 651, dated 27 August 2024).

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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