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# Modulatory Effects of IL-24 on Autophagy and Apoptosis in Large Cell Lung Carcinoma

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## ABSTRACT

Large cell lung carcinoma (LCLC) is a highly aggressive, undifferentiated subtype of non-small cell lung cancer characterized by rapid metastasis and resistance to traditional systemic chemotherapies. Targeted immunotherapies using cytokines like Interleukin-24 (IL-24) represent promising avenues to selectively induce cancer-specific cell death. This study evaluated the concentration-dependent cytotoxic and molecular modulatory effects of IL-24 on patient-derived primary LCLC cell lines. Primary LCLC cultures established from ultrasound-guided tru-cut biopsies were treated with varying concentrations of recombinant human IL-24 protein. Cell viability and growth inhibition rates were quantified using the MTT colorimetric assay. Transcriptional variations in the autophagic flux marker (LC3) and the initiator of intrinsic apoptosis (caspase-9) were evaluated using quantitative real-time PCR (RT-qPCR). MTT assay profiling demonstrated a strong concentration-dependent cytotoxic effect after 48 hours of treatment, yielding a half-maximal inhibitory concentration (IC50) of 22.9 pg/mL. Cell viability sharply collapsed from 82.5% at a sub-threshold dose of 5 pg/mL down to 14.7% at the maximum concentration of 100 pg/mL. RT-qPCR tracking showed a robust, dose-dependent upregulation of LC3 across all tested doses, indicating immediate activation of the autophagic pathway. Conversely, apoptotic response tracking revealed a distinct threshold activation; caspase-9 expression remained low at 10 pg/mL but co-elevated drastically with LC3 at higher concentrations of 50 pg/mL and 100 pg/mL. These findings validate that IL-24 exerts potent anti-cancer activity in primary LCLC by triggering excessive, toxic autophagy that drives past a cytoprotective survival threshold, successfully engaging the ATG4/ATG5 molecular switch to orchestrate a fatal transition into the intrinsic mitochondrial apoptotic pathway. Consistently supported by quantitative alignment between gene upregulation and loss of viability, IL-24 proves to be a compelling therapeutic candidate that warrants advanced in vivo evaluation.

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## INTRODUCTION

Lung carcinoma is one of the most prevalent and lethal malignancies worldwide, accounting for a significant proportion of cancer-related mortality [1]. Non-small cell lung cancer (NSCLC) comprises most of these cases. large cell lung carcinoma (LCLC) is one of non-small lung carcinoma that represents a

distinct, highly aggressive, and undifferentiated subtype. LCLC is clinically characterized by its rapid proliferation, early metastasis, and characteristically poor clinical prognosis, making it a formidable challenge in pulmonary oncology [2].

Historically, the standard of care for advanced LCLC has depend on traditional cytotoxic chemotherapies, particularly platinum-based chemotherapy regimens. While these chemotherapeutic agents effectively disrupt cellular division and temporarily reduce tumor burden, but their lack of target specificity results in many serious systemic adverse effects. Patients frequently suffer from severe toxicities, including myelosuppression, nephrotoxicity, and gastrointestinal distress [3]. Furthermore, the long-term efficacy of chemotherapy is frequently compromised by the development of multidrug resistance by cancer cells, severely limiting survival outcomes and highlighting the urgent need for more targeted interventions [4].

To address the limitations of conventional chemotherapy, cancer treatment has increasingly shifted toward targeted immunotherapies. Unlike traditional agents that attack all rapidly dividing cells, immunotherapy enhances and facilitates the patient’s immune system to recognize and eliminate malignant cells [5]. This strategy offers the potential for more specific and durable antitumor responses while reducing off-target systemic toxicity. Among immunotherapeutic approaches, cytokines—particularly interleukins—have emerged as important molecular tools. Interleukins are powerful regulators of the immune response that can modify the tumor microenvironment, activate cytotoxic T cells, and directly induce tumor cell death [6].

Among the array of immunomodulatory cytokines, Interleukin-24 (IL-24), also known as melanoma differentiation-associated gene-7 (mda-7), has distinguished itself as a multidimensional and highly potent anti-cancer therapeutic [7]. A unique hallmark of IL-24 is its "bystander effect" and its profound cancer-specific cytotoxicity; it selectively induces cell death in diverse tumor lineages without exerting harmful effects on normal, healthy cells. Mechanistically, IL-24 represses protective mitochondrial proteins such as BCL-2, promotes the liberation of BAX, and orchestrates DNA damage and growth suppression [8,9]. IL-24 initiates tumor destruction by triggering excessive, toxic autophagy that eventually reaches a critical threshold, prompting a calpain-mediated cleavage of ATG5. This event acts as a physiological switch, decisively shifting the cellular response from cytoprotective autophagy to irreversible intrinsic apoptosis [10].

Despite the well-documented efficacy of IL-24 across various solid tumors, its precise molecular kinetics and autophagic thresholds within the highly aggressive LCLC subtype remain insufficiently characterized. Therefore, the recent study is hypothesized that IL-24 functions as a potent, concentration-dependent therapeutic agent in large cell lung carcinoma primary cell line by driving autophagic flux past the cytoprotective threshold, ultimately orchestrating a fatal switch to the intrinsic apoptotic pathway.

The ercent study was conducted to evaluate the targeted in vitro cytotoxicity of IL-24 on LCLC cells. Specifically, this study aims to define the chemotherapeutic concentration gradient required to suppress cell viability via MTT assay and to elucidate the molecular relation between excessive autophagy and cell death via quantifying the co-elevation of LC3 (as a marker of autophagic flux) and caspase-9 (as the primary initiator of intrinsic apoptosis).

## MATERIALS AND METHODS

### Preparation Primary Cell culture of tru-cut large cell lung carcinoma biopsies

Primary large cell lung carcinoma cell lines were established from patient tru-cut core biopsy specimens obtained under ultrasound guidance and local anesthesia, following previously described approaches for primary tumor cell culture establishment (Leake et al., 1987; Dairkee et al., 1995; Speirs, 2004). Immediately after collection, tissue fragments were transferred into RPMI-1640 medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), and fluconazole (10 µg/mL). Samples were subsequently washed several times with phosphate-buffered saline (PBS) containing antibiotic-antifungal agents to minimize contamination.

The biopsy tissues were mechanically minced using a sterile scalpel blade and centrifuged at 2000 rpm for 5 min. The resulting fragments were then subjected to enzymatic dissociation in 0.25% collagenase and incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. Gentle mechanical pipetting was performed every 10 min to facilitate tissue digestion and cell release. Enzymatic activity was terminated by adding complete RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

Following centrifugation at 1000 rpm for 5 min, the cellular pellet was resuspended in fresh RPMI-1640 medium and seeded into T25 culture flasks. Cultures were maintained at 37 °C in a humidified incubator with 5% $CO^2$

### Primary Cell Exposure to IL-24:

Recombinant human IL-24 protein (MBS2097224, Mybiosource, USA) was used to evaluate its biological activity on primary lung cancer cells, specifically large cell carcinoma cells. Cell suspensions were prepared at a density of 1×10^5cells/mL, and 120 µL of the suspension was seeded into each well of sterile 96-well culture plates.

Cells were exposed to different concentrations of IL-24 (10, 25, 50 and 100 pg/mL), with each concentration tested in triplicate. Treated cultures were incubated for 24 h at 37 °C in a humidified atmosphere containing 5% CO_2. Following incubation, cellular responses to IL-24 exposure were assessed for comparative analysis among the tested concentrations.

### MTT Assay

Cell viability and cytotoxic activity of IL-24 were evaluated using the MTT assay according to the method described by Hayon et al. (2003). Briefly, primary large cell lung carcinoma cells were seeded into 96-well microtiter plates at a density of $1 \times 10^5$cells/mL in DMEM supplemented culture medium and allowed to attach under standard incubation conditions. Different concentrations of IL-24 (5, 10, 50, 75, and 100 pg/mL) were prepared in serum-free medium and added to the wells in triplicate. Control wells received serum-free medium only. Cells were incubated for 48 h at 37 °C in a humidified atmosphere containing 5% $CO_2$.

Following treatment, 30 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl) − 2,5-diphenyltetrazolium bromide)was added to each well and incubated for 1 h to allow the formation of intracellular formazan crystals by metabolically active cells. Subsequently, 30 µL of dimethyl sulfoxide (DMSO) was added to dissolve the formed crystals, and the plates were incubated in the dark at room temperature for 30 min. Absorbance was then measured at 630 nm using a microplate reader.

The percentage of growth inhibition (cytotoxicity) was calculated using the following equation:

$$\text{Inhibition Rate (IR\%)} = \frac{A - B}{A} \times 100$$

where *A*represents the optical density (OD) of the control group and *B*represents the OD of the treated group.

**Gene Expression Analysis**

Total RNA was extracted from primary large cell lung carcinoma cells using a spin-column–based RNA extraction kit according to the manufacturer's instructions. Briefly, fresh or frozen cell pellets were homogenized in lysis buffer supplemented with β-mercaptoethanol and Proteinase K, followed by incubation at 56 °C to ensure complete cellular disruption and protein digestion. The lysate was centrifuged, and the supernatant was transferred to spin columns for RNA purification through sequential binding, washing, and DNase I treatment steps to eliminate genomic DNA contamination. Purified RNA was finally eluted in RNase-free elution buffer and stored at −20 °C until further analysis.

Lyophilized LC3 and Caspase 9 primers were reconstituted in nuclease-free dd H2O to prepare a 100 µM stock solution, while a working concentration of 10 µM was subsequently prepared and maintained at −20 °C. Complementary DNA (cDNA) synthesis was performed using RT AddScript Master Mix in a final reaction volume of 20 µL containing RNA template, random hexamer primers, and nuclease-free water. Reverse transcription conditions included priming at 25 °C for 10 min, cDNA synthesis at 50 °C for 60 min, and enzyme inactivation at 80 °C for 5 min.

Quantitative real-time PCR (RT-qPCR) was carried out using GoTaq qPCR Master Mix in a total reaction volume of 20 µL containing forward and reverse primers, cDNA template, and nuclease-free water. Amplification was performed using an initial hot-start activation step at 95 °C for 10 min, followed by 40 amplification cycles consisting of denaturation at 95 °C for 10 sec, annealing/data collection at 60 °C for 30 sec, and extension at 72 °C for 30 sec. A final dissociation step at 72 °C for 2 min was included to confirm amplification specificity.

Gene expression levels of LC3 and Caspase-9 were evaluated in control cells (without IL-24 treatment) and in cells treated with IL-24 at concentrations of 10, 50, and 100 pg/mL to assess the effect of IL-24 on autophagy- and apoptosis-related gene regulation in primary large cell lung carcinoma cells.

**RESULTS**

**Cytotoxic Effects of IL-24 on Primary Large Cell Lung Carcinoma Cells**

The cytotoxic activity of IL-24 against primary large cell lung carcinoma cells was assessed using the MTT assay following exposure to serial concentrations of IL-24 (5, 10, 50, 75, and 100 pg/mL). The results demonstrated a concentration-dependent inhibitory effect of IL-24 on tumor cell proliferation after 48 h of treatment.

Analysis of cell viability data revealed that the half maximal inhibitory concentration (IC50) of IL-24 against primary large cell lung carcinoma cells was 22.9 pg/mL (Fig. 1). This finding indicates that IL-24 exhibited a marked antiproliferative and cytotoxic effect on the cultured carcinoma cells within the tested concentration range.

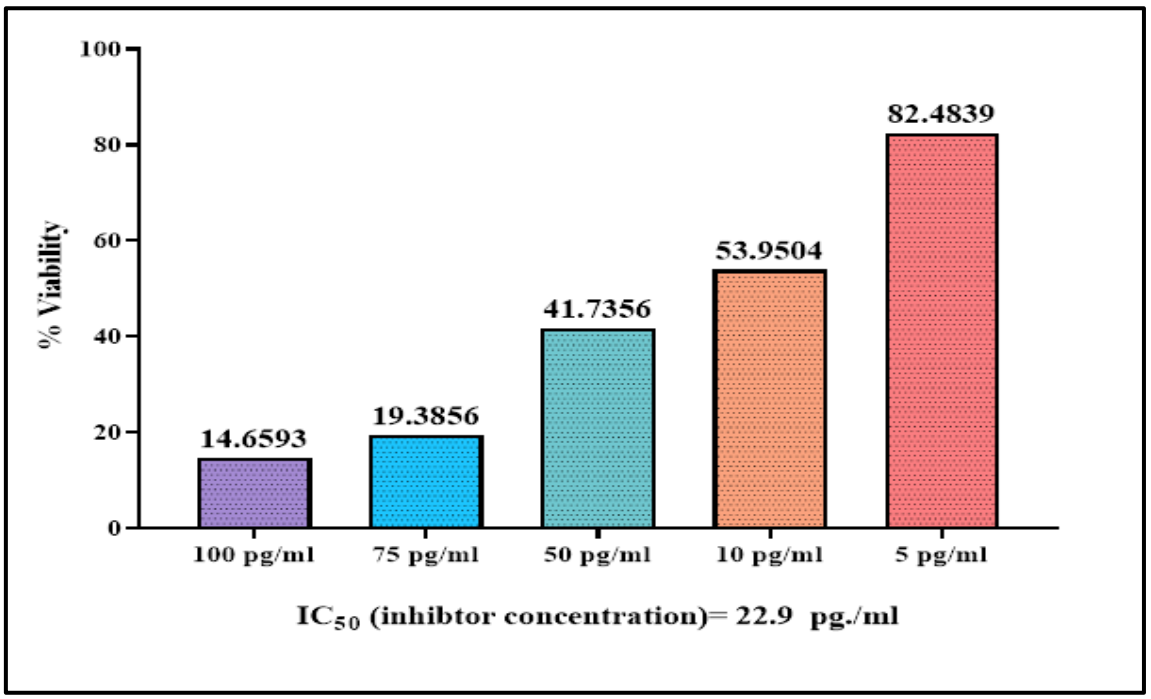


**Fig. 1.** Cytotoxicity percentage and $LC_{50}$ value of IL-24 against primary large cell lung carcinoma cells.

**LC3 and Caspase-9 Gene Expression in Large Cell Lung Carcinoma**

To investigate the molecular mechanisms underlying IL-24-induced cell death in primary large cell lung carcinoma cells, the gene expression levels of LC3 (an autophagy marker) and caspase-9 (an intrinsic apoptosis initiator) were quantified via RT-qPCR following treatment with different concentrations of IL-24 (10, 50, and 100 pg/mL). The analysis of LC3 gene expression revealed a statistically significant, concentration-dependent upregulation across all treated groups compared to the untreated control cells (Fig. 2). This progressive increase indicates a robust activation of the autophagic pathway even at the lowest administered dose.

Conversely, the apoptotic response showed a distinct activation threshold; caspase-9 expression at the lowest concentration of 10 pg/mL did not show a significant difference compared to the control group. However, treatment with higher concentrations (50 and 100 pg/mL) induced a highly significant increase in caspase-9 transcriptional activity (Fig. 3). The most significant elevation for both genes was consistently recorded at the maximum concentration of 100 pg/mL. The recent findings demonstrate that while autophagy is triggered at lower thresholds, higher concentrations of IL-24 drive a concurrent, dose-dependent enhancement of both autophagic and apoptotic pathways in primary large cell lung carcinoma cells.

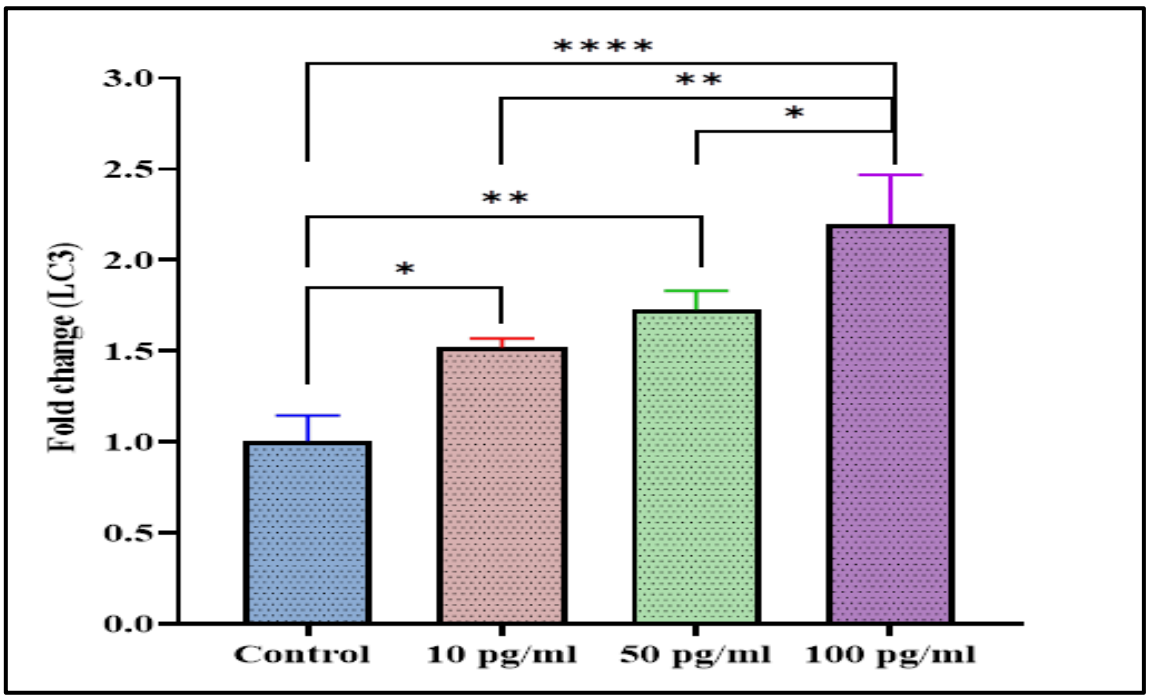


**Fig. 2.** Effect of IL-24 on LC3 Expression in Large Cell Lung Carcinoma Cells at Different Concentrations.

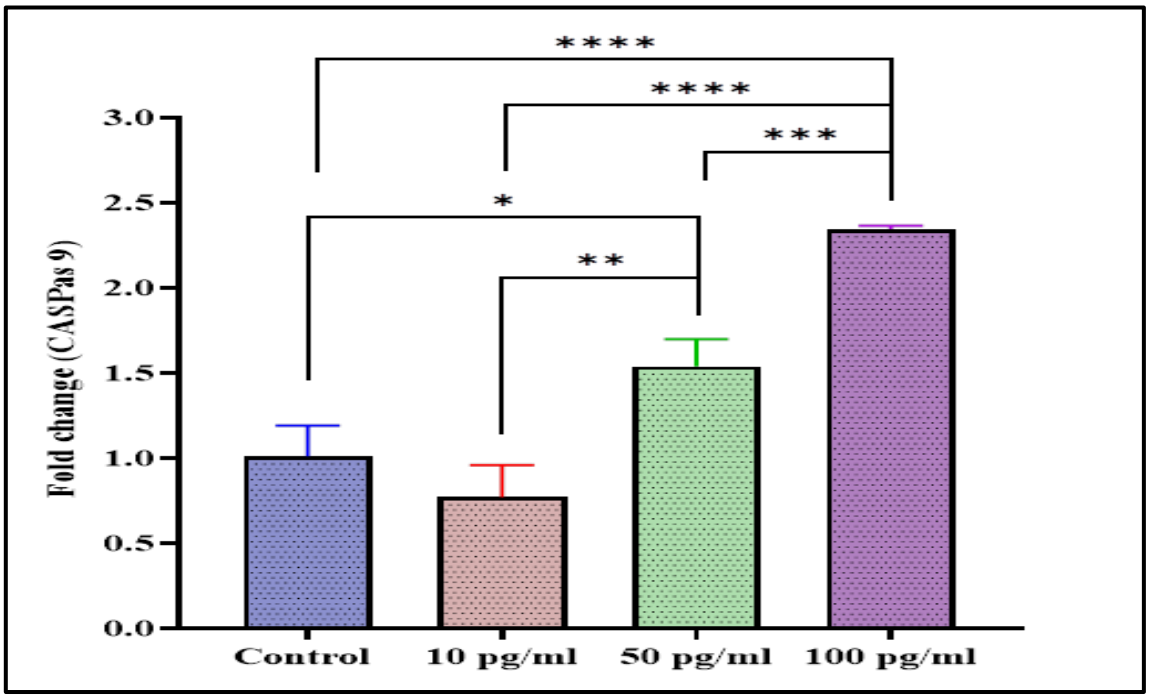


**Fig. 3.** Effect of IL-24 on Casp 9 Expression in Large Cell Lung Carcinoma Cells at Different Concentrations.

## DISCUSSION

IL-24 (MDA-7) was selected as the therapeutic agent in this study based on its established ability to selectively kill cancer cells by converting autophagy into a lethal process. IL-24 engages its receptors to activate a ROS-dependent signaling cascade that upregulates Beclin-1, initiating toxic autophagy, while calpain-mediated cleavage of ATG5 drives the critical shift from cytoprotective to cytotoxic autophagy [7]. In the present study, gene expression profiling of

large cell lung carcinoma cells exposed to IL-24 at 10, 50, and 100 pg/mL alongside untreated controls revealed a clear dose-dependent increase in LC3 expression, significant only at 50 and 100 pg/mL, indicating that IL-24 overwhelms the autophagic survival threshold exclusively at higher doses. As a well-recognized autophagosome membrane marker, LC3 upregulation faithfully captures the escalating autophagic activity driven by IL-24, where LC3-II accumulation was shown to precede the onset of terminal apoptosis [13].

Excessive autophagy is linked to apoptosis through ATG4 and ATG5, which function as key molecular switches. Calpain cleaves ATG5 into a 24-kDa fragment that redirects autophagic signaling toward apoptosis, and inhibition of autophagy with 3-methyladenine enhances adenoviral MDA-7/IL-24–induced apoptosis, suggesting that autophagy is initially protective before becoming cytotoxic [10]. In addition, calpain-mediated ATG5 truncation commits cells to mitochondrial apoptosis in response to anticancer stimuli [14]. Once this switch occurs, BAX is released from BCL-2 sequestration. IL-24 suppresses BCL-2 expression, whereas BCL-2 overexpression reduces sensitivity to IL-24-induced cell death [8]. MDA-7/IL-24 also promotes mitochondrial dysfunction by downregulating BCL-2 and BCL-xL and upregulating BAX and BAK, thereby triggering cytochrome c release and caspase-9 activation [7]. Moreover, IL-24 suppresses pro-survival proteins such as Mcl-1, further shifting the intracellular balance toward apoptosis [15].

The activation of this intrinsic apoptotic pathway is directly noted in the gene expression data of recent study, where caspase-9 co-elevates with LC3 specifically at 50 and 100 pg/mL while remaining low at 10 pg/mL and in the untreated control, forging a clear molecular link between IL-24-induced autophagic excess and intrinsic apoptosis. Critically, these molecular events are functionally confirmed by MTT cytotoxicity data: untreated cells maintained ~100% viability with no LC3 or caspase-9 induction; at 5 pg/mL, viability remained relatively high at ~82.5%, consistent with a sub-threshold response; at 50 pg/mL, concurrent with significant gene upregulation, viability fell to ~41.7%; and at 100 pg/mL, where both genes peaked, viability collapsed to ~14.7%, producing an LC50 of 22.9 pg/mL. The quantitative agreement between gene expression changes and cell death magnitude across all treatment conditions provides compelling evidence of a coherent, concentration-driven autophagy-to-apoptosis axis. The therapeutic potency of IL-24 in lung cancer specifically is further documented by Panneerselvam et al. (2020), who reported analogous growth inhibition and DNA damage responses in lung cancer models [9].

These findings are consistent with evidence from multiple cancer models that support the same mechanism. In oral squamous cell carcinoma, IL-24 induced LC3-II-associated autophagy and that autophagy inhibition with 3-methyladenine enhanced apoptosis, as measured by the MTT assay [16]. In glioblastoma, adenoviral IL-24 suppressed proliferation and induced apoptosis through LC3-II-dependent autophagy and caspase activation, confirmed by MTT assay and flow cytometry [17]. Emdad et al. (2019) concluded that MDA-7/IL-24 exerts antiproliferative effects through cytotoxic autophagy and intrinsic apoptosis, accompanied by increased LC3 processing and reduced MCL-1, BCL-xL, and BCL-2 expression [7]. The clinical relevance of targeting autophagic thresholds to overcome chemoresistance is also supported by the meta-analysis of Xu et al. (2018) [18]. Overall, the molecular and cytotoxic evidence in this study supports IL-24 as a strong inducer of excessive autophagy-driven intrinsic apoptosis in large cell lung carcinoma, with LC3 and caspase-9 acting as closely linked markers of cell death, consistent with the marked loss of viability observed at 100 pg/mL.

## CONCLUSION

In conclusion, IL-24 exerted concentration-dependent cytotoxic effects on large cell lung carcinoma cells by promoting excessive autophagy-linked apoptosis. This was evidenced by the concurrent upregulation of LC3 and caspase-9 at 50 and 100 pg/mL, indicating activation of the ATG4/ATG5-regulated autophagy-apoptosis switch and the intrinsic mitochondrial pathway. These molecular changes were supported by MTT assay results, which showed a progressive reduction in cell viability to 14.7% at 100 pg/mL and an LC50 of 22.9 pg/mL. Together, these findings identify IL-24 as a promising anticancer agent that merits further in vivo evaluation.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

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## REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, CA: A Cancer J Clinic. 2021;71(3):209–249. https://doi.org/10.3322/caac.21660
2. Fasano M, Della Corte CM, Papaccio F, Ciardiello F, Morgillo F. 'Pulmonary Large-Cell Neuroendocrine Carcinoma: From Epidemiology to Therapy J Thorac Oncol, 2015;10(8):1133–1141. https://doi.org/10.1097/JTO.0000000000000589
3. Oun R, Moussa YE, Wheate NJ. 'The side effects of platinum-based chemotherapy drugs: a review for chemists', Dalton Transac. 2018;47(19):6645–6653. https://doi.org/10.1039/c8dt00838h
4. Thorburn A, Thamm DH, and Gustafson DL. Autophagy and cancer therapy. Molecular Pharmacol. 2014;85(6):830–838. https://doi.org/10.1124/mol.114.091850
5. Waldmann TA. Cytokines in Cancer Immunotherapy, Cold Spring Harbor Perspectives. Biol. 2018;10(12):a028431. https://doi.org/10.1101/cshperspect.a028472
6. Berraondo P, Sanmamed MF, Ochoa MC, Etxeberria I, Aznar MA, Pérez-Gracia JL, Rodríguez-Ruiz ME, Ponz-Sarvise M, Castañón E, and Melero I. Cytokines in clinical cancer immunotherapy. British J Canc. 2019;120(1):6–15. https://doi.org/10.1038/s41416-018-0328-y
7. Emdad L, Bhutia SK, Das SK, Sarkar D, and Fisher PB. Recent insights into apoptosis and toxic autophagy: the roles of MDA-7/IL-24, a multidimensional anti-cancer therapeutic. Seminars Canc Biol. 2019;66:140–154. https://doi.org/10.1016/j.semcancer.2019.07.013
8. Hu Z, Zhang Z, Guise T. and Seth P. MDA-7/IL-24 induces Bcl-2 denitrosylation and ubiquitin-degradation involved in cancer cell apoptosis. PLoS ONE. 2012;7(5):e37200. https://doi.org/10.1371/journal.pone.0037200
9. Panneerselvam J, Shanker M, Jin J, Branch CD, Muralidharan R, Zhao YD, Archibald SJ, Hubin TJ, Bhutia SK, and Ramesh R. IL-24 inhibits lung cancer growth by suppressing GLI1 and inducing DNA damage. Cancers. 2020;12(1):84. https://doi.org/10.1371/journal.pone.0122439
10. Bhutia SK, Dash R, Das SK, Azab B, Su ZZ, Lee SG, Grant S, Yacoub A, Dent P,

Curiel DT, Sarkar D, and Fisher PB. Mechanism of autophagy to apoptosis switch triggered in prostate cancer cells by antitumor cytokine melanoma differentiation-associated gene 7/interleukin-24. Cancer Res. 2010;70(9):3667–3676. https://doi.org/10.1158/0008-5472.CAN-09-3647

11. Johnson JI, Decker AM, Zaharevitz D, Rubinstein LV et al., 'Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials', British J Canc. 2001;84(10):1424–1431. https://doi.org/10.1054/bjoc.2001.1796

12. Gazdar AF, Girard L, Lockwood WW, Lam WL, Minna JD. Lung cancer cell lines as tools for biomedical discovery and research. J Nation Can Instit. 2010;102(17):1310-21. https://doi.org/10.1093/jnci/djq279

13. Bhutia SK, Das SK, Azab B, Dash R, Su ZZ, Lee SG, Dent P, Curiel DT, Sarkar D, and Fisher PB. Autophagy switches to apoptosis in prostate cancer cells infected with melanoma differentiation associated gene-7/interleukin-24 (mda-7/IL-24). Autophagy. 2011;7(9):1076–1077. https://doi.org/10.4161/auto.7.9.16163

14. Lian F, Zhong Y, Liu J, Chen X, Huang M, Zhang X, and Li J. The role of autophagy in cancer: from molecular mechanism to therapeutic window', Front Immunol. 2025;16:1528230. https://doi.org/10.3389/fimmu.2025.1528230

15. Park MA, Yacoub A, Sarkar D, Emdad L, Rahmani M, Spiegel S, Koumenis C, Graf M, Curiel DT, Grant S, Fisher PB. PERK-dependent regulation of MDA-7/IL-24-induced autophagy in primary human glioma cells. Autophagy. 2008;4(4):513-5. https://doi.org/10.4161/auto.5725

16. Li J, Yang D, Wang W, Piao S, Zhou J, Saiyin W, Zheng C, Sun H, Li Y. Inhibition of autophagy by 3-MA enhances IL-24-induced apoptosis in human oral squamous cell carcinoma cells. J Experim Clinic Cancer Res. 2015;34(1):97. https://doi.org/10.1186/s13046-015-0211-0

17. Babazadeh SM, Zolfaghari MR, Zargar M, Baesi K, Hosseini SY, and Ghaemi A. Interleukin-24-mediated antitumor effects against human glioblastoma via upregulation of P38 MAPK and endogenous TRAIL-induced apoptosis and LC3-II activation-dependent autophagy. BMC Cancer. 2023;23(1):519. https://doi.org/10.1186/s12885-023-11021-y

18. Xu R, Ji Z, Xu C, and Zhu J. The clinical value of using chloroquine or hydroxychloroquine as autophagy inhibitors in the treatment of cancers: a systematic review and meta-analysis. Medicine. 2018;97(46):e12912. https://doi.org/10.1097/MD.0000000000012912