Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/2025.24205

Evaluating the Efficacy of Astragaloside IV and Neferine Combination on Lung Cancer Through Inactivation of the Wnt/β-Catenin Signaling Pathway

Yichen Mi

Southlands Christian School, Rowland Heights, USA
miyichen887@gmail.com

Abstract: Lung cancer is a serious disease that ends numerous lives throughout the world. Surgery and chemotherapy are often used in treating cancer nowadays, but they are overly aggressive especially for the elderly and can result in unsatisfactory outcomes like painful side-effects, drug resistance, etc. With the modernization of traditional Chinese medicine (TCM) and increasing evidence that indicates TCM has essential anticancer components. Astragaloside IV and Neferine are considered as two main effective ingredients in *Astragalus membranaceus* and *Nelumbo nucifera. Astragalus membranaceus* and *Nelumbo nucifera have* traditionally been used for various diseases such as fever, inflammation, insomnia, nervous disorders, et al. Thus, as TCM, Astragaloside IV and Neferine can be a potential new weapon for lung cancer which has much less side-effects. In this research, we studied the effect of Astragaloside IV and Neferine on the Wnt /β-catenin signaling pathway which is closely related to the occurrence of lung cancer.

Keywords: Lung cancer, Astragaloside IV, Neferine, Wnt /β-catenin signaling pathway

1. Introduction

Lung cancer is classified as one kind of chronic non-communicable disease with a bad prognosis, which can be further divided into two main divisions: small-cell lung cancer and non-small-cell lung cancer. Almost 80% of the patients with lung cancer could be diagnosed with NSCLC, and only 10%–15% of the patients can survive for 5 years or longer [1]. According to the Cancer Statistics Center of the American Cancer Society, lung cancer has the highest mortality rate among all cancers, with way ahead deaths [2,3]. Currently, lung cancer is treated using chemotherapy. Though considered one of the strongest treatments in killing cells that cause cancer, it results in several side effects like extreme exhaustion, loss of appetite, and also falling off of hair. Results in most cases are always disappointing; therefore, a dire need to develop new effective approaches in fighting lung cancer.

Traditional Chinese medicine (TCM) is a subset of traditional medicine with more than 3500 years of history of Chinese medical practice. TCM has been traditionally used to treat many diseases, including cancer in China. Although the mechanism of TCM has not been fully researched, TCM is a trend and more scientists are interested in the effectiveness of TCM. Based on research, TCM can be a promising therapy for lung cancer [4]. Neferine, a natural product extracted from plants, is considered a potential candidate for cancer treatment [5]. Astragaloside-IV, a key compound derived from *Astragalus membranaceus* Bunge, has been shown to exert antitumor effects by inhibiting cell

DOI: 10.54254/2753-8818/2025.24205

proliferation, invasion, and metastasis across different types of cancer [6]. In this research, we will evaluate specifically on the effect of Astragaloside IV and Neferine extract from The plant *Nelumbo nucifera and Astragalus membranaceus* on lung cancer.

Since the first member of the Wnt family was identified in 1982,[7] studies on Wnt signaling have been steadily increasing. Notably, the Wnt/β-catenin pathway, implicated in regulating embryonic development and the regeneration of adult tissue homeostasis, is strongly related to disease when dysregulated and therefore might be a promising target for therapy. Many signaling pathways have been implicated in the initiation and development of LC, among which one of the most critical is the Wnt ligand/β-catenin signaling pathway [8]. Pathologically activated Wnt/β-catenin signaling pathways mediate β-catenin's nuclear accumulation, bringing about an overexpression of transcription for a lot of oncogenes, such as c-Myc and CyclinD-1, and ultimately leading to carcinogenesis and development. The cause of abnormal Wnt/β-catenin pathway activation results in the disordered expression and transcription of the related proteins and genes, promoting proliferation, differentiation, migration, and invasion, as well as resistance to apoptosis of lung cancer cells [9]. In this research, we aim to study the effect of Astragaloside IV and Neferine combination on lung cancer by inactivating or blocking the Wnt /β-catenin signaling pathway.

2. Hypothesis

I predict that increasing concentration of Astragaloside IV and Neferine combination compared to either one alone will lead to increasing death of A549 lung cancer cells, reduced tumor size in A549 mouse xenografts, and increased phosphorylation of B-catenin leading to decreased Wnt activated cell proliferation, thus negatively regulating the Wnt/β-catenin pathway.

3. Experimental design

Measuring cell viability by CKK8 Assay and xenograft tumor size by weight or size by caliper. Measure quantity of Phosphorylated β-catenin by western blot. Positive Control: Atezolizumab Negative Control: PBS

4. Methods

4.1. Cell viability measurement by CCK8 assay

The cell viability of cultured cells was assessed using the Cell Counting Kit-8 (CCK8) assay. Cells were seeded in a 96-well plate at a density of 5,000 cells per well and allowed to adhere overnight. After treatment with Astragaloside IV (10 mol, 100 mol, 500 mol, 1000 mol) and a combination of Astragaloside IV and Neferine (5+5 mol, 50+50μL, 250 mol+250 mol, 500+500 μL) for 6 hours, 12 hours, 24 hours, 48hours, 10 μL of CCK8 solution (Dojindo, Japan) was added to each well. The plates were incubated at 37°C for 2 hours. The absorbance at 450 nm was measured using a microplate reader (Bio-Rad, USA). Cell viability was calculated as a percentage relative to the control group, with all assays performed in triplicates [10].

4.2. Measurement of xenograft tumor size by caliper and weight

For in vivo experiments, xenograft tumors were established by subcutaneously injecting 5 × 10^6 cells into the flanks of six-week-old nude mice. Mice were treated with Astragaloside IV (20 mg/kg, 50 mg/kg, 100 mg/kg, 500 mg/kg), and their combinations (10 mg/kg + 10 mg/kg, 25 mg/kg + 25 mg/kg, 50 mg/kg + 50 mg/kg 250 mg/kg+250 mg/kg) daily for 2, 4, and 6 weeks. Tumor growth was monitored by measuring tumor size with a caliper twice a week. Tumor volume (V) was calculated using the formula: V = (length × width^2)/2. At the endpoint of the experiment, mice were euthanized,

DOI: 10.54254/2753-8818/2025.24205

and tumors were excised and weighed. Tumor weight was recorded as a direct measure of tumor burden [11].

4.3. Western blot analysis of phosphorylated β-catenin

To measure the levels of phosphorylated β-catenin, cells were lysed in a RIPA buffer containing protease and phosphatase inhibitors (Sigma-Aldrich, USA). The lysates were centrifuged at 12,000 × g for 15 minutes at 4°C, and the supernatants were collected. Protein concentrations were determined using the BCA protein assay kit (Pierce, USA). Equal amounts of protein (30 μg) were separated by SDS-PAGE on a 10% gel and transferred to PVDF membranes (Millipore, USA). Membranes were blocked with 5% nonfat dry milk in TBS-T (Tris-buffered saline with 0.1% Tween 20) for 1 hour at room temperature and then incubated overnight at 4°C with a primary antibody against phosphorylated β-catenin (Ser33/37/Thr41) (1:1000, Cell Signaling Technology, USA). After washing, membranes were incubated with HRP-conjugated secondary antibodies (1:2000, Cell Signaling Technology, USA) for 1 hour at room temperature. Bands were visualized using an ECL detection kit (GE Healthcare, USA) and quantified using ImageJ software. β-Actin (1:5000, Abcam, UK) was used as a loading control [12].

4.4. Controls and statistical analysis

Control groups were included to validate the experimental outcomes. Positive control for the CCK8 assay and Western blot analysis was Atezolizumab-treated cells, while the negative control was cells treated with PBS.

All experiments were performed in triplicates. Data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using GraphPad Prism 8 software. An unpaired Student's t-test was used for comparing two groups, while one-way ANOVA followed by Tukey's post hoc test was applied for multiple group comparisons. A p-value of less than 0.05 was considered statistically significant.

5. Possible results

Table 1: Possible result

Combination Result # (CR#)	Do Astragaloside IV and Neferine combination decrease viability more than Astragaloside IV by CCK8 assay?	Do Astragaloside IV and Neferine combination decrease tumor size more than Astragaloside IV by xenograft tumor weight?	Do Astragaloside IV and Neferine increase phospho-b-catenin more than Astragaloside IVwestern blot?	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial
5	+	-	-	Partial
6	-	+	-	Partial
7	-	-	+	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates the measurement changes the same as atezolizumab positive control, "-" indicates the measurement changes the same as the DMSO/PBS negative control

DOI: 10.54254/2753-8818/2025.24205

Below is the analysis of the possible results shown in Table 1.

5.1. Combination of possible result 1 (CR1)

Astragaloside IV decreased cell viability on CCK8 assay, but showed a decrease in xenograft tumor weight indicating reduced tumor size than the use of Astragaloside IV alone. Western blot analysis revealed that phosphorylated β-catenin levels were higher after combined drug treatment than with a single drug, Astragaloside IV.

5.2. Combination of possible result 2 (CR2)

The utilization of Astragaloside IV together with Neferine leads to an astonishing reduction in cell viability than when only used Astragaloside IV alone without any other medicine as shown by the CCK8 results. Xenograft Tumor Weight Measurement shows that there is a decrease in tumor size if Astragaloside IV is administered jointly with Neferine but not when it is used singly. Unlike astragalosides monotherapy, western blot analysis does not show an increase in phosphorylated β-catenin levels following combination drug treatment.

5.3. Combination of possible result 3 (CR3)

Neferine combined with Astragaloside IV significantly decreases cell viability compared to using Astragaloside IV alone according to CCK8 assay result. However, tumor size does not lower down measured by xenograft tumor weight when both drugs are given instead of using only one which is Astragaloside IV. Phosphorylated β-catenin levels increase more after combination drug treatment than for those treated with only Astragaloside IV as indicated by western blot analysis.

5.4. Combination of possible result 4 (CR4)

CCK8 test outcomes show no significant decrease in cell viability when both drugs are used together; Astragaloside IV and Neferine. They should have been compared to Astragaloside IV alone. Also, a reduction in tumor size was observed from xenograft tumor weight upon combined administration of Astragaloside IV and Neferine relative to its use alone. However, none of them displayed any increase in phosphorylated β-catenin levels from combined drug administration unlike those by Astaxanthin itself according to Western blotting analysis.

5.5. Combination of probable result 5 (CR5)

The outcomes of the CCK8 assay also indicate that Astragaloside IV alone use resulted in much fewer viable cells than its combination with Neferine. Also, combined drug treatment using Astragaloside IV and Neferine did not reduce tumor size any more than Astragaloside IV alone when measured by xenograft tumor weight. In addition, Western blotting shows no increase in phosphorylated β-catenin levels due to combined drug therapy, unlike in the case of using only Astragaloside IV.

5.6. Combination of likely result 6 (CR6)

No CCK8 assays have shown significant drops on cell viability through treatment with both drugs at once; However this is not true for Astragaloside IV and Neferine which are found to be less effective when used together compared to when they are used individually. From xenograft tumor weight a reduction in tumor size can be observed after combining administration of Astragaloside IV and Neferine as opposed to its application alone. But there was no increase in phosphorylated β-catenin

DOI: 10.54254/2753-8818/2025.24205

levels from combined drug administration as revealed by Western blot analysis unlike those by Astaxanthin itself.

5.7. Combination of probable result 7 (CR7)

According to CCK8 assay results, there is no significant decrease in cell viability with the combined drug treatment (Astragaloside IV & Neferine) as against only using Astragaloside IV alone. Measurement of xenograft tumor weight does not show any decrease in tumor size with the combined drug treatment compared to only using Astragaloside IV alone. Moreover, Western blot analysis performed on these samples demonstrated an increase in levels of phosphorylated β-catenin when treated with combination rather than treating only with astragaloside IV.

5.8. Combination of possible result 8 (CR8)

Results from CCK8 assay reveal that there is no significant reduction in cell viability for combined drug treatment containing Astragaloside IV and Neferine as compared to astragaloside iv alone. Measurement of xenograft tumor weight indicates that there is no decrease in tumor size after combining drugs used as opposed to astragaloside iv alone. On the other hand, Western blot fails to show any reduction on phosphorylated β-catenin level despite combining both compounds but it only does so for one compound, i.e., astragaloside IV.

6. Discussion

CR1 states that when Astragaloside IV and Neferine are combined, there is a drop in cell viability; tumor size shrinks while phosphorylated β-catenin levels increase than when Astragaloside IV alone is used. It fully supports the hypothesis and the combination effectively inhibits Lung Cancer cell growth. It is highly possible that the Increased phosphorylation of β-catenin reduces the accumulation of β-catenin and transcription of oncogenes, such as c-Myc and CyclinD-1. Thus inhibited the cancer cell proliferation. Cell viability may decrease as the concentration of Astragaloside IV and Neferine increases. This indicates that the cell death is concentration-dependent and supports the hypothesis that higher drug concentrations are more effective at inhibiting cancer cell proliferation and growth. Cell viability may decrease as the treatment duration with Astragaloside IV and Neferine increases. This indicates that the cell death is treatment duration-dependent, supporting the hypothesis that prolonged exposure enhances drug efficacy. The future study shall focus on different concentrations to determine the optimal combination for clinical use.

CR2 reveals that while Astragaloside IV and Neferine combination alone does not increase phosphorylated β-catenin levels, it significantly reduces cell viability as well as tumor size. This supports the idea that anti-tumour effects can be enhanced through combination therapy. Cell viability may decrease as the concentration of Astragaloside IV and Neferine increases. This indicates that the cell death is concentration-dependent and supports the hypothesis that higher drug concentrations are more effective at inhibiting cancer cell proliferation and growth. Cell viability may decrease as the treatment duration with Astragaloside IV and Neferine increases. This indicates that the cell death is treatment duration-dependent, supporting the hypothesis that prolonged exposure enhances drug efficacy. However, lack of increase in β-catenin signaling implies involvement of other alternative pathways like PI3K/AKT/mTOR pathway for observed efficacy and to detect the level of phospho AKT and mTOR. Therefore, more studies need to be done investigating these alternative mechanisms and also evaluate long-term effects on resistance development or growth inhibition of such combined treatments.

CR3 indicates a significant decrease in cell viability following treatment with astragaloside iv Neferine combination however no significant reduction was observed in terms of tumor size but

DOI: 10.54254/2753-8818/2025.24205

increased levels were seen regarding phospho β-catenin. It partially supports the hypothesis. Cell viability may decrease as the concentration of Astragaloside IV and Neferine increases. This indicates that the cell death is concentration-dependent and supports the hypothesis that higher drug concentrations are more effective at inhibiting cancer cell proliferation and growth, but the tumor size didn't reduce as the treatment duration with Astragaloside IV and Neferine increases. This indicates that the cell death is treatment duration-dependent, supporting the hypothesis that prolonged exposure enhances drug efficacy. The result might mean that the drugs did not effectively reach the tumor in sufficient concentrations due to poor vascularization of tumor, dense extracellular matrix, high interstitial fluid pressure, etc. The future research can focus on investigating different strategies to deliver Astragaloside IV and Neferine, such as using liposomes or niosome as drug carriers.

CR4 shows that there was no significant decrease in terms of cell viability but still registered a decline in tumor size and increase in levels of phosphorylated β-catenin with combination between Astragaloside IV and Neferine meaning this therapy may act mainly in vivo possibly through activating immune system or systemic effect which do not directly affect cell death in vitro. The upregulation of β-catenin signaling also indicates involvement during anti cancer effects witnessed. Cell viability may not change as the concentration of Astragaloside IV and Neferine increases. This suggests that the drug combination does not affect cell proliferation at the tested concentrations or that the cells have developed resistance. Cell viability may decrease as the treatment duration with Astragaloside IV and Neferine increases. This indicates that the cell death is treatment duration-dependent, supporting the hypothesis that prolonged exposure enhances drug efficacy. More investigation ought to be investigating the tumor reduction in vivo mechanisms, such as immune system activation, changes in the tumor microenvironment, and systemic effects that contribute to tumor reduction.

CR5 indicates that a combination of Astragaloside IV and Neferine brings a considerable effect on cell viability in a dose-dependent manner in vitro. However, tumor sizes reduce, and the levels of phosphorylated β-catenin increase. This suggests that either the drugs are not effectively delivered to the tumor tissue or alternative pathways, such as the PI3K/AKT/mTOR pathway, are involved. As the concentration of Astragaloside IV and Neferine increases, cell viability decreases, supporting the hypothesis that higher drug concentrations can effectively inhibit cancer cell growth and proliferation. The viability of cells subjected to prolonged treatment with Astragaloside IV and Neferine does not seem to be affected, indicating that a longer incubation period may not be necessary for the drug combination to impact viability, or the cells may develop survival mechanisms against extended exposure. Future research will focus on improving drug delivery using different carriers, such as liposomes or niosomes, and investigating other potential pathways, like the PI3K/AKT/mTOR pathway, through western blot analysis.

The claim for CR6 is that there was no significant fall in cell viability, yet shrinkage in tumor size with no change in the levels of phosphorylated β-catenin post-treatment. The partial confirmation of the hypothesis here would seem to indicate that the Astragaloside IV-Neferine combination may affect tumor growth by pathways other than through the killing of the cancerous cells per se. The increased concentration of Astragaloside IV and Neferine could be lowering the viability of cells, which may mean that the death is concentration dependent, hence supporting the hypothesis that the more the concentration of the drug, the more the retardation of the growth and proliferation of the cancerous cells. Increased duration of treatment may not alter cell viability by Astragaloside IV and Neferine treatment. Other mechanisms may thus be by immune modulation or disruption in the tumor microenvironment. Further experiments will therefore be directed to other pathways of investigation, other immune responses, and changes in the tumor microenvironment as a mechanism of action for the reduced sizes observed in tumors.

DOI: 10.54254/2753-8818/2025.24205

CR7 presents no significant decline in cell viability or tumor size increase in phosphorylated β-catenin levels. Thus, this supports partially the hypothesis that while the combination of Astragaloside IV and Neferine caused an alteration in the level of phosphorylated β-catenin, it still did not result in diminished viability of cancer cells or tumor size reduction. Since the Wnt/β-catenin signaling pathway is an established reliable target in treating lung cancer, mechanisms of resistance to drugs may develop in tumor cells and allow them to survive, thus sustaining tumor volume. In such a case, an increase in the concentration of both Astragaloside IV and Neferine would affect cell viability. It follows therefore that a combination of the drugs at concentrations tested was without effect on cell proliferation, or the cells have simply acquired resistance. Increased treatment time with Astragaloside IV and Neferine may thus not change cell viability. That would mean that this combination does not take longer to disclose its action on cell viability, or the cells have devised ways to survive the extended treatment. Thus, the search for possible mechanisms of resistance, including efflux pumps, alterations in metabolic pathways, or changes in drug targets, shall be the focus of future studies.

Furthermore, there was no significant loss in cell viability, tumor size, or rise in the level of phosphorylated β-catenin after combined treatment with Astragaloside IV and Neferine in CR8, exactly opposite to what was hypothetically speculated. Thus, this suggests that the experimental conditions are not sufficient to affect either the Wnt/β-catenin signaling pathways or the survival and proliferation of malignant cells. More specifically, viability may not change as the concentrations of Astragaloside IV and Neferine are increased. The combination of drugs, within the applied concentration, has not influenced the proliferation of cells; either the cells may acquire resistance. There may not be a difference in the viability of the cells even after the prolonged period of the treatment with Astragalus IV and Neferine. This, in turn, means that the combination does not require extended exposure to viability or that the cells have developed mechanisms for survival in response to such long treatments. Future studies will be directed toward other possible drug combinations and dosing, along with investigating other targets relevant to the strategies of lung cancer treatment.

7. Conclusion

Now Astragaloside IV and Neferine as TCM can offer a new therapy with less side-effects for lung cancer, leading developments and improvements in treatment of cancer. Future research may focus on the application of Astragaloside IV and Neferine on other cancers and investigation of similar mechanisms.

References

[1] *C.J Beadsmoore, N.J Screaton,(Jan, 2003) Classification, staging and prognosis of lung cancer, 45(1) P8-17 DOI: 10.1016/s0720-048x(02)00287-5*

[2] *American Cancer Society, explore cancer statistics, https://cancerstatisticscenter.cancer.org/?_ga=1.105656131.1125562482.1469021664#/*

[3] *American Cancer Society, explore cancer statistics, https://cancerstatisticscenter.cancer.org/?_ga=1.105656131.1125562482.1469021664#/*

[4] *Te-Mao Li, Yang-Hao Yu, Fuu-Jen Tsai, Chi-Fung Cheng, Yang-Chang Wu (March 2018) Characteristics of Chin ese herbal medicine usage and its effect on survival of lung cancer patients in Taiwan 213 https://doi.org/10.1016/j.jep.2017.10.031*

[5] *Dasari, Subramanyam et al. "Neferine Targets the Oncogenic Characteristics of Androgen-Dependent Prostate Cancer Cells via Inducing Reactive Oxygen Species." International journal of molecular sciences vol. 24,18 14242. 18 Sep. 2023, doi:10.3390/ijms241814242*

[6] *Zhou, Liangxing et al. "Anticancer effects and mechanisms of astragaloside-IV (Review)." Oncology reports vol. 49,1 (2023): 5. doi:10.3892/or.2022.8442*

DOI: 10.54254/2753-8818/2025.24205

[7] *Roel Nusse, Harold E. Varmus, (November 1982) Many tumors induced by the mouse mammary tumor virus conta in a provirus integrated in the same region of the host genome, 31(1), 99-109 https://doi.org/10.1016/0092-8674(8 2)90409-3*

[8] *Liu, J., Xiao, Q., Xiao, J. et al. Wnt/β-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Sig Transduct Target Ther 7, 3 (2022). https://doi.org/10.1038/s41392-021-00762-6*

[9] *W. Zhu, H. Wang, D. Zhu Wnt/β-catenin signaling pathway in lung cancer Med. Drug. Disc., 13 (2022), p. 100113, 10.1016/j.medidd.2021.100113*

[10] *Cai, Ling et al. "Comparison of Cytotoxicity Evaluation of Anticancer Drugs between Real-Time Cell Analysis and CCK-8 Method." ACS omega vol. 4,7 12036-12042. 11 Jul. 2019, doi:10.1021/acsomega.9b01142*

[11] *Liu, Yihan et al. "Patient-derived xenograft models in cancer therapy: technologies and applications." Signal transduction and targeted therapy vol. 8,1 160. 12 Apr. 2023, doi:10.1038/s41392-023-01419-2*

[12] *Mahmood, Tahrin, and Ping-Chang Yang. "Western blot: technique, theory, and troubleshooting." North American Journal of Medical Sciences vol. 4,9 (2012): 429-34. doi:10.4103/1947-2714.100998*