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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ALTERATIONS IN THE APOPTOSIS-ASSOCIATED GENE EXPRESSIONS IN LUNG CANCER CELLS IN THE PRESENCE OF IXAZOMIB AND GIVINOSTAT

İXAZOMİB VE GİVİNOSTAT VARLIĞINDA AKCİĞER KANSERİ HÜCRELERİNDE APOPTOZLA İLİŞKİLİ GEN EKSPRESYON DÜZEYLERİNDEKİ DEĞİŞİKLİKLER

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ABSTRACT

Objective: *Proteasome inhibitors and histone deacetylase (HDAC) inhibitors represent promising therapeutic agents in lung cancer, particularly by enhancing cancer cell sensitivity to treatment. Among these, the combination of ixazomib, a proteasome inhibitor, and givinostat, an HDAC inhibitor, has demonstrated synergistic cytotoxicity in non-small cell lung cancer (NSCLC) cell lines. This strategy holds potential for overcoming therapeutic resistance. Furthermore, macrophage co-culture systems have been shown to modulate gene expression patterns in lung cancer cells. In this context, the present study aimed to investigate the effects of ixazomib and givinostat, individually and in combination, along with differentiated M1 macrophages, on the expression of apoptosis-related genes in NSCLC cells.*

Material and Method: *The IC_{50} values of ixazomib and givinostat in A549 lung cancer cells were determined using the MTT assay after 24 hours of treatment with a range of concentrations (400, 100, 10, and 1 µM). Based on the determined IC_{50} values, A549 cells were treated with ixazomib alone or in combination with givinostat for 24 hours. In parallel, A549 cells treated with the ixazomib–givinostat combination were co-cultured with THP-1 monocyte–derived M1 macrophages. Total RNA was then isolated, and RT-PCR analysis was performed to evaluate alterations in apoptosis-related gene expression.*

Result and Discussion: *Based on the results of the anti-proliferative effect analysis, the IC50 values for ixazomib and givinostat were determined to be 2.42 µM and 7.32 µM, respectively. According to the RT-PCR results, variations of up to 48.99-fold were observed in the NFKB2, NFKBIA, NFKBIB, RELB, IL-6, IL-8, TP53, PIK3CA, and BCR genes. Significant alterations were particularly observed in genes involved in apoptosis-related pathways, especially in the presence of M1 macrophages. Additional investigation is warranted to thoroughly explore the heightened inflammatory response observed in lung cancer cells in the presence of these drugs.*

Keywords: *Apoptosis, givinostat, ixazomib, lung cancer, M1 macrophage*

ÖZ

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Amaç: *Proteazom inhibitörleri ve histon deasetilaz (HDAC) inhibitörleri, özellikle kanser hücrelerinin tedaviye duyarlılığını artırmaları açısından akciğer kanserinde umut vadeden tedavi ajanları olarak değerlendirilmektedir. Bu ajanlar arasında, bir proteazom inhibitörü olan ixazomib ile bir HDAC inhibitörü olan givinostat'ın kombinasyonu, küçük hücreli olmayan akciğer kanseri (KHOAK) hücre hatlarında sinerjistik sitotoksisite göstermiştir. Bu tedavi yaklaşımı, terapötik dirençlerin aşılması açısından potansiyel taşımaktadır. Ayrıca, makrofaj ile ortak kültür sistemlerinin, akciğer kanseri hücrelerinde gen ekspresyon paternlerini değiştirdiği gösterilmiştir. Bu bağlamda, bu çalışma, ixazomib ve givinostat'ın ayrı ayrı ve birlikte uygulanmasının yanı sıra farklılaşmış M1 makrofajların etkisinin, KHOAK hücrelerinde apoptozla ilişkili genlerin ekspresyonu üzerindeki etkilerini araştırmayı amaçlamıştır.*

Gereç ve Yöntem: *İxazomib ve givinostat'ın A549 akciğer kanseri hücrelerindeki IC_{50} değerleri, farklı konsantrasyonlarda (400, 100, 10 ve 1 µM) 24 saatlik uygulamayı takiben MTT testi kullanılarak belirlenmiştir. Belirlenen IC_{50} değerlerine göre A549 hücreleri 24 saat boyunca yalnızca ixazomib veya ixazomib+givinostat kombinasyonu ile muamele edilmiştir. Paralel olarak, ixazomib–givinostat kombinasyonu ile muamele edilen A549 hücreleri, THP-1 monositlerinden türetilmiş M1 makrofajlar ile ko-kültüre edilmiştir. Ardından total RNA izole edilmiş ve apoptozla ilişkili gen ekspresyonundaki değişimleri değerlendirmek amacıyla RT-PCR analizleri gerçekleştirilmiştir.*

Sonuç ve Tartışma: *Anti-proliferatif etki analizine dayalı olarak, ixazomib ve givinostat için IC_{50} değerleri sırasıyla 2.42 µM ve 7.32 µM olarak belirlenmiştir. RT-PCR sonuçlarına göre, NFKB2, NFKBIA, NFKBIB, RELB, IL-6, IL-8, TP53, PIK3CA ve BCR genlerinde 48.99 kata varan değişiklikler gözlemlenmiştir. Özellikle M1 makrofajların varlığında, apoptozla ilişkili yollaklarda yer alan genlerde anlamlı düzeyde değişiklikler saptanmıştır. Bu ilaçların varlığında akciğer kanseri hücrelerinde gözlenen artmış inflamatuar yanıtın detaylı olarak incelenmesi için ek çalışmalara ihtiyaç vardır.*

Anahtar Kelimeler: *Apoptoz, akciğer kanseri, givinostat, ixazomib, M1 makrofaj*

INTRODUCTION

Lung cancer remains one of the leading causes of cancer-related mortality worldwide, with non-small cell lung cancer (NSCLC) accounting for approximately 85% of all cases [1] . Despite advances in targeted therapies and immunotherapies, treatment resistance and tumor recurrence continue to pose significant clinical challenges [2,3]. These limitations underscore the need for novel combination strategies that target both cancer cells and their supportive microenvironment.

The ubiquitin-proteasome system (UPS) plays a central role in maintaining protein homeostasis by regulating the degradation of intracellular proteins involved in cell cycle control, apoptosis, DNA repair, immune responses, and cellular signaling, and its dysregulation has been implicated in cancer progression by promoting tumor cell proliferation, immune evasion, metastasis, and altering the tumor microenvironment [4]. Proteasome inhibitors such as ixazomib have been shown to induce apoptosis by disrupting protein degradation and promoting cellular stress. Although the development of UPS inhibitors has faced challenges, ixazomib stands out as a promising anti-cancer agent due to its oral bioavailability, target specificity, and capacity to bypass drug resistance, demonstrating clinical success in multiple myeloma and showing potential in treating other types of cancer [5,6]. However, their efficacy as monotherapies in solid tumors remains limited. On the other hand, histone deacetylase (HDAC) inhibitors like givinostat can modulate gene expression by altering chromatin structure, leading to the reactivation of tumor suppressor genes and sensitization of cancer cells to chemotherapeutic agents [7,8]. Givinostat is an orally bioavailable and potent pan-HDAC inhibitor that promotes a relaxed chromatin structure and facilitates transcriptional activation by inhibiting HDAC, while also exhibiting anti-inflammatory properties through the suppression of pro-inflammatory cytokines such as *Tumor Necrosis Factor-alpha (TNF-α)*, *Interleukin-1 alpha (IL-1α)*, *Interleukin-1 beta (IL-1β)*, *Interleukin-6 (IL-6)*, and *Interferon-gamma (IFN-γ)* [9–11]. The combination of ixazomib and givinostat is a rational therapeutic strategy in lung cancer, particularly NSCLC, as it synergistically disrupts proteostasis and epigenetic regulation—enhancing apoptosis, inhibiting NF-κB signaling, and potentially overcoming drug resistance in aggressive tumor subtypes.

In addition to intrinsic tumor signaling, the tumor microenvironment-particularly immune cells

such as macrophages-plays a pivotal role in shaping therapy responses. Macrophages are highly plastic immune cells that can adopt a classically activated (M1) or alternatively activated (M2) phenotype, depending on local signals [12]. M1 macrophages exhibit pro-inflammatory and anti-tumoral properties, and their presence has been associated with enhanced therapeutic outcomes [13,14]. However, the impact of M1 macrophage polarization on gene expression in lung cancer cells under chemotherapeutic stress remains largely unexplored.

In this study, we aimed to investigate the combined effects of the proteasome inhibitor ixazomib and the HDAC inhibitor givinostat on the gene expression profile of A549 NSCLC cells, with a particular focus on apoptosis- and survival-related genes. In addition, we evaluated how co-culture with M1-polarized macrophages modulates these molecular responses. This integrated approach provides insight into the interplay between cancer therapies and the immune microenvironment at the transcriptional level and may inform future combination strategies in lung cancer treatment. The study was designed in a stepwise manner, beginning with cytotoxicity assessment to define IC_{50} values, followed by transcriptional analysis under drug treatment and immune co-culture conditions.

MATERIAL AND METHOD

A549 and THP-1 Cell Culture

The A549 human lung cancer cell line was cultured in Kaighn's Modification of Ham's F-12 Medium (F-12K), supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, at 37°C in a humidified incubator with 5% CO_2. THP-1 human monocyte cells were maintained in RPMI-1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin under the same incubation conditions. When the cells reached a proliferation density of 70-80%, they were either passaged into new flasks or prepared as cell stocks for future experiments. Before conducting experiments, the cell count was determined using the Cedex XS Analyzer (Roche Innovatis, Germany) following trypan blue staining to ensure the required cell numbers.

MTT Cell Viability Assay

The IC_{50} values of ixazomib and givinostat on A549 lung cancer cells were determined using the colorimetric MTT assay in 96-well plates. This assay assesses cell viability based on the reduction of yellow tetrazolium salt (MTT) to purple formazan crystals by metabolically active cells. The resulting color change provides a quantitative indication of cell viability and proliferation, with higher absorbance values corresponding to increased metabolic activity [15].

A549 cells were seeded at a density of $1x10^4$ cells per well in 96-well plates. After 24 hours of incubation, the cells were treated with varying concentrations (400, 100, 10, and 1 µM) of ixazomib and givinostat. Following a 24-hour exposure period, 10 µl of MTT solution was added to each well, and the plates were incubated for an additional 3 hours. Absorbance was measured at 540 nm using a Cytation 3 Cell Imaging Multi-Mode Reader (BioTek, USA). The absorbance values, which are directly proportional to the number of viable cells, were used to calculate cell viability. Results were expressed as a percentage relative to the untreated control group [16,17].

Polarization of the Macrophages into M1 Subtype

THP-1 cells were differentiated into M1 macrophages to simulate the pro-inflammatory tumor microenvironment and examine the effects of macrophage–tumor cell interactions on A549 lung cancer cells. To induce differentiation into a macrophage-like phenotype, THP-1 cells were treated with 100 ng/mL PMA in serum-free medium (SFM) for 24 hours. After differentiation, the adherent cells were allowed to rest in fresh culture medium (without PMA) for an additional 48 hours to reach the resting (M0) macrophage state. The macrophages were then polarized into the M1 phenotype by exposure to 20 ng/ml LPS and IFN-γ for 24 hours. To remove residual cytokines, the polarized macrophages were thoroughly washed and incubated in fresh medium for 24 hours [12]. Finally, M1 macrophage morphology was assessed by imaging with a Leica DM inverted light microscope.

Co-culture of A549 and M1 Macrophages

A549 non-small lung cancer cells and M1-polarized THP-1 macrophages were co-cultured in a 6-well transwell plate to investigate macrophage–tumor cell interactions. Briefly, M1 macrophages were polarized as previously described and then seeded at a density of 2.5×10^5 cells per well in the upper chamber of the Transwell inserts (0.4 µm pore size). A549 cells were seeded in the lower chamber at a density of 5×10^5 cells per well. The cells were co-cultured and treated with the determined IC_{50} concentrations of ixazomib and givinostat for 24 hours in a humidified incubator at 37°C with 5% CO_2. After the co-culture period, A549 cells from the lower chamber were collected for RNA extraction, and gene expression was analyzed using RT-PCR. To ensure accurate analysis, cell viability was confirmed via trypan blue staining prior to RNA extraction [18].

Quantitative Analysis of Apoptosis-Related Gene Expression via RT-PCR

Following exposure to the determined IC_{50} concentrations for 24 hours, total RNA was isolated from A549 cells treated with ixazomib alone, ixazomib + givinostat, and THP-1 monocytes differentiated into M1 macrophages and co-cultured with this combination. RT-PCR experiments were then performed to assess changes in the expression of key inflammatory and tumor suppressor genes related to apoptosis, including *Nuclear Factor Kappa B Subunit* 2 (*NFKB2), Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (NFKBIA), Nuclear Factor of Kappa light polypeptide gene enhancer in B-cells inhibitor, beta* (*NFKBIB), v-rel reticuloendotheliosis viral oncogene homolog B* (*RELB), IL-6, IL-8, Tumor Protein 53* (*TP53), Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha* (*PIK3CA),* and *Breakpoint Cluster Region* (*BCR).* Approximately 5×10^5 A549 cells were collected, and total RNA was isolated using the EcoPURE Total RNA Kit, following the manufacturer's protocol (EcoTech Biotechnology, Türkiye).

RNA concentration was measured by spectrophotometry at 260 nm and 280 nm using a NanoDrop 2000 (Thermo Scientific, USA). cDNA synthesis was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Germany) following the manufacturer's protocol. The resulting cDNA was amplified on the LightCycler 480 RT-PCR instrument (Roche, Germany) with the LightCycler 480 SYBR Green I Master (Roche, Germany). Primer sequences are listed in Table 1. PCR mixes and cDNA were loaded into 96-well plates, and appropriate SYBR Green I detection settings were applied. The plate was then placed in the LightCycler 480 for quantitative PCR, with ACTB as the reference gene for normalization [17,18].

Table 1. Forward and reverse sequences of the genes

Gene	Forward Sequence (5'→3')	Reverse Sequence (5'→3')
ACTB	GGCCAGGTCATCACCATT	GGATGCCACAGGACTCCAT
NFKB2	CCCATCCATGACAGCAAAT	CTTGTCACAAAGCAGATAAACTTCA
NFKBIA	GCTGATGTCAATGCTCAGGA	ACACCAGGTCAGGATTTTGC
NFKBIB	AGAGTGAGGAGGACTGGAAGC	CTCCACATCTTTGTGGATAACG
RELB	AAGAAAAAGCCGGCCATC	CACGGTGCCAGAGAAGAAGT
IL-6	CCAGAGCTGTGCAGATGAGT	GGGTCAGGGGTGGTTATTG
IL-8	TAGCCAGGATCCACAAGTCC	CTGTGAGGTAAGATGGTGGCTA
TP53	AGGCCTTGGAACTCAAGGAT	CCCTTTTTGGACTTCAGGTG
PIK3CA	TCAGTGGCTCAAAGACAAGAAC	TCCAGCACATGAACGTGTAAA
BCR	TGAGCCAAACTGGAACGAG	CACTTTTCATAGCACAGTATCCTCA

Statistical Analysis

Experimental data were imported into GraphPad Prism 8.4.2, where replicate values were averaged, and standard deviations were calculated. All graphs were generated using the same software, and statistical analyses were performed using one-way ANOVA followed by Tukey's post hoc test. Results are presented as the mean ± standard deviation (SD) of three independent experiments (n = 8 for cell viability assays; n = 3 for RT-PCR experiments).

RESULT AND DISCUSSION

Proteasome inhibitor-based therapies face several limitations: many patients either do not respond or relapse; solid tumors are particularly resistant. These drugs can cause significant side effects like peripheral neuropathy and gastrointestinal issues. Despite preclinical promise, their clinical efficacy in solid tumors is limited, possibly due to low immunoproteasome levels. Their mechanisms remain incompletely understood, especially regarding conflicting effects on NF-κB signaling. Additionally, their broad, non-selective activity and challenges in drug development hinder clinical progress [19,20]. Therefore, combining proteasome inhibition with epigenetic modulation has emerged as a promising strategy to overcome therapeutic limitations. To enhance the therapeutic response, the combination of proteasome inhibitors with HDACi has attracted attention as a rational approach, targeting complementary mechanisms such as protein degradation and chromatin remodeling.

In this study, we explored the molecular effects of the proteasome inhibitor ixazomib and the HDACi givinostat, alone and in combination, in A549 NSCLC cells. IC_{50} values were determined to guide treatment concentrations, ensuring that the observed effects on gene expression were pharmacologically relevant and not due to overt cytotoxicity. Both drugs exhibited dose-dependent effects, with their cytotoxicity aligning with previous reports on proteasome inhibition [12] and HDAC inhibition [21,22] in NSCLC models.

The effects of ixazomib and givinostat on A549 cell viability were assessed using the MTT assay to evaluate the cytotoxicity of these compounds at various concentrations. A549 cells were exposed to ixazomib and givinostat at concentrations of 400, 100, 10, and 1 µM, and cell viability was measured after 24 hours. The results revealed a dose-dependent decrease in cell viability, with ixazomib showing viability rates of 94.36%, 92.36%, 90.97%, and 81.09% at 400, 100, 10, and 1 µM concentrations, respectively (Figure 1).

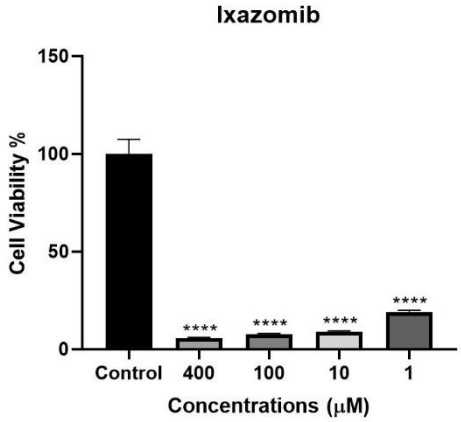

Figure 1. Cell viability of A549 cells following 24-hour treatment with ixazomib at concentrations of 400, 100, 10, and 1 µM. Results are expressed as mean ± SD from three independent experiments (n = 8 per group). Statistical significance versus control is indicated as ****$p < 0.0001$

Similarly, treatment with givinostat resulted in a dose-dependent decrease in cell viability, with viability rates of 95.93%, 76.83%, 50%, and 41.76% as the concentration increased (Figure 2). Based on these results, the IC_{50} values for ixazomib and givinostat were determined to be 2.42 µM and 7.32 µM, respectively, highlighting their anti-proliferative effects. These findings suggest that both ixazomib and givinostat exert dose-dependent cytotoxic effects on A549 cells, with givinostat showing a more pronounced impact at lower concentrations.

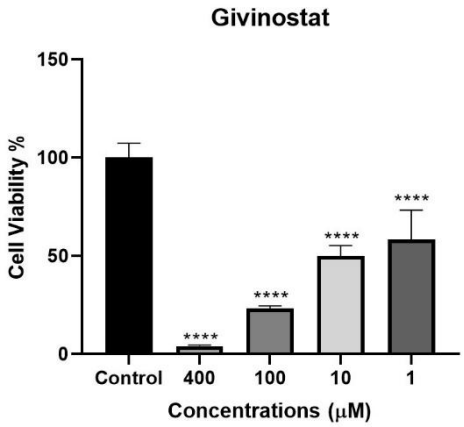

Figure 2. Cell viability of A549 cells following 24-hour treatment with givinostat at concentrations of 400, 100, 10, and 1 μM. Results are expressed as mean ± SD from three independent experiments (n = 8 per group). Statistical significance versus control is indicated as ****p < 0.0001

Macrophages are highly plastic immune cells capable of altering their phenotype and function in response to microenvironmental cues. They are broadly categorized into M1 (pro-inflammatory) and M2 (anti-inflammatory) subtypes, depending on the nature of the stimulus. Lipopolysaccharide (LPS), a microbial component, induces polarization toward the M1 phenotype [23]. M1 macrophages are typically medium-sized, amoeboid cells displaying multiple cytoplasmic projections. Upon adhesion, they acquire a rounded and flattened morphology. Their pronounced adhesive properties, mediated by integrin αD expression, result in reduced migratory capacity relative to M2 macrophages [24–26]. In the present study, although phenotypic marker analysis was not repeated, the polarized morphology of THP-1-derived macrophages was consistent with our previous findings [12]. Newly acquired microscopic images (Figure 3) further supported the successful induction of the M1 phenotype under the experimental conditions used.

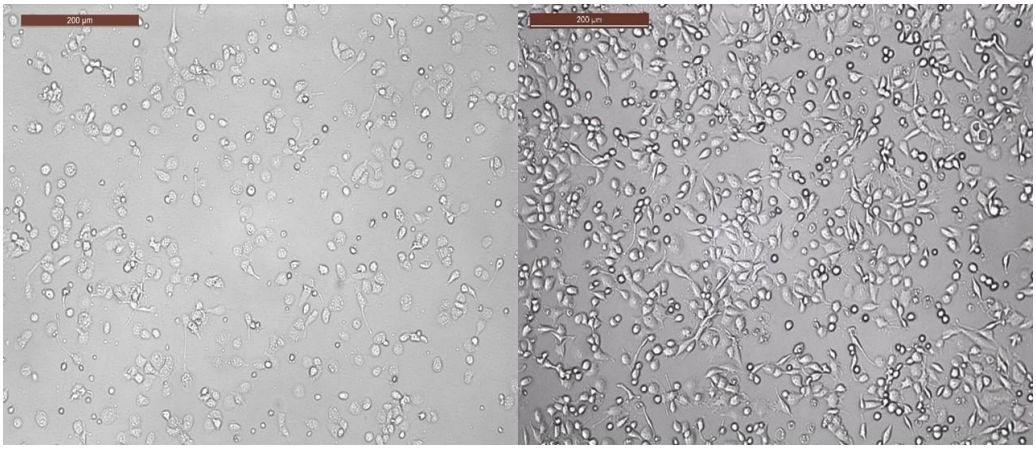

Figure 3. THP-1 origin macrophage (M0) (left) and M1 polarized macrophage (right) cells observed under a light microscope (10X)

In this study, RT-PCR analysis was employed to assess the effects of ixazomib and givinostat treatment on the expression levels of various genes in A549 cells. The fold change values for gene expression in the treated groups revealed distinct molecular responses to the treatments (Table 2).

Table 2. Fold change of mRNA expression levels of apoptosis-related genes

Gene name	Gene symbol	Fold change (mean±standard deviation)			
		Control	Ixazomib	Ixazomib+ Givinostat	Ixazomib+Givinostat +M1
NFKB inhibitor Alpha	NFKBIA	1±0.25	0.66±0.11	0.26±0.07	0.16±0.03
NFKB inhibitor beta	NFKBIB	1±0.35	1.74±0.20	8.53±0.40	4.77±0.14
NFKB subunit 2	NFKB2	1±0.25	0.68±0.21	0.73±0.15	1.96±0.34
RELB proto-oncogene	RELB	1±0.29	1±0.42	1.46±0.31	2.09±0.14
Interleukin-6	IL-6	1±0.23	0.68±0.14	1.13±0.31	0.91±0.41
Interleukin-8	IL-8	1±0.41	3.90±0.16	21.78±0.53	25.21±0.71
Tumor Protein P53	TP53	1±0.23	0.78±0.68	0.22±0.10	0.14±0.11
Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	PIK3CA	1±0.13	0.48±0.02	1.38±0.26	3.52±0.59
BCR activator of RhoGEF and GTPase	BCR	1±0.12	3.58±0.17	48.99±0.97	3.97±0.43

Notably, the expression of NFKBIA was significantly reduced in the ixazomib and combination treatment groups. NFKBIB showed a slight increase upon ixazomib treatment, which was more pronounced when combined with givinostat and then decreased with the addition of M1 polarization. The expression of NFKB2 and RELB was elevated in all treatment groups compared to the control. The expression of IL-6 decreased with ixazomib treatment but was restored with givinostat and slightly decreased with M1 polarization.

A marked increase in the expression of IL-8 was observed, particularly in combination treatments involving ixazomib and givinostat. TP53 expression was reduced across all treatment groups. The expression of PIK3CA and BCR decreased with ixazomib treatment, but both genes showed significant increases with givinostat and M1 polarization, with BCR experiencing a notably high fold change (Figure 4).

RT-PCR analysis revealed significant alterations in the expression of genes involved in apoptosis, inflammation, and survival. Ixazomib reduced NFKBIA expression, suggesting a disruption in IκBα-mediated inhibition of NF-κB, while NFKB2 and RELB were upregulated across treatment groups. Interestingly, givinostat appeared to partially restore IL-6 expression after ixazomib-induced suppression, consistent with the dual regulatory roles of HDAC inhibitors on cytokine expression. The combination treatment markedly increased IL-8 expression, which could reflect a compensatory inflammatory response or a paracrine effect relevant to tumor-immune interactions [27,28]. Of particular note, the expression of TP53 was consistently downregulated, indicating possible transcriptional repression under treatment stress or an adaptive survival mechanism [29] Importantly, the downregulation of TP53 at the mRNA level does not necessarily contradict the induction of apoptotic responses. Previous reports have demonstrated that proteasome inhibitors can induce apoptosis in a p53-independent manner in cancer cells, including p53-deficient models where caspase activation occurs despite lack of functional TP53 [30]. Additionally, histone deacetylase inhibitors have been shown to regulate apoptosis through p53-independent pathways involving BAX and other mitochondrial effectors [31]. Finally, transcriptional downregulation of TP53 may not reflect functional protein activity, as p53 stabilization and activation are primarily controlled by post-translational modifications under stress conditions [32].

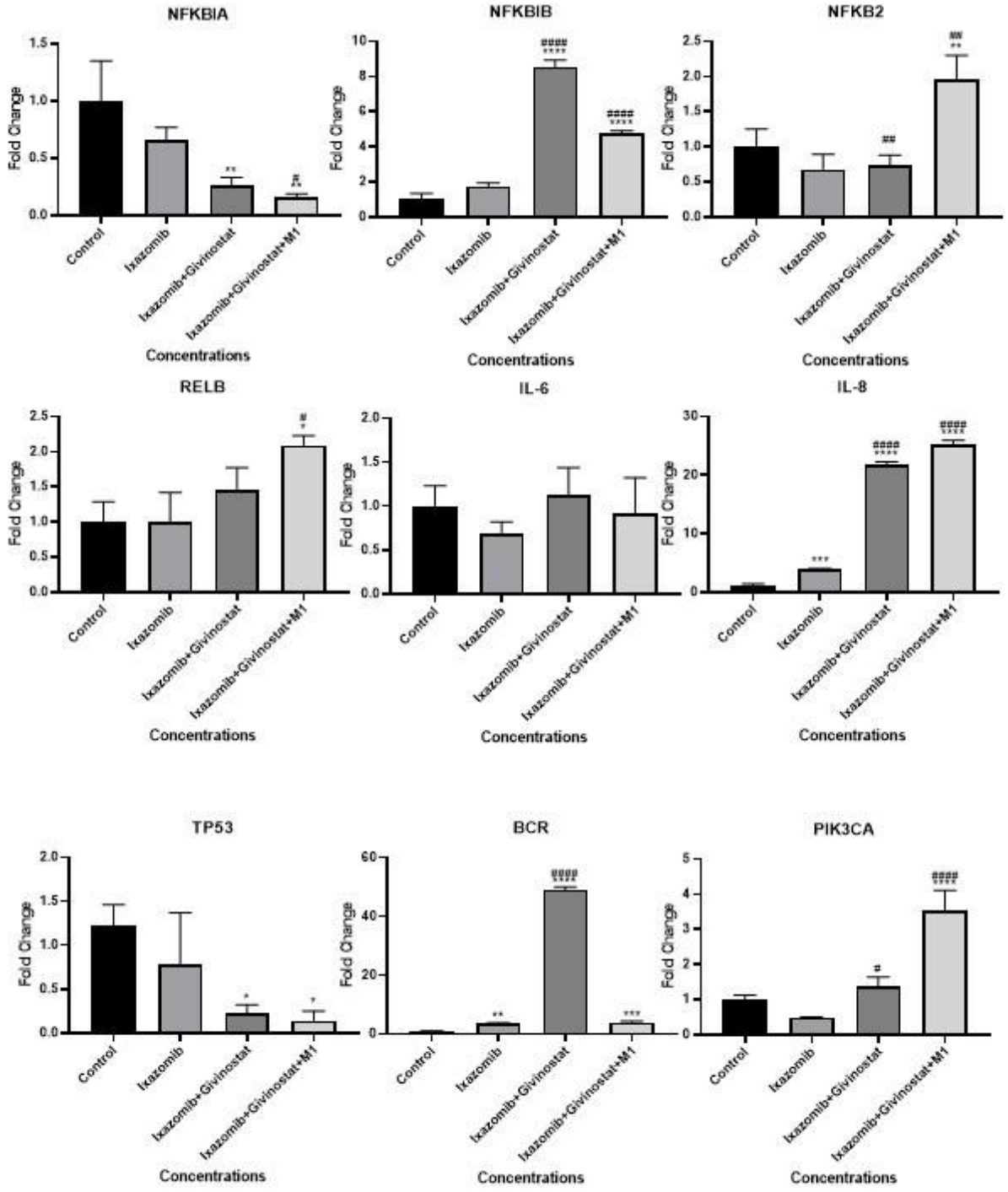

Figure 4. Fold change in gene expression levels in A549 cells determined by RT-PCR analysis. Gene expression was normalized to ACTB and expressed relative to the control group. Data are presented as mean ± SD from two independent experiments (n = 3 per group). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance is indicated as *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus untreated control; #p < 0.05, ##p < 0.01, ###p < 0.001, ####p < 0.0001 versus ixazomib-treated group

Meanwhile, the expression of PIK3CA and BCR genes was significantly increased with givinostat and M1 macrophage co-culture, suggesting that epigenetic modulation and immune signals may

converge on survival and proliferation pathways. These findings align with literature suggesting enhanced PI3K signaling in response to HDACi exposure in certain cellular contexts [33,34]. Notably, the pronounced increase observed in BCR expression should be considered in the context of the limited number of biological replicates. Although the upregulation was consistent across experiments, confirmation in further studies is warranted.

The addition of M1-polarized macrophages further modulated gene expression patterns, underscoring the influence of the immune microenvironment on cancer cell responses [12]. Some pro-inflammatory genes were downregulated, while others were amplified, implying a complex crosstalk between tumor and immune components. This co-culture system provides a more physiologically relevant model for evaluating potential combination therapies [35,36].

In conclusion, ixazomib and givinostat significantly alter the expression of genes involved in inflammation (e.g., IL-6, IL-8), cell survival (PIK3CA), and apoptosis (TP53) in NSCLC cells. Changes in NF-κB–related genes, including NFKBIA downregulation and RELB and NFKB2 upregulation, indicate modulation of the NF-κB pathway rather than a clearly defined activation or inhibition state. This gene expression pattern suggests a complex regulatory response that may involve non-canonical NF-κB signaling or compensatory feedback mechanisms, consistent with previous reports showing context-dependent effects of proteasome and HDAC inhibitors. Moreover, co-culture with M1 macrophages further modulated gene expression levels, underscoring the influence of the immune microenvironment. Overall, these findings highlight the potential of combining proteasome and HDAC inhibitors with immune-based approaches in lung cancer models, particularly those with defined mutations such as TP53.

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AUTHOR CONTRIBUTIONS

Concept: E.K.T., S.E.Ö.; Design: E.K.T.; Control: S.E.Ö.; Sources: E.K.T., S.E.Ö.; Materials: E.K.T., S.E.Ö.; Data Collection and Processing: E.K.T., S.E.Ö.; Analysis and Interpretation: E.K.T., S.E.Ö.; Literature Review: E.K.T.; Manuscript Writing: E.K.T.; Critical Review: E.K.T., S.E.Ö.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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