

Curcumin inhibits the growth via Wnt/ β -catenin pathway in non-small-cell lung cancer cells

J.-Y. WANG¹, X. WANG², X.-J. WANG³, B.-Z. ZHENG¹, Y. WANG²,
X. WANG⁴, B. LIANG^{2,5}

¹Department of Radiotherapy, Shanxi Province Tumor Hospital, Taiyuan, China

²Key Laboratory of Cardiovascular Medicine and Clinical Pharmacology of Shanxi Province, Taiyuan, China

³Oncology Department, Shanxi Province Hospital of Traditional Chinese Medicine, Tai Yuan, China

⁴Department of Respiration, The Second Hospital of Shanxi Medical University, Taiyuan, China

⁵Department of Cardiology, The Second Hospital of Shanxi Medical University, Taiyuan, China

Abstract. – OBJECTIVE: In recent decades, the death rate from lung cancer appears to be an increasing yearly trend, particularly for non-small-cell lung cancer (NSCLC). Curcumin is a yellow pigment found in turmeric rhizomes, reported to exhibit various anti-inflammatory, anti-angiogenic, anti-proliferative, and antioxidant properties. Many reports have suggested that curcumin could induce apoptosis in malignant cells, and therefore, has great potential in tumor treatment. However, little is known about the effect of curcumin on NSCLC or its associated mode of action. Therefore, in this study, we explored curcumin's effect on NSCLC and investigated its associated mechanism.

MATERIALS AND METHODS: The non-small-cell lung cancer (NSCLC) cell line A549 was cultured and subjected to MTT and clonogenic survival assays to assess cell proliferation. Reactive oxygen species (ROS) levels were measured using a Fluostar Omega Spectrofluorimeter. Superoxide dismutase (SOD) and γ -glutamyl cysteine synthetase (γ -GCS) activity in A549 cells were both determined by a commercial determination kit. Expression levels of p-GSK3 β (Ser9), c-Myc, cyclin D1, β -catenin α -tubulin, and proliferating cell nuclear antigen (PCNA) were analyzed by Western blot.

RESULTS: Results of the MTT and clonogenic survival assay indicated that curcumin reduced A549 proliferation. ROS levels and SOD and γ -GCS activities were detected. Curcumin decreased intracellular ROS levels and increased SOD and γ -GCS activity. Meanwhile, the ROS inhibitor N-Acetylcysteine (NAC) reversed the decrease in ROS levels and the increase in SOD and γ -GCS activity. These results indicate that oxidative stress is involved in the curcumin-induced reduction of A549 viability. Curcumin also strongly inhibited β -catenin and p-GSK3 β (Ser9) protein expression, as well as the expres-

sion of downstream cyclin D1 and c-Myc. Similarly, NAC reversed the inhibition of β -catenin and p-GSK3 β (Ser9) protein expression, as well as the expression of downstream cyclin D1 and c-Myc.

CONCLUSIONS: We showed that curcumin inhibits NSCLC proliferation via the Wnt/ β -catenin pathway.

Key Words:

Non-small-cell lung cancer cell line (NSCLC), Curcumin, Proliferation, Oxidative stress, Wnt/ β -catenin signaling pathway.

Introduction

Lung cancer is statistically one of the most common diseases worldwide, and presents a serious threat to human health and life^{1,2}. In recent decades, lung cancer death rates have increased yearly in an apparent trend, particularly non-small-cell lung cancer (NSCLC)³. NSCLC is the most common type of lung cancer. At present, chemotherapy is one of the most common methods for NSCLC treatment^{4,5}. However, many chemotherapeutic drugs produce debilitating side effects on the human body, and hence, are limited in clinical application.

Recent attention has focused on phytochemicals as anticancer agents. Curcumin is a yellow pigment found in turmeric rhizomes, reported to exhibit various anti-inflammatory, anti-angiogenic, anti-proliferative, and antioxidant properties. More importantly, it was found to not induce cytotoxic effects in healthy cells^{6,7}. Many reports^{8,9} have suggested that curcumin may induce

apoptosis in malignant cells, indicating potential use in the treatment of tumors. Apoptosis is a programmed cell death process related to tumor malignancy, and is very important in embryonic development and tissue homeostasis. Little is currently known about the effects of curcumin on NSCLC, or the mechanism through which such effects would be induced. Therefore, in this study, we explored the effects of curcumin on NSCLC and investigated the associated mechanisms.

Oxidative stress is one of the most common causes of cellular damage, mostly due to the build-up of reactive oxygen species (ROS) free radicals through impairments to antioxidative enzymes, such as superoxide dismutase (SOD). Dysregulated oxidative metabolism may lead to cell death, an important reason for increased proliferation, with several studies¹⁰⁻¹² showing the involvement of oxidative stress in cell proliferation. Therefore, we explored the relationship between oxidative stress and cell proliferation in curcumin-treated NSCLC cells.

The Wnt/ β -catenin pathway determines the development of vertebrates and invertebrates by regulating cell destiny¹³. Wnt is a secreted glycoprotein which binds to Frizzled receptors. β -catenin, which is deregulated in many cancers, is a multifunctional adaptor protein/transcription factor. In the absence of Wnt binding (off-state), β -catenin is downregulated via a degradation complex including GSK-3, CK1, Axin, APC, and PP2A^{14,15}. Previous studies have reported that the Wnt/ β -catenin signaling pathway is abnormally active in hepatic carcinoma¹⁶. However, little is known about the mechanism of Wnt/ β -catenin signaling in lung cancer cells.

In this study, we explored the effect of curcumin on proliferation in NSCLC A549 cells and investigated its associated mechanism. Results indicated that curcumin could inhibit NSCLC proliferation induced by oxidative stress-mediated upregulation of the Wnt/ β -catenin pathway.

Materials and Methods

Materials

Dulbecco's Modified Eagle Medium (DMEM) and fetal bovine serum (FBS) were obtained from GIBCO (Grand Island, NY, USA). Penicillin/streptomycin, pancreatin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Dimethyl sulfoxide (DMSO) and

Hoechst 33342 were purchased from Sigma-Aldrich (St. Louis, MO, USA). The scavenger of ROS (N-acetyl-L-cysteine, NAC), BCA protein kit, Western lysis buffer and enhanced chemiluminescence kit were from Beyotime Biotechnology (Shanghai, China). β -catenin and Cyclin D1 antibodies were obtained from Abcam (Cambridge Science, London, UK). Antibodies for phosphor-GSK3 β (Ser 9) and c-Myc were purchased from BBI (Sangon Biotech, Shanghai, China). α -tubulin and PCNA were from Sigma-Aldrich (St. Louis, Mo, USA).

Cell Culture and Drug Treatments

Non-small-cell lung cancer cell line (A549) was purchased from the Institute of Biochemistry and Cell Biology (SIBS, CAS, Shanghai, China). A549 was cultured in DMEM medium (Grand Island, NY, USA). Media was supplemented with 10% FBS (Grand Island, NY, USA) and 1% penicillin/streptomycin (Solarbio, Beijing, China). Cells were incubated under a humidified atmosphere with 5% CO₂ at 37°C.

MTT Assay

Non-small-cell lung cancer cell line (A549) was seeded in 96-well plates at a density of 8000 cells per well. After 24 h, the cells were treated with curcumin (2.5, 5, 10, 20, 40, 60, 80 μ M) for 24 h. Control group was treated with phosphate-buffered saline (PBS) in medium. MTT (0.5 mg/mL in PBS, 20 μ L) was added to each well and the plates were incubated for 4 h at 37°C. Then, the medium was removed and 150 μ L dimethyl sulfoxide (DMSO) was added to each well for 10 min to dissolve the purple formazan crystals. Absorbance at 570 nm was measured using a microplate reader. The percentage of viability was determined based on the following formula: A570 Experiment/A570 Control $\times$ 100%. All assays were performed in three independent experiments.

Clonogenic Survival Assay

A549 was plated in 12-well plates (2000 cells per well). After 24 h, cells were treated with curcumin (5, 10, 20 μ M) for 7 days. Control group was treated with phosphate-buffered saline (PBS) in medium. Then cells were fixed with 200 μ L methyl alcohol (-20°C) at 4°C for 5 min and stained with 200 μ L 0.1% crystal violet. The plates were observed under a stereomicroscope (Olympus SZX16, Tokyo, Japan). The number of colonies (more than 50 cells each) was counted at

10 different microscopic fields. Colony formation rate was calculated based on the following formula: Colony formation rate = (Colonies/Seeded cells) $\times$ 100%.

Measurement of ROS Generation

DCFH-DA is a cell-permeable, nonfluorescent probe. DCFH-DA is cleaved by intracellular esterase and then turns into a highly fluorescent dichlorofluorescein upon reaction with H_2O_2 . The generation of ROS was determined by DCFH-DA. A549 were seeded in 6-well plate at a density of 1×10^6 cells/well and treated with curcumin (5, 20, and 60 μ M) with or without NAC (5 mmol, 2 h) for 24 h. Control group was treated with phosphate-buffered saline (PBS) in medium. Then, cells were stained with DCFH-DA (10 μ mol/L) for 30 min at 37°C. The generation of ROS was determined by dichlorofluorescein fluorescence. Cells were collected and the fluorescence intensity in the cells was measured using a fluorescence microplate reader (Varioskan Flash, Thermo Scientific, Waltham, MA, USA) with excitation 488 nm and emission 525 nm.

Determinations of Oxidative Stress-Related Parameters

SOD activity and γ -GCS activity in cells were detected by commercial determination kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). A549 were seeded in 6-well plate at a density of 1×10^6 cells/well and with curcumin (5, 20, and 60 μ M) with or without NAC (5 mmol, 2 h) for 24 h. Control group was treated with phosphate-buffered saline (PBS) in medium. Cells were collected and dissolved in physiological solution. Then, cells were disrupted using ultrasound equipment and centrifuged at 6000 rpm for 10 min. The supernatants were used to determine enzyme activity.

Western Blot Analysis

The treated A549 cells were lysed with Western lysis buffer (containing 1% PMSF) at 4°C for 10 min and centrifuged under a high speed refrigerated (Eppendorf, Germany) at 13000 rpm, 4°C for 15 min. The precipitate was collected. The concentration of protein was determined by bicinchoninic acid (BCA) protein kit (Beyotime Biotechnology, Shanghai, China). An equal amount of protein (60 μ g) was loaded and resolved by 12% SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and transferred onto PVDF membranes (polyvinylidene difluo-

ride membranes). The membrane was blocked for 1 h in Tris-buffered saline and Tween-20 (TBS, containing 5% milk and Tween-20). The membrane was incubated with anti-p-GSK3 β (Ser9), anti-c-Myc, anti-Cyclin D1, anti- β -catenin, anti- α -tubulin, and anti-PCNA overnight at 4°C. After washing, the membrane was incubated with the appropriate HRP-conjugated secondary antibody (Abcam, Cambridge, UK). For measurement of β -catenin, the cytoplasmic protein and the nuclear protein were extracted and treated according to the instructions of the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime Biotech Inc., Nantong, China). Immune-reactive proteins were detected using Enhanced chemiluminescence (ECL) kit (Tottenham, UK).

Statistical Analysis

The results were carried out using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). All data were derived from three independent experiments and presented as the mean $\pm$ SD. Then, the data was tested by ANOVA with Tukey's multiple comparison tests. Comparisons between two groups were evaluated using Student's *t*-test. A value of less than 0.05 ($p < 0.05$) and 0.01 ($p < 0.01$) were considered statistically significant and highly significant, respectively.

Results

Curcumin Reduces A549 Cell Proliferation

To investigate the effect of curcumin on the proliferation of an NSCLC cell line (A549), we used an MTT assay to measure cell viability after treatment with curcumin (2.5, 5, 10, 20, 40, 60, 80 μ M) for 24 h. Compared with the control group, curcumin significantly decreased A549 viability in a dose-dependent manner (Figure 1A). Furthermore, the effect of curcumin on the ability of individual A549 cells to aggregate into viable colony clusters was determined by a clonogenic survival assay. As shown in Figure 1B, the colony formation rate of A549 was decreased after curcumin treatment when compared with the control group. Taken together, curcumin reduces A549 proliferation.

Oxidative Stress is Involved in A549 Proliferation, and is Affected by Curcumin Treatment

Oxidative stress is reportedly involved in cell proliferation^{11,12}. Therefore, we suggested that

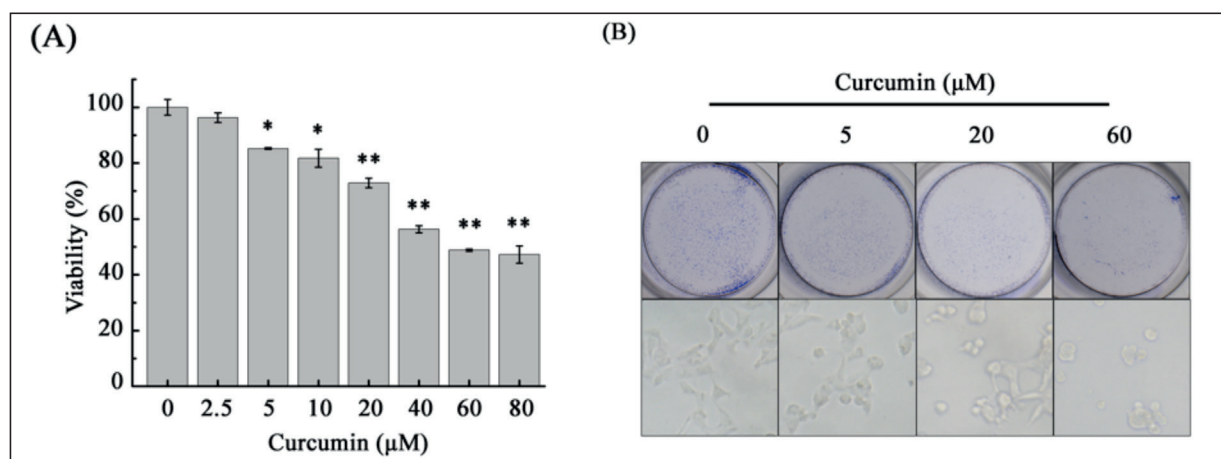


Figure 1. Effect of curcumin on viability in A549 cells. **A**, A549 cells were seeded in 96-well plates at a density of 8000 cells per well and treated with curcumin (2.5, 5, 10, 20, 40, 60, 80 μ M) for 24 h. The cell viability was determined by MTT assay; **B**, A549 cells were plated in 12-well plates (2000 cells per well). After 24 h, cells were treated with curcumin (5, 10, 20 μ M) for 7 d. The ability of individual cells to aggregate into viable colony clusters was detected by a clonogenic survival assay. In **(A)** and **(B)**, values are percent as the mean $\pm$ SD of three independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ vs. control.

increased oxidative stress may be involved in A549 proliferation, and reduced by curcumin treatment. ROS, which can regulate apoptosis and proliferation in tumor cells, is one of the most important factors in oxidative stress. SOD and γ -glutamyl cysteine synthetase (γ -GCS) are important antioxidant enzymes that are also involved in oxidative stress. To detect the effect of curcumin on oxidative stress in A549, cells were treated with curcumin (5, 20, and 60 μ M) with or without the ROS inhibitor N-Acetylcysteine (NAC) (5 mmol, 2 h) for 24 h. The control group was treated with phosphate-buffered saline (PBS) in medium. Results indicated that curcumin decreased the levels of ROS and increased the level of SOD and γ -GCS. After pre-treatment with NAC, the reduction in ROS and SOD and the increase in γ -GCS were all reversed (Figures 2A, B and C). Meanwhile, the decrease in A549 viability induced by curcumin was also reversed after NAC treatment (Figure 2D). These results indicate that oxidative stress plays an important role in curcumin-induced reduction of A549 viability.

Curcumin Inhibits the Oxidative Stress-Mediated Wnt/ β -Catenin Pathway

The Wnt/ β -catenin pathway is reported activated in abnormal cells¹⁷. The previous results showed that curcumin inhibited the growth of the NSCLC cell line, A549. Therefore, we investigated the relationship between the Wnt/ β -catenin pathway and proliferation in curcumin-treated A549 cells. A549 cells were seeded in 60 mm

dishes (1×10^6 cells/dish). After incubation for 24 h, A549 cells were treated with 60 μ M curcumin for 24 h. As shown in Figures 3A and B, curcumin strongly inhibited β -catenin and p-GSK3 β (Ser 9) protein expression. Meanwhile, the expression of downstream cyclin D1 and c-Myc were also significantly decreased following curcumin treatment. These results indicate that curcumin inhibits the Wnt/ β -catenin pathway. Over the last few decades, the Wnt/ β -catenin pathway has been reported to be activated through ROS-mediated induction. The preceding data indicates that both the Wnt/ β -catenin pathway and ROS are involved in curcumin-induced reduction of A549 cell viability. Hence, we explored whether the curcumin-mediated inhibition of the Wnt/ β -catenin pathway was mediated by ROS. A549 cells were seeded in 60 mm dishes (1×10^6 cells/dish). After 24 h incubation, A549 cells were treated with curcumin (60 μ M, 24 h) and NAC (5 mmol, 2 h) in DMEM medium composite processing. Results showed that NAC significantly reversed the curcumin-induced reduction in β -catenin and p-GSK3 β (Ser 9) proteins. Meanwhile, the reduction in downstream cyclin D1 and c-Myc protein was also reversed markedly after pre-treatment with NAC (Figure 3A and B). These results indicate the involvement of oxidative stress in curcumin-induced inhibition of the β -catenin pathway.

A hallmark event of active Wnt/ β -catenin signaling is the translocation of β -catenin into the nucleus^{18,19}. Hence, we took one further step in

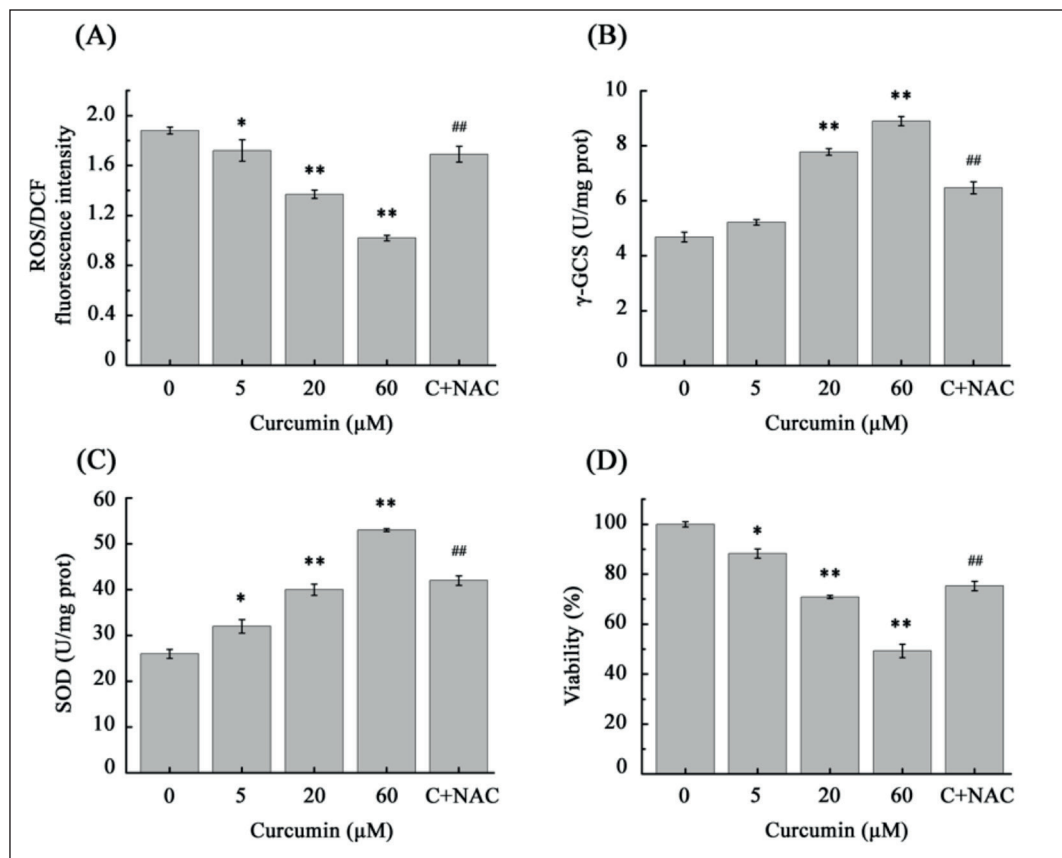


Figure 2. Effect of oxidative stress on curcumin-induced proliferation in A549 cells. A549 cells were seeded in 6-well plate at a density of 1×10^6 cells/well and treated with curcumin (5, 20 and 60 μM) with or without NAC (5 mmol, 2 h) for 24 h. Control group was treated with phosphate-buffered saline (PBS) in medium. **A**, Cells were stained with DCFH-DA (10 $\mu\text{mol/L}$) at 37°C for 30 min. The generation of ROS was determined by dichlorofluorescein fluorescence; Meanwhile, cells were collected and dissolved in physiological. Then cells were disrupted using ultrasound equipment and centrifuged at 6000 rpm for 10 min. The supernatants were used to determine γ -GCS activity (**B**) and SOD activity (**C**). **D**, The cell viability was determined by MTT assay. In (**A**), (**B**), (**C**) and (**D**), values are percent as the mean $\pm$ SD of three independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ vs. control; $0.01 < \#p < 0.05$ and $##p < 0.01$ vs. curcumin alone.

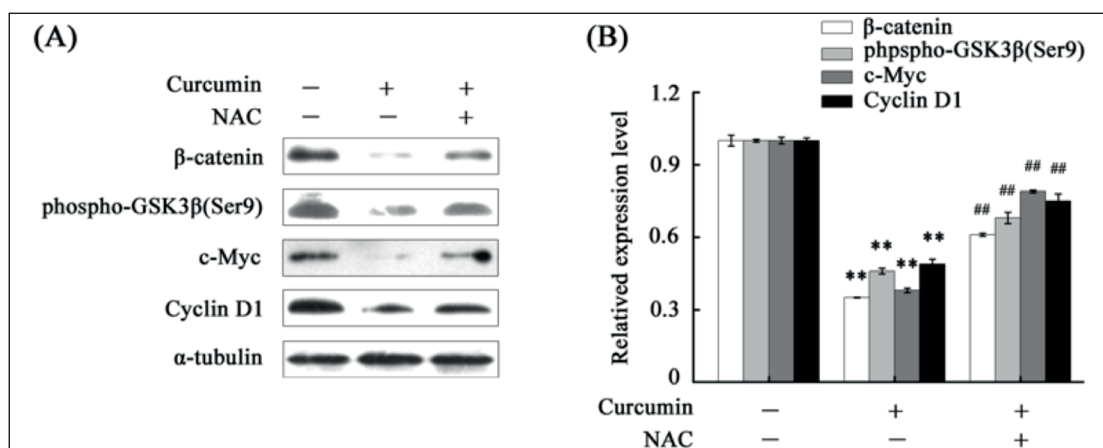


Figure 3. Curcumin inhibited the Wnt/ β -catenin pathway mediated by oxidative stress. A549 cells were seeded in 60 mm dishes (1×10^6 cells/dish) and incubated overnight. After 24 h, A549 cells were treated with curcumin (5, 20 and 50 μM) with or without NAC (5 mmol, 2 h) for 24 h. **A**, The expression of β -catenin, phosphor-GSK3 β (Ser9), c-Myc and Cyclin D1 were detected using Western blot. **B**, Ratio of values of β -catenin, phosphor-GSK3 β (Ser9), c-Myc and Cyclin D1. In (**A**) and (**B**), values are percent as the mean $\pm$ SD of three independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ vs control; $0.01 < \#p < 0.05$ and $##p < 0.01$ vs curcumin alone.

exploring the effect of curcumin on β -catenin expression. Results indicated that curcumin inhibited β -catenin expression in both the nucleus and cytoplasm. Furthermore, curcumin-induced reduction of β -catenin in the nucleus and cytoplasm were both reversed significantly following pre-treatment with NAC (Figures 4A and B). These findings show that curcumin-mediated inhibition of the Wnt/ β -catenin pathway is mediated by oxidative stress.

To further elucidate the role of the Wnt/ β -catenin signaling pathway in curcumin-induced inhibition of A549 viability, A549 cells were treated with β -catenin siRNA. As shown in Figure 5A, β -catenin siRNA reduced β -catenin expression and inhibited Wnt/ β -catenin signaling. Meanwhile, β -catenin siRNA further aggravated the inhibition of A549 cell growth by curcumin (Figure 5B). These results were similar to our previous data, which showed the involvement of the Wnt/ β -catenin signaling pathway in curcumin-mediated inhibition of A549 cell viability.

Discussion

Lung cancer is one of the most common cancer types, and is a leading cause of cancer-related death. The rate of decline in the NSCLC mortality rate still lags considerably behind that of other prevalent cancers such as breast, prostate, colon, and rectal, among others^{20,21}. NSCLC accounts

for more than 80% of all lung cancer cases. Curcumin, which is a phyto-polyphenolic pigment, derives from turmeric (*Curcuma longa* L.). Curcumin has been shown to have multiple anticancer effects including inhibition of proliferation, angiogenesis, DNA topoisomerase II, as well as induction of apoptosis^{8,22}. Though curcumin is reported to inhibit cancer cell proliferation, its effect on NSCLC has not been widely investigated. Therefore, we studied the effect of curcumin on NSCLC proliferation and found that curcumin reduces NSCLC proliferation in a dose-dependent manner.

Oxidative stress, mostly caused by the excessive formation of free radicals, is reportedly involved in cell proliferation¹². An increase in oxidative stress has been implicated in the aging process and results from an imbalance between the generation and clearance of ROS²³. The relationship between oxidative stress and curcumin-induced inhibition of cell proliferation is unknown. Hence, whether oxidative stress is involved in curcumin-induced inhibition of proliferation was researched in NSCLC cells. Results indicated that curcumin decreased the oxidative stress in A549 cells. Meanwhile, NAC reversed the effect of curcumin on oxidative stress. Furthermore, NAC also reversed the curcumin-induced reduction of cell viability. Taken together, the inhibition of proliferation induced by curcumin is mediated by oxidative stress.

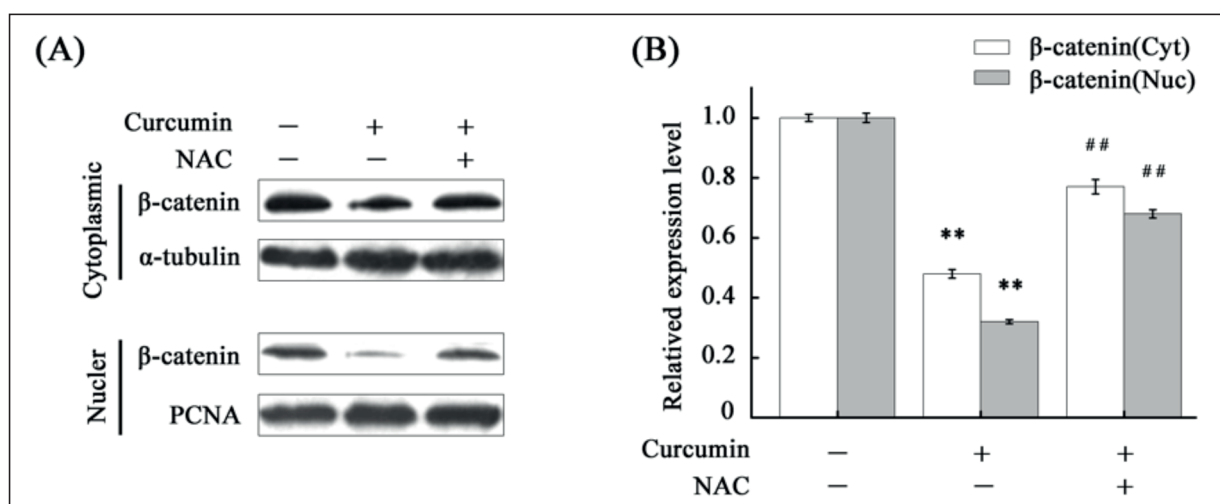


Figure 4. Curcumin inhibited the Wnt/ β -catenin pathway mediated by oxidative stress in nucleus and cytoplasm. A549 cells were seeded in 60 mm dishes (1×10^6 cells/dish) and incubated overnight. After 24 h, A549 cells were treated with curcumin (5, 20 and 50 μ M) with or without NAC (5 mmol, 2 h) for 24 h. **A**, The expression of β -catenin was detected using Western blot in nucleus and cytoplasm. **B**, Ratio of values of β -catenin. In **(A)** and **(B)**, values are percent as the mean $\pm$ SD of three independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ vs control; $0.01 < \#p < 0.05$ and $##p < 0.01$ vs. curcumin alone.

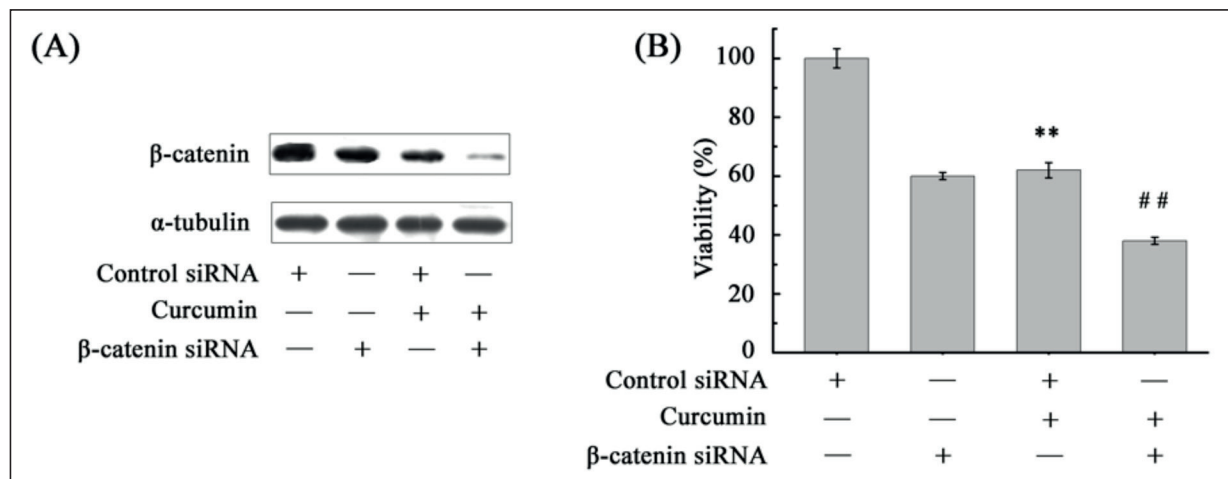


Figure 5. β-catenin siRNA inhibited Wnt/β-catenin signaling pathway induced by curcumin. A549 cells were seeded in 60 mm dishes (1×10^6 cells/dish) and incubated overnight. After 24 h, A549 cells were treated with curcumin (5, 20 and 50 μM) with or without siRNA for 24 h. The expression of β-catenin was detected using Western blot (A) and Real-time PCR (B). In (A) and (B), values are percent as the mean $\pm$ SD of three independent experiments. $0.01 < *p < 0.05$ and $**p < 0.01$ vs. control; $0.01 < \#p < 0.05$ and $\#\#p < 0.01$ vs. curcumin alone.

The Wnt/β-catenin pathway has been reported to regulate cell density^{24,25}. The Wnt/β-catenin pathway is active in abnormal cells²⁶. Following treatment with curcumin, the level of β-catenin, p-GSK3β (Ser 9) and the downstream cyclin D1, c-Myc in A549 cells was detected. Results found curcumin inhibited Wnt/β-catenin signaling pathway associated proteins. Furthermore, NAC reversed the effect of curcumin on the Wnt/β-catenin signaling pathway. All these illustrate that curcumin inhibits the Wnt/β-catenin pathway, in a mechanism mediated by oxidative stress in A549 cells.

Conclusion

The effect of curcumin on the proliferation of the NSCLC cell line A549 was determined using the MTT and clonogenic survival assays. Both assays indicated that curcumin is an inhibitor of A549 cell growth. Furthermore, the results implicated oxidative stress in the curcumin-induced reduction of A549 cell viability. The Wnt/β-catenin pathway is reportedly activated in abnormal cells. Hence, we explored the relationship between the Wnt/β-catenin pathway and curcumin-mediated proliferation. We observed that the Wnt/β-catenin pathway was involved in curcumin-induced A549 proliferation. Simultaneously, the role of ROS in the Wnt/β-catenin pathway was also detected. We indicated that oxidative stress is involved in

the curcumin-induced inhibition of the β-catenin pathway. Our data therefore proved that curcumin inhibits the Wnt/β-catenin pathway, which is mediated by oxidative stress, in NSCLC A549 cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

- 1) JEMAL A, SIEGEL R, XU J, WARD E. Cancer statistics 2010. *CA Cancer J Clin* 2010; 23: 53-58.
- 2) PARKIN DM, BRAY F, PISANI P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55: 74-108.
- 3) SAAD AS, GHALI RR, SHAWKI MA. A prospective randomized controlled study of cisplatin versus carboplatin-based regimen in advanced squamous nonsmall cell lung cancer. *J Cancer Res Ther* 2017; 13: 198-203.
- 4) WANG Y, HU L, ZHANG X, ZHAO H, XU H, WEI Y, JIANG H, XIE C, ZHOU Y, ZHOU F. Downregulation of mitochondrial single stranded DNA binding protein (SSBP1) induces mitochondrial dysfunction and increases the radiosensitivity in Non-Small Cell Lung Cancer Cells. *J Cancer* 2017; 8: 1400-1409.
- 5) SONG L, LI D, GU Y, WEN ZM, JIE J, ZHAO D, PENG LP. MicroRNA-126 targeting PIK3R2, inhibits NSCLC A549 cell proliferation, migration, and invasion by regulation of PTEN/PI3K/AKT pathway. *Clin Lung Cancer* 2016; 17: 65-75.
- 6) LAI CS, WU JC, YU SF, BADMAEV V, NAGABHUSHANAM K, HO CT, PAN MH. Tetrahydrocurcumin is more ef-

- fective than curcumin in preventing azoxymethane-induced colon carcinogenesis. *Mol Nutr Food Res* 2011; 55: 1819-1828.
- 7) KIM T, DAVIS J, ZHANG AJ, HE X, MATHEWS ST. Curcumin activates AMPK and suppresses gluconeogenic gene expression in hepatoma cells. *Biochem Bioph Res Co* 2009; 388: 377-382.
- 8) KARUNAGARAN D, RASHMI R, KUMAR TR. Induction of apoptosis by curcumin and its implications for cancer therapy. *Curr Cancer Drug Targets* 2015; 5: 117-129.
- 9) SALVIOLI S, SIKORA E, COOPER EL, FRANCESCHI C. Curcumin in cell death processes: a challenge for CAM of age-related pathologies. *Evid Based Complement Alternat Med* 2007; 4: 181-190.
- 10) AZMANDIAN J, MANDEARY A, POOTARI M, NEMATOLAH MH, EBADZADEH MR, HABIBZADEH SD, DEGHANI MH, ETMINAN A, FAZELI F, MOUSAVI M. Role of donors and recipients' glutathione S-transferase gene polymorphisms in association of oxidative stress with delayed graft function in kidney allograft recipients. *Iran J Kidney* 2017; 11: 241-248.
- 11) LU Y, PAN Y, SHENG N, ZHAO AZ, DAI J. Perfluorooctanoic acid exposure alters polyunsaturated fatty acid composition, induces oxidative stress and activates the AKT/AMPK pathway in mouse epididymis. *Chemosphere* 2016; 158: 143-153.
- 12) LIU XR, LI YQ, HUA C, LI SJ, ZHAO G, SONG HM, YU MX, HUANG Q. Oxidative stress inhibits growth and induces apoptotic cell death in human U251 glioma cells via the caspase-3-dependent pathway. *Eur Rev Med Pharmacol Sci* 2015; 19: 4068-4075.
- 13) CUI Z, CUI Y, YANG S, LUO G, WANG Y, LOU Y, SUN X. KLK4 silencing inhibits the growth of oral squamous cell carcinoma through Wnt/ β -catenin signaling pathway. *Cell Biol Int* 2017; 41: 392-404.
- 14) JIN XT, SONG L, ZHAO JY, LI ZY, ZHAO MR, LIU WP. Dichlorodiphenyltrichloroethane exposure induces the growth of hepatocellular carcinoma via Wnt/ β -catenin pathway. *Toxicol Lett* 2014; 225: 158-166.
- 15) LEE H, BAC S, KIM K, KIM W, CHUNG SI, YANG Y, YOON Y. Shikonin inhibits adipogenesis by modulation of the Wnt/ β -catenin pathway. *Life Sci* 2011; 88: 294-301.
- 16) DU YY, LIU X, SHAN BE. Periplocin extracted from cortex periplocae induces apoptosis of SW480 cells through inhibiting the Wnt/ β -catenin signaling pathway. *J Am Soc Nephrol* 2009; 20: 1997-2008.
- 17) SONG L, LIU D, HE J, WANG X, DAI Z, ZHAO Y, KANG H, WANG B. SOX1 inhibits breast cancer cell growth and invasion through suppressing the Wnt/ β -catenin signaling pathway. *APMIS* 2016; 124: 547-555.
- 18) CHEN JF, LUO X, XIANG LS, LI HT, ZHA L, LI N, HE JM, XIE GF, XIE X, LIANG HJ. EZH2 promotes colorectal cancer stem-like cell expansion by activating p21cip1-Wnt/ β -catenin signaling. *Oncotarget* 2016; 7: 41540-41558.
- 19) HU Z, XIE L. LHX6 inhibits breast cancer cell proliferation and invasion via repression of the Wnt/ β -catenin signaling pathway. *Mol Med Rep* 2015; 12: 4634-4639.
- 20) WANG JY, HONG X, CHEN GH, LI QC, LIU ZM. Mucosolvan serves to optimize perioperative airway management of NSCLC patients in fast track surgery: a randomized placebo controlled study. *Eur Rev Med Pharmacol Sci* 2015; 21: 115-119.
- 21) ZHANG J, QU Y, ZHENG L, ZHANG X, XIA R, KONG F, SHEN Y, WANG S, LIN L. Clinical implication of insulin-like growth factor II mRNA-binding protein 3 expression in non-small cell lung carcinoma. *Oncol Lett* 2015; 9: 1927-1933.
- 22) ANAND P, SUNDARAM C, JHURANI S, KUNNUMAKKARAA B, AGGARWAL BB. Curcumin and cancer: an "old-age" disease with an "age-old" solution. *Cancer Lett* 2008; 267: 133-164.
- 23) PATEL B, KUMAR P, BANERJEE R, BASU M, PAL A, SAMANTA M, DAS S. Lactobacillus acidophilus attenuates aeromonas hydrophila induced cytotoxicity in catla thymus macrophages by modulating oxidative stress and inflammation. *Mol Immunol* 2016; 75: 69-83.
- 24) SUN TJ, TAO R, HAN YQ, XU G, LIU J, HAN YF. Therapeutic potential of umbilical cord mesenchymal stem cells with Wnt/ β -catenin signaling pathway pre-activated for the treatment of diabetic wounds. *Eur Rev Med Pharmacol Sci* 2014; 18: 2460-2464.
- 25) WANG Y, WANG W, QIU E. Protection of oxidative stress induced apoptosis in osteosarcoma cells by dihydromyricetin through down-regulation of caspase activation and up-regulation of Bcl-2. *Saudi J Biol Sci* 2017; 24: 837-842.
- 26) GAN F, HU Z, HUANG Y, XUE H, HUANG D, QIAN G, HU J, CHEN X, WANG T, HUANG K. Overexpression of pig selenoprotein S block OTA-induced promotion of PCV2 replication by inhibiting oxidative stress and p38 phosphorylation in PK15 cells. *Oncotarget* 2016; 7: 20469-20485.