

Research on the Inhibition of Cancer Cell Metastasis by Graphene Oxide Suppresses the Translation of the Snail mRNA

Xinyu Wei¹, Can Luo¹, Xiaohong Ming¹, Xianbo Jia¹, Ping Long¹, Lei Feng¹, Ming Zhu¹, Xiaolin Hu¹, Ming Li², Hui Li¹

¹Department of Hematology, Tianyou Hospital, Wuhan University of Science and Technology, Wuhan, Hubei, 430000, People's Republic of China;

²Department of Laboratory Medicine, Tianyou Hospital, Wuhan University of Science and Technology, Wuhan, Hubei, 430000, People's Republic of China

Correspondence: Hui Li, Email lhlhklal988@126.com

Introduction: Lung cancer is the most common malignant tumor worldwide and often presents with advanced metastasis. This study explores the effects of graphene oxide (GO) on lung cancer cell motility, investigates underlying mechanisms, and identifies potential therapeutic targets.

Methods: The effects of GO on the viability and motility of lung cancer cells A549 and H226, and normal bronchial epithelial cells BEAS-2B, were assessed using cytotoxicity, scratch, and Transwell assays. Mechanisms were explored by measuring intracellular ROS, EMT-related and TGF- β pathway protein expression, cellular TGF- β release, and Snail mRNA levels, suggesting potential new targets.

Results: Cytotoxicity, scratch, and Transwell experiments indicated that GO had cytotoxic effects on A549, H226, and BEAS-2B, and the effects increased with increasing GO concentration and culture time. A specific concentration of GO could significantly inhibit the cell motility of A549 and H226 within a specific time window. The results of the molecular mechanism experiment showed that within the selected GO concentration and time window, there was no significant change in intracellular reactive oxygen species (ROS); the epithelial-associated protein E-cadherin increased, the EMT regulatory protein Snail decreased, the level of TGF- β secreted by cells did not change, and the expression level of Snail mRNA increased.

Conclusion: GO increases Snail mRNA but suppresses its translation, reducing EMT protein Snail and increasing E-cadherin, which further decreases tumor cell motility, offering a novel therapeutic strategy for addressing distant metastasis in lung cancer.

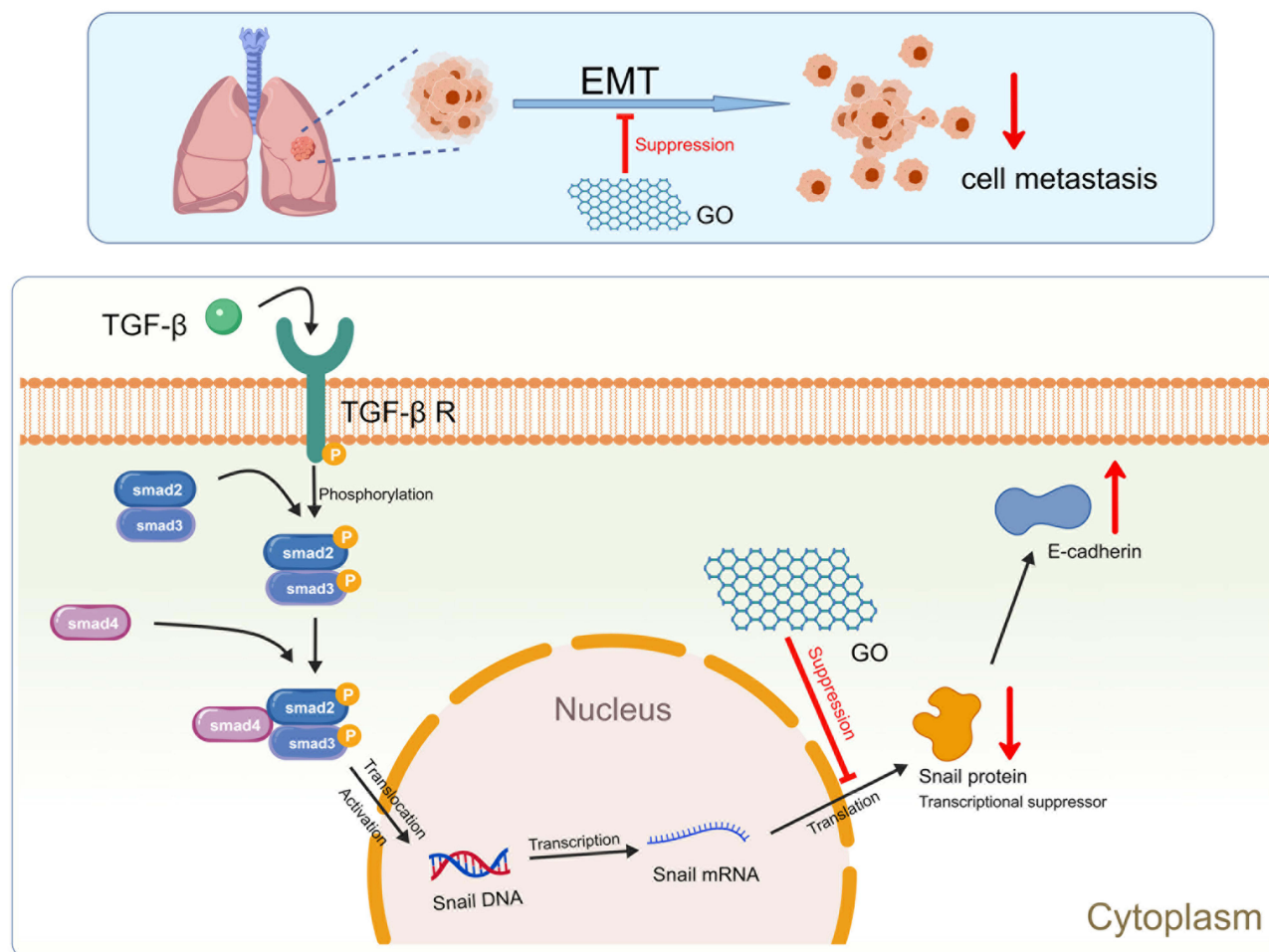
Keywords: graphene oxide, metastasis of lung cancer cells, epithelial-mesenchymal transition, Snail mRNA, protein synthesis

Introduction

Lung cancer, recognized as one of the deadliest malignant tumors globally with high rates of illness and death, poses a major challenge for both clinical and scientific researchers.¹ For metastatic lung cancer, the main treatment options include chemotherapy, targeted therapy, and immunotherapy.² However, only a small number of patients with specific genetic mutations, such as those in the EGFR and ALK genes, are suitable for targeted therapy.³ Therefore, creating new therapeutic strategies to prevent tumor cell metastasis is critically important.

The main process behind lung cancer cell metastasis is epithelial-mesenchymal transition (EMT). E-cadherin, an essential protein that maintains tight junctions between epithelial cells, when down-regulated or lost, causes these cells to detach from each other, which is a hallmark of EMT.⁴ Additionally, increased levels of certain mesenchymal-associated proteins, such as N-cadherin and Vimentin, promote cell movement and invasion. This process is regulated by factors such as Snail, Slug, Twist1, and ZEB, which suppress E-cadherin and induce N-cadherin and Vimentin, promoting cell migration and tumor spread.⁵ Due to its role in cancer progression, EMT is a major drug target. The targeted therapy Trametinib plays an important role in treating metastatic lung cancer by inhibiting the ERK pathway.⁶

Graphical Abstract



Graphene, a novel nanomaterial, gained prominence among researchers after its discovery in 2004. Since then, it has been extensively studied across various fields, including electronic devices, conductors, energy materials, and catalytic processes.⁷ Graphene oxide (GO), prepared through the oxidation of graphene, has a large specific surface area that enables it to host a variety of metals, biomolecules, DNA, RNA, and drugs.^{8,9} Furthermore, GO has numerous hydrophilic groups on its surface, allowing stable dispersion in aqueous solutions and exhibiting excellent biocompatibility, which supports cellular uptake.¹⁰ These biological properties of GO offer significant advantages for studying its interactions with tumor cells.

Tian¹¹ found that graphene oxide nanosheets insert into actin filament gaps, segregating actin tetramers and disrupting filaments to inhibit tumor cell migration. Our investigation identified a new mechanism: GO inhibits tumor cell migration by suppressing Snail mRNA translation and reducing Snail protein levels. This reversal of E-cadherin inhibition increases E-cadherin protein expression, maintains the epithelial state, inhibits EMT, and reduces cell metastasis capacity. We identified a safe graphene oxide concentration and exposure time that inhibits cell metastasis without harming normal cells, providing a reference for future experimental design. This study also uncovers a novel mechanism by which graphene oxide reduces cell metastasis, supporting the development of anti-cancer nanomedicines.

Materials and Methods

Materials

Graphene oxide (GO) was sourced as a 10 mg/mL aqueous dispersion (100 mL) from Suzhou Carbonfund Graphene Technology Co. Lung bronchial epithelial cells BEAS-2B, lung adenocarcinoma cells A549, and lung squamous cell carcinoma cells H226 were obtained from the experimental group at the College of Life and Science, Wuhan University of Science and Technology, and were maintained throughout the experiment. Dulbecco's modified Eagle medium (DMEM) and Roswell Park Memorial Institute (RPMI) 1640 were acquired from Thermo Fisher (New York, NY, USA). 1×PBS and extra-grade fetal bovine serum (FBS) were procured from Biosharp (Anhui, China). Serum-free non-programmed cell freezing solution (phenol red-free), 0.25% trypsin digestion solution, Cell Proliferation Grade Toxicity Assay Kit (CCK-8), Reactive Oxygen Species Detection Kit (ROS), Western primary antibody dilution, Western secondary antibody dilution, RIPA lysate, protein phosphatase inhibitor complex (100×), and protease inhibitor mixtures were obtained from Meilunbio Corporation (USA). Cell culture chambers with a pore size of 8.0 μm were sourced from Ladselect (USA). Primary antibodies ERK1/2 and P-ERK were acquired from Proteintech (USA). Primary antibodies E-cadherin, N-cadherin, and vimentin were obtained from UpingBio (China). Secondary antibodies, including anti-mouse and anti-rabbit, were sourced from Wuhan Kerui Biotech (Wuhan, China). Primary antibodies smad2/3, p-smad2/3, and Snail were obtained from Affinity Biosciences (USA). The human TGF-β assay kit was acquired from Beyotime (China). Primers and the One Step RT-qPCR kit were obtained from Sangon Biotech (Shanghai, China).

Cell Culture and Passage

A549 and H226 lung cancer cell lines were cultured in DMEM supplemented with 10% FBS, while the BEAS-2B bronchial epithelial cell line was maintained in 1640 medium containing 10% FBS. Cells were seeded in 10-cm dishes and incubated in a humidified incubator at 37°C with 5% CO₂. They were passaged when cell confluence reached approximately 80–90%.

Cell Viability

The Cell Counting Kit-8 (CCK-8) was used to evaluate cell viability. A549, H226, and BEAS-2B cells were seeded at a density of 5000 cells per well in 96-well plates, with 100 μL of medium in each well, and cultured for 12 hours. The cells were treated with graphene oxide (GO) at concentrations of 0 μg/mL, 10 μg/mL, 30 μg/mL, 50 μg/mL, 100 μg/mL, 150 μg/mL, and 200 μg/mL for 24 and 48 hours at 37 °C. After treatment, the medium was replaced with 100 μL of FBS-free medium, and 10 μL of CCK-8 reagent was added to each well, followed by a 2-hour incubation. The absorbance was measured at 450 nm to determine cell viability.

Cell Scratch Assay

A549, H226, and BEAS-2B cells were seeded in 6-well plates (2 mL each) at a density of 6×10^5 cells per well and incubated at 37°C for 24 hours. Cell confluence, observed under a microscope, exceeded 80%. The cells were treated with graphene oxide (GO) at concentrations of 0 μg/mL, 50 μg/mL, 100 μg/mL, and 200 μg/mL. A 200 μL pipette tip was used to draw a straight line in the center of each well, and images were taken at 0, 24, and 48 hours to monitor the healing of the scratch. The healing distance was then calculated as a ratio relative to the measurements at 0 hours.

Transwell Migration

The migratory ability of lung cancer cells and normal lung cells was evaluated using 24-well Transwell plates. Cells treated with different concentrations of GO for 24 hours were resuspended in serum-free medium at a density of 2×10^5 cells/mL. Then, 200 μL of this cell suspension was added to the upper chamber, while 600 μL of medium containing 20% FBS was placed in the lower chamber. After 24 hours, the cells that migrated through the membrane pores were fixed with 4% paraformaldehyde, stained with crystal violet, and the non-migrated cells in the upper chamber were removed using a cotton swab. The migrated cells were counted under a microscope.

ROS

Cells were inoculated at a density of 3×10^4 cells per well in 24-well plates and treated with graphene oxide (GO) at 0 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, and 200 $\mu\text{g/mL}$ for 24 and 48 hours. Following treatment, cells were rinsed three times with serum-free medium. DCFH-DA (10 mM) was diluted 1:1000 in serum-free medium, and 200 μL of the solution was added to each well. The cells were then incubated at 37°C for 30 minutes. Subsequently, the samples were examined under a fluorescence inverted microscope to capture images for documentation. Fluorescence intensity was analyzed using ImageJ software and normalized against the control group.

Western Blot

Cells were lysed in a RIPA mixture (250 μL ; RIPA lysate: 100 mM PMSF: protease inhibitor mixture: protein phosphatase inhibitor complex = 1000:10:10:10), followed by fragmentation using an ultrasonic cell crusher for 5 minutes. The lysate was then centrifuged at 10,000 g for 15 minutes at 4°C in a high-speed centrifuge. Protein concentration was assessed using the BCA kit (Biosharp, China), and the concentration of each group was standardized with RIPA mix. Subsequently, $5\times$ SDS-protein loading buffer was added at a 4:1 ratio, and the samples were heated to 100°C for 10 minutes. Proteins (about 15 μg per lane) were separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes. The PVDF membranes were incubated with a primary antibody followed by a peroxidase-conjugated secondary antibody. Finally, the protein bands were developed using Feikert Ultra Sensitive ECL solution (Meilunbio, USA) and visualized with a ChemiDoc (BIO-RAD, USA). The resulting protein bands were analyzed using Image J to obtain grayscale values, which were normalized to the control.

ELISA-Kit

Cells were treated with various concentrations of GO, after which the upper medium layer was collected and centrifuged at 4000 rpm for 20 minutes, discarding the lower precipitate. A volume of 100 μL from each sample was mixed with 20 μL of HCl; after 10 minutes, 20 μL of NaOH was added. Subsequently, 50 μL of the sample or standard was transferred to a 96-well plate containing TGF- β substrate, followed by the addition of 100 μL of horseradish peroxidase (HRP)-labeled antibody. The plate was incubated at 37°C in the dark for 1 hour. After five washes with $1\times$ washing solution, 100 μL of a 1:1 mixture of substrates A and B was added, and the mixture was incubated at 37°C in the dark for 15 minutes. Following another five washes with $1\times$ washing solution, an additional 100 μL of the 1:1 mixture of substrates A and B was added, and the incubation was continued at 37°C in the dark for another 15 minutes. The optical density (OD) was measured at 450 nm using an enzyme meter, allowing the construction of a standard curve to calculate the concentration of TGF- β in the supernatant.

RT-qPCR

Cells were lysed with a lysis solution, transferred to the upper layer of an RNA extraction tube, and centrifuged at 12,000 g for 1 minute to remove the liquid from the lower chamber. Wash solutions 1 and 2 were added, and the washing procedure was repeated three times. Subsequently, RNA Binding Solution was used to elute the RNA bound to the binding column, which was then transferred to a new enzyme-free centrifuge tube. The RNA concentration in each tube was analyzed using an RNA detector. Following this, $2\times$ Onestep RT-qPCR Master Mix, forward primer, reverse primer, RT enzyme mix, RNA template, and RNase-free ddH $_2\text{O}$ were added according to the specified ratios. The mixture was then placed in a PCR instrument (BIO-RAD, USA), where the temperature and duration for the denaturation-annealing-extension program were configured, and the program was executed for 40 cycles. After completing the 40 cycles, fluorescence levels were measured, and data were collected.

Statistical Analysis

All data are expressed as mean $\pm$ S.E.M. Multiple groups (with varying GO concentrations) were compared using a one-way ANOVA. Comparison of the two groups was statistically analyzed using unpaired Student's *T*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Result

Acquisition and Characterization of Graphene Oxide

Graphene oxide (GO) is a 10 mg/mL aqueous dispersion (100 mL) sourced from Suzhou Carbon Feng Graphene Technology Co., LTD. We performed scanning electron microscopy (SEM) and elemental analysis of the acquired samples (Figure 1) to simultaneously examine GO morphology. The particle size of the obtained GO ranged from 100 nm to 300 nm, exhibiting a sheet-like morphology with partial folding and overlap (Figure 1A). The carbon content of the synthesized graphene oxide was measured at 64.54%, while the oxygen content was found to be 29.49% (Figure 1B and C). Upon magnification using scanning electron microscopy (SEM) at a scale of 200 nm, the folded regions of the graphene oxide thin sheet exhibited a three-dimensional structure resembling a pyramid (Figure 1D). The elemental analysis results, presented in bar and pie charts, indicate that the concentrations of other elements are negligible compared to those of carbon and oxygen (Figure 1E). This observation confirms that the samples utilized in this experiment fulfil the necessary experimental criteria.

The Toxic Effect of Graphene Oxide on Cells

To assess the biocompatibility of graphene oxide (GO) in this study, A549 cells, derived from a lung adenocarcinoma cell line, and H226 cells, derived from a lung squamous cell carcinoma cell line, were utilized alongside BEAS-2B cells from a normal bronchial epithelial cell line.

Detection of cell activity using CCK-8 indicated that there was no significant decrease in cell viability after 24 hours of GO treatment (Figure 2A–C). A549 cells exhibited a substantial reduction in viability only at a concentration of 100 μ g/mL after 48 hours of GO exposure (Figure 2A). In contrast, H226 cells showed a significant change at 30 μ g/mL (Figure 2B), while BEAS-2B cells demonstrated a statistically significant decrease in viability at a lower concentration of 10 μ g/mL (Figure 2C). These results suggest that GO exerts an inhibitory effect on the survival of A549, H226, and

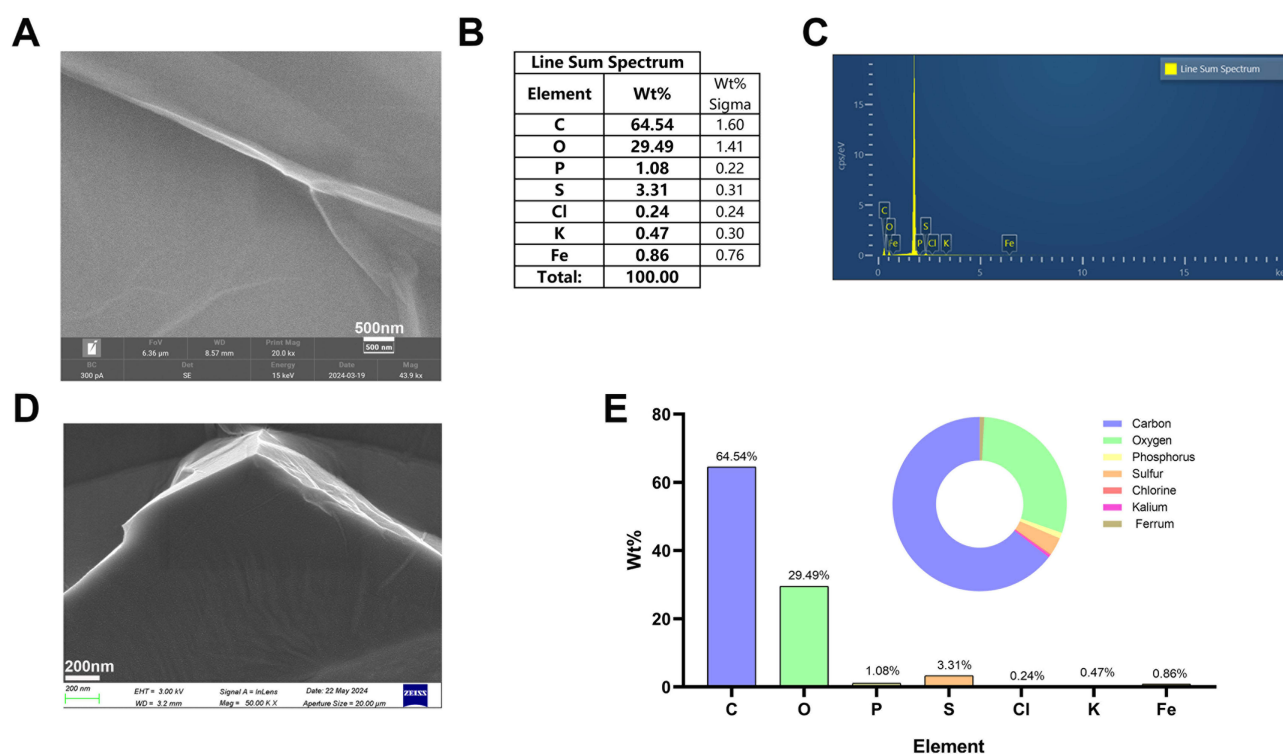


Figure 1 Characterization Study of graphene oxide: (A) Electron microscopy reveals GO as sheet-like with partial folding and overlap (scale: 500 nm). (B and C) Elemental analysis detects carbon, oxygen, phosphorus, sulfur, chlorine, potassium, and iron; Wt% denotes weight percentage, Wt% sigma its standard deviation. (D) Some sheets fold into three-dimensional structures (scale: 200 nm). (E) Bar and pie charts display element proportions, with Carbon at 64.54%, Oxygen at 29.49%, Phosphorus at 1.08%, Sulfur at 3.31%, Chlorine at 0.24%, Kalium at 0.47% and Ferrum at 0.86%.

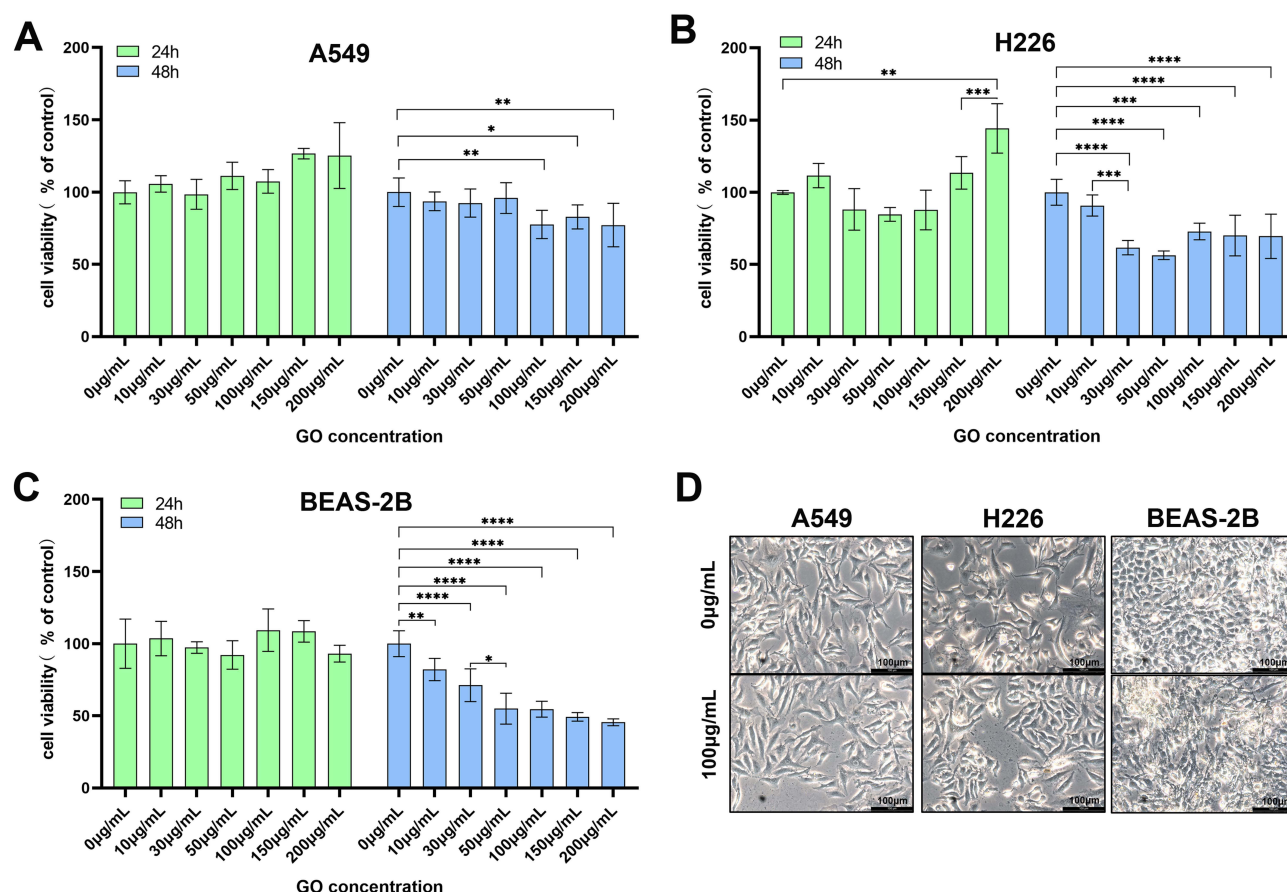


Figure 2 Effect of GO on Cell Viability and cell status: (A–C) A549, H226 and BEAS-2B cells were co-cultured with different concentrations of GO for 24h and 48h, and then the cell viability was detected by CCK-8. Values represent the means $\pm$ S.E.M. ($n \geq 3$), * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$. (D) A549, H226, and BEAS-2B cells were co-cultured with 0 µg/mL and 100 µg/mL GO for 48h, and cell photographs were taken (scale: 100 µm).

BEAS-2B cells, with this effect being both time-dependent and concentration-dependent. Consequently, the safe range for GO-treated cells is established as up to 24 hours.

Regarding cell morphology, treatment with GO at 100 µg/mL for 48 hours did not result in significant changes in the appearance of A549 and H226 cells, which maintained their spindle shape or irregular polymorphism; however, a reduction in extracellular pseudopods was observed. Additionally, compared to the control group (0 µg/mL), a notable increase in vacuoles containing brown substances was evident within the cells, suggesting the presence of vesicles formed by cytotropic graphene oxide (Figure 2D). BEAS-2B cells also exhibited decreased cell density following GO treatment, accompanied by increased cell death and fragmentation (Figure 2D).

The Influence of Graphene Oxide on Cell Motility

The inhibitory effect of graphene oxide (GO) on cell motility was initially assessed through cell scratch assays. The results indicated that (Figure 3A–C), at various concentrations of GO treatment, the cell migration distance of all three cell types significantly decreased at 24 hours compared to the control group (0 µg/mL). Notably, at 48 hours, A549 cells exhibited a more pronounced decline in cell migration distance relative to the 24-hour mark (Figure 3D), whereas H226 and BEAS-2B cells demonstrated a recovery in their cell migration distance (Figure 3E and F). This observation may be attributed to the fact that, at 48 hours, the cell migration distance is influenced by both cell migration and proliferation. In contrast, at 24 hours, the limited number of cell clones means that the cell migration distance is primarily determined by cell migration.

To eliminate interference, the Transwell experiment was conducted. The results indicated that after 24 hours of treatment with GO, the perforation efficiency of A549 and H226 cells was significantly reduced (Figure 4A and B). At

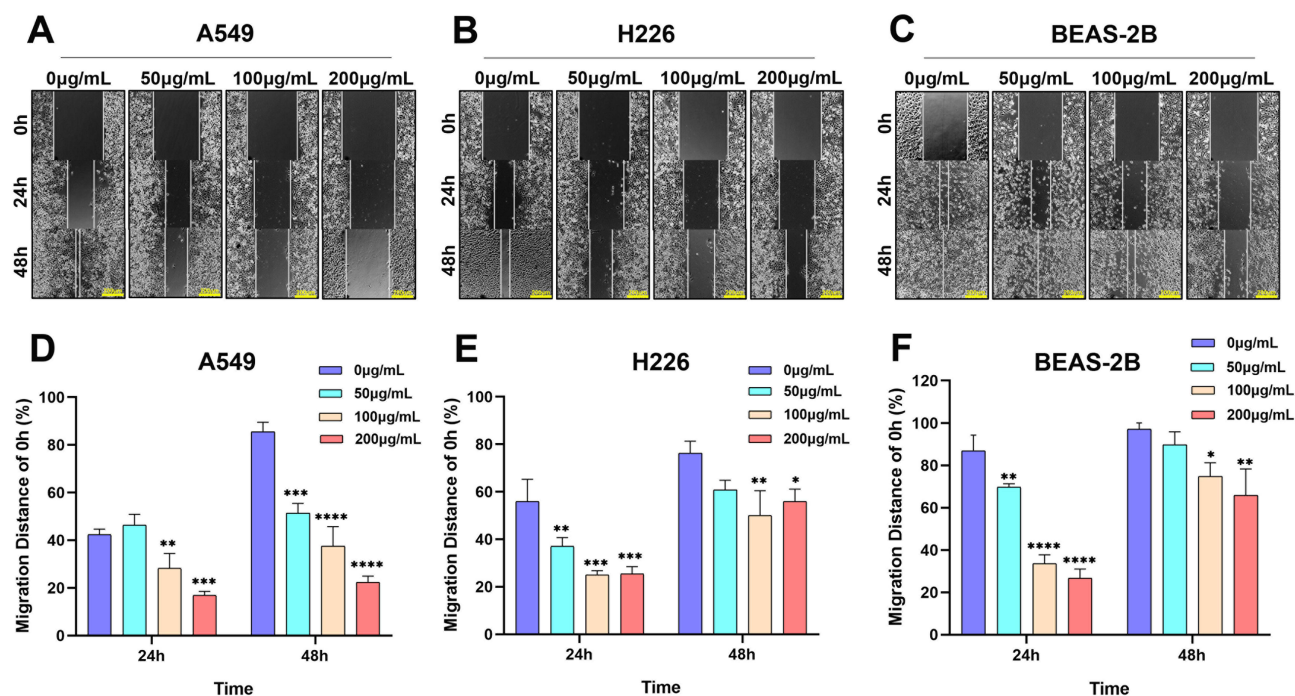


Figure 3 The effect of GO on the healing of cell scratches: (A–C) Cell images of scratch healing degree of A549, H226, and BEAS-2B cells co-cultured with GO at different concentrations at 24h and 48h (scale: 200 µm). (D–F) Analyzing the cell migration distance of 0h (%). Values represented the means $\pm$ S.E.M. (n=3), * P <0.05, ** P <0.01, *** P <0.001, **** P <0.0001.

a concentration of 50 µg/mL, the perforation efficiency of these cells had already decreased markedly (Figure 4C and D). Thus, it can be concluded that the metastasis of tumor cells co-treated with GO is significantly inhibited, even at a relatively low concentration of 50 µg/mL. Notably, under the same experimental conditions, almost no perforated cells were detected in BEAS-2B. This observation may be attributed to the fact that BEAS-2B cells are normal bronchial epithelial cells with limited cell migration capacity. The low baseline migration of BEAS-2B limits the comparison.

The Effect of Graphene Oxide on the Level of Reactive Oxygen Species (ROS) within Cells

In the subsequent study, we evaluated whether GO induced apoptosis or necrosis by measuring intracellular ROS levels. The results indicated that at 24 hours, ROS levels in A549, H226, and BEAS-2B cells treated with 50 µg/mL and 100 µg/mL did not differ significantly from those in the control group (0 µg/mL) (Figure 4E–G). In A549 cells, a statistically significant increase in ROS levels (P < 0.05) was observed only after treatment with GO at 100 µg/mL and 200 µg/mL for 48 hours (Figure 4H). For H226 cells, a statistically significant increase in ROS (P < 0.01) was noted solely at 200 µg/mL for 48 hours (Figure 4I). A substantial increase in ROS was detected in BEAS-2B cells at 200 µg/mL for both 24 hours and 48 hours (Figure 4J). These findings suggest that 50 µg/mL and 100 µg/mL at 24 hours are safe concentrations for cells. Under these conditions, GO inhibits tumor cell migration without inducing toxic effects on normal cells.

We observed that the fluorescence intensity in BEAS-2B cells was significantly higher than that in A549 and H226 cells (Figure 4E–G). The fluorescence levels in A549 and H226 cells were notably low, which hindered the identification of fluorescently positive cells. This observation may be attributed to the inherent characteristics of tumor cells. Specifically, following these gene mutations, tumor cells exhibit lower intracellular levels of reactive oxygen species (ROS) than normal cells. This alteration facilitates the survival and proliferation of tumor cells.

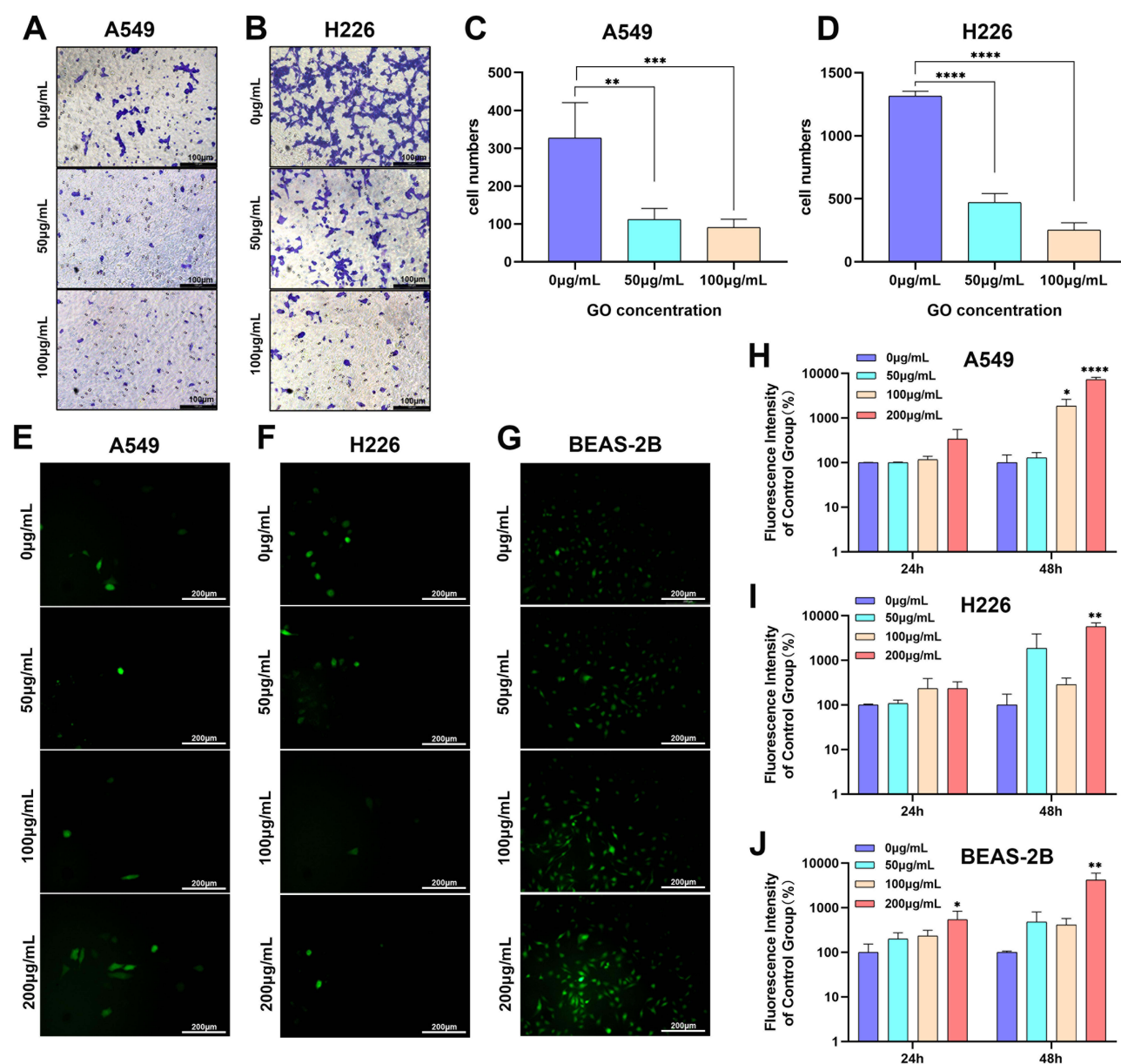


Figure 4 Transwell assay results, and fluorescence detection results of intracellular reactive oxygen species (ROS): **(A and B)** After co-culturing A549 and H226 cells with GO for 24h, resuspend them in the upper cell chamber for 24h, fix and stain the cells, and take cell photographs (scale: 100 µm). **(C and D)** Bar chart made after cell counting of cells in the field of view. Values represented the means $\pm$ S.E.M. ($n=3$), $**P<0.01$, $***P<0.001$, $****P<0.0001$. **(E–G)** A549, H226, and BEAS-2B cells were treated with GO for 24h, respectively. DCFH-DA probes were used, and fluorescence was captured under an inverted microscope (scale: 200 µm). **(H–J)** After 24h and 48h, bar charts were drawn after fluorescence quantitative analysis of cells within the field of view. Values represented the means $\pm$ S.E.M. ($n=3$), $*P<0.05$, $**P<0.01$, $***P<0.001$, $****P<0.0001$.

The Influence of Graphene Oxide on the Expression Levels of TGF- β Signaling Pathway Proteins and EMT-Related Proteins within Cells

In lung cancer cells, the epithelial-mesenchymal transition (EMT) mainly occurs through the classical TGF- β pathway. As a result, subsequent research used Western blot analysis to examine the levels of signaling proteins in this pathway. The findings demonstrated that the β -tubulin protein band appeared at 52KDa, E-cadherin at 130KDa, N-cadherin at 120KDa, and Vimentin at 55KDa. Additionally, the P-smad2/3 protein band was observed at 60KDa. Smad2/3 also appeared at 60KDa, and snail at 30KDa (Figure 5A–C). Notably, β -tubulin, serving as an internal reference protein, exhibited consistent expression across nearly all cells. This expression was unaffected by graphene oxide (GO) exposure. Subsequent analysis indicated a significant increase in E-cadherin and a decrease in Snail in A549 cells following treatment with 50 µg/mL for

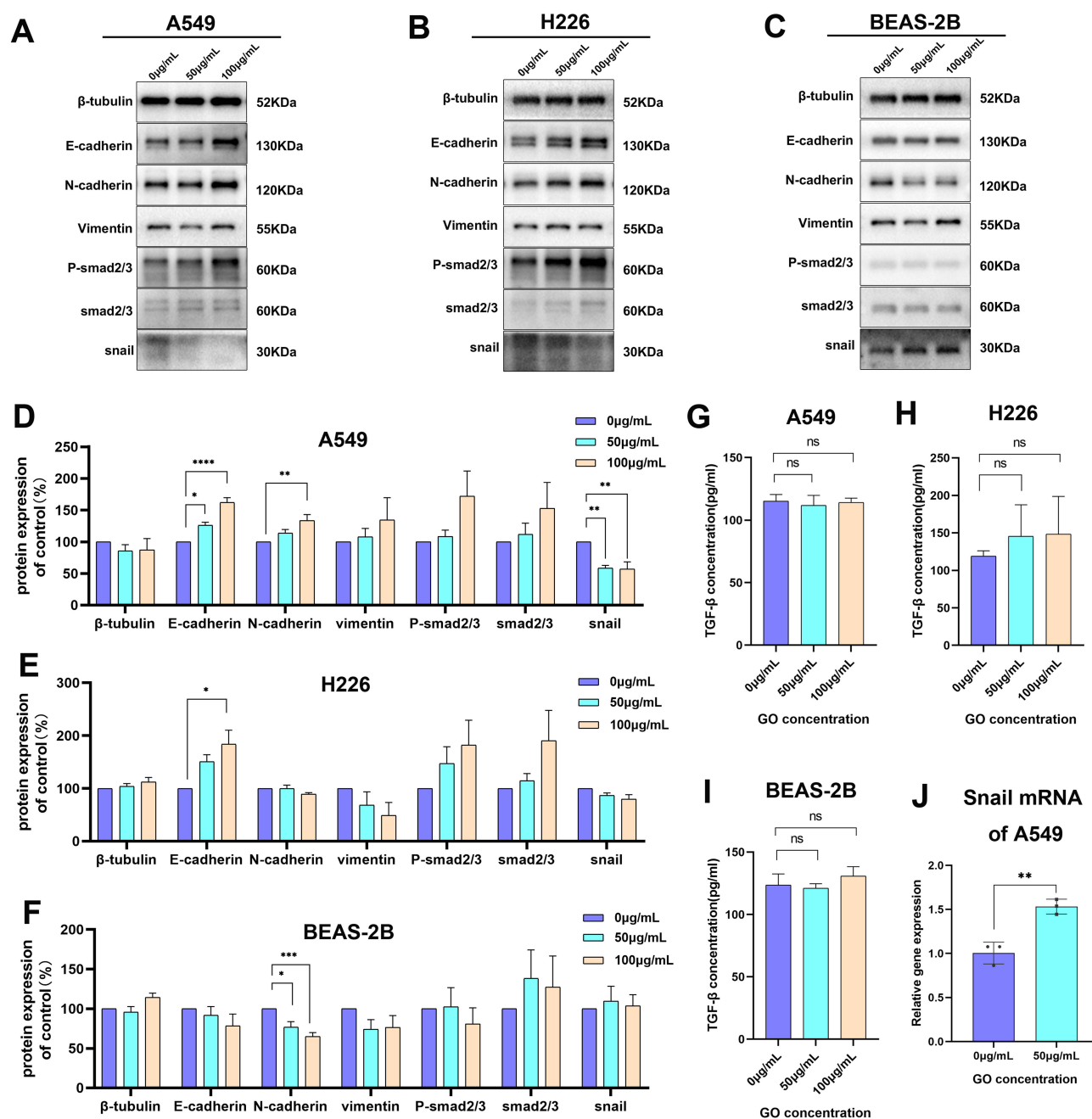


Figure 5 Western Blot, ELISA Kits, and RT-qPCR: (A–C) WB band images of A549, H226, and BEAS-2B. The left side shows the protein name, and the right side shows the protein molecular weight. The bands from left to right are 0 µg/mL, 50 µg/mL, and 100 µg/mL, respectively. (D–F) Illustrate A549, H226, and BEAS-2B cells co-cultured with 50 µg/mL and 100 µg/mL GO. The resulting bands were quantified for gray value, summarized in bar charts, and normalized to the control group (0 µg/mL). Values represent the means ± S.E.M. (n = 3), *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (G–I) TGF-β levels in the upper culture medium of A549, H226, and BEAS-2B cells after 24h of GO at 0 µg/mL, 50 µg/mL, and 100 µg/mL, where the unit of the Y-axis is pg/mL. Values represent the means ± S.E.M. (n = 3), ns: P>0.05. (J) The level of mRNA transcribed from the snail gene in A549 cells after 0 µg/mL and 50 µg/mL GO treatment for 24h (Snail mRNA). Values represent the means ± S.E.M. (n = 3), **P<0.01.

24 hours. Moreover, treatment with 100 µg/mL for the same duration resulted in increased E-cadherin, N-cadherin, and decreased Snail (Figure 5D). In H226 cells, E-cadherin levels rose only at a concentration of 100 µg/mL over 24 hours (Figure 5E). Conversely, BEAS-2B cells exhibited reduced N-cadherin expression (Figure 5F). These outcomes suggest that exposure to 50 µg/mL of graphene oxide for 24 hours effectively impedes cellular EMT progression.

The Effect of Graphene Oxide on the Secretion Level of TGF- β by Cells

Previous experiments have demonstrated that GO does not influence the upstream signaling factors of the TGF- β pathway. To further confirm that GO does not affect TGF- β release from cells, we used an ELISA kit to measure TGF- β levels released by cells. The experimental results indicate that, following GO treatment, A549, H226, and BEAS-2B cells did not show statistically significant changes in TGF- β release levels (Figure 5G–I).

The Effect of Graphene Oxide on the Expression Level of Snail mRNA in Lung Cancer Cells

In previous research, we demonstrated that GO reduced the level of Snail protein in A549 cells. To understand the changes in Snail at the genetic level, we used RT-qPCR to measure Snail mRNA levels. The results showed that Snail mRNA levels in A549 cells treated with GO at 50 $\mu\text{g/mL}$ for 24 hours were significantly higher than the control group (0 $\mu\text{g/mL}$) (Figure 5J). Notably, while Western blot analysis revealed a decrease in Snail protein levels in A549 cells (Figure 5D), RT-qPCR results showed a significant increase in Snail mRNA. This discrepancy suggests that GO likely inhibits the translation of Snail mRNA while potentially promoting Snail gene transcription through alternative pathways.

Discussion

Lung cancer, recognized as the malignant tumor with the highest incidence and mortality rates globally, presents a significant challenge that necessitates urgent attention in both clinical and basic research domains.¹² Currently, treatment options include surgical resection, radiotherapy, and chemotherapy. In advanced stages of the disease, distant metastasis represents an inevitable pathological process for patients.¹³ In addition to pulmonary dissemination, the emergence of secondary tumors in organs such as the brain, liver, and bones often results in a poor prognosis.¹⁴ For managing metastatic lesions, current clinical practice predominantly relies on chemotherapy and targeted therapy. However, the use of targeted drugs is considerably limited; it is constrained by specific gene mutation lineages (such as EGFR and ALK), and most patients ultimately develop acquired drug resistance after a brief initial response, which significantly reduces their survival. Consequently, there is an urgent need to explore new intervention strategies aimed at curbing tumor metastasis to enhance the prognosis of lung cancer.

In recent research, numerous novel treatment and diagnostic techniques have been developed successively. Ma and Du created a pH-responsive neutrophil membrane-camouflaged nanoplateform (MGF@laN NPs) for the simultaneous delivery of gallium (Ga^{3+}) and manganese (Mn^{2+}) ion complexes.¹⁵ This platform prolongs in vivo circulation, specifically targets tumor cells and metastatic microenvironments, and releases Ga^{3+} and Mn^{2+} to induce tumor cell apoptosis and impede metastasis. Longjian Huang, Shang Qiu, and Zhao Liu devised a label-free, poly(A)-regulated self-assembly technique for surface-enhanced Raman spectroscopy.¹⁶ Carcinoembryonic antigen (CEA) detection is achieved through Raman spectroscopy, demonstrating exceptional sensitivity and analytical efficacy in CEA quantification.

Epithelial-mesenchymal transition (EMT) is a critical biological process in which highly polarized and tightly connected epithelial cells transform into mesenchymal cells.¹⁷ This transition enhances their migratory and invasive capabilities in response to specific signaling cues. During this process, cells downregulate epithelial markers, including E-cadherin, while expressing interstitial markers, such as N-cadherin and vimentin.¹⁸ This results in weakened cell junctions and a reorganization of the cytoskeleton, facilitating increased motility.¹⁹ EMT plays a critical role in physiological processes, including embryonic development and wound healing; however, it is also exploitatively utilized by cancer cells as an initial step for detachment from the primary tumor, invasion, and subsequent distant metastasis.²⁰ Consequently, it represents a significant target in anti-cancer research. The most prevalent signaling pathway involved in EMT is the TGF- β pathway. Specifically, upon binding of extracellular TGF- β to the TGF- β receptor on the cell membrane, intracellular Smad2/3 is recruited by the activated TGF- β receptor and subsequently phosphorylated to form p-Smad2/3.²¹ This phosphorylated form of Smad2/3 is the active variant, which then associates with free Smad4 to create a complex that translocates to the nucleus, where it activates the Snail gene. Upon activation of the Snail gene, it primarily acts as a transcriptional suppressor. The most notable and significant target of this gene is E-cadherin, whose

deletion marks a critical event in EMT. The findings of this study demonstrate that GO can inhibit the EMT transformation of tumor cells when administered at a concentration of 50 µg/mL for 24 hours, thereby reducing cell migration. Furthermore, it does not exhibit cytotoxicity in normal BEAS-2B cells. Yet, this alteration is only evident within a brief period (24h). Over an extended duration (48h), the detrimental impact of GO on healthy cells escalates notably (Figure 2B). Hence, in studies involving GO as a cancer-fighting medication, particular emphasis must be placed on its enduring impact and biological safety.

The effects of graphene oxide (GO) on tumor cells have primarily been observed in the induction of apoptosis and the inhibition of cell migration in prior studies. For example, GO induces apoptosis and cell cycle arrest in the neuroepithelioma cell line PC12 by altering the phosphorylation levels of MEK1/2, ERK, and p-90RSK within the ERK pathway.²² Additionally, GO promotes the formation and aggregation of autophagosomes in breast cancer cell lines MDA-MB-231 and ZR-75-1, thereby initiating autophagy in these cancer cells.²¹ The nanocomplex RGO/Cu inhibits the migratory capacity of MCF in breast cancer cells by downregulating the expression of the cathepsin D and MMP9 genes.²² Notably, a previous study demonstrated that low concentrations (10 µg/mL) of GO enhanced the metastatic ability of A549 cells by facilitating the EMT process; however, this effect was inhibited at higher concentrations (20 µg/mL).²³ In contrast, the current study elucidates the mechanism by which high concentrations of GO inhibit the metastatic potential of A549 cells: GO suppresses cell migration by inhibiting Snail mRNA translation. Based on earlier research, it is hypothesized that GO may enter the cytoplasm via endocytosis or directly through the cell membrane, thereby inhibiting the synthesis of Snail proteins.

In recent years, an increasing number of researchers have integrated drugs with nanomaterials to create multi-functional complexes, yielding a diverse array of satisfactory results. Lixing Xu's study achieved the successful synthesis of a gastric acid pH-responsive hydrogel comprising curcumin/sodium alginate/polyaspartic acid@CaCO₃ (Cur/SA/PC).²⁴ This hydrogel enables sustained curcumin release, offering a potential treatment for gastric ulcers. Moreover, the SA/PC hydrogel demonstrates the ability to create a protective barrier, thereby mitigating the development of alcohol-induced acute gastric ulcers. A nanoscale drug delivery system utilizing polylactic-co-glycolic acid (PLGA) nanoparticles and a hyaluronic acid (HA) hydrogel has demonstrated sustained in vitro release of esketamine for at least 21 days, resulting in enduring and safe analgesic effects in mice.²⁵ Heng Wang developed Ber-loaded sEVs derived from hUC-MSCs and incorporated them into GelMA hydrogel for targeted delivery to the damaged tissue, facilitating localized drug release and augmenting therapeutic outcomes.²⁶

Meanwhile, many studies have examined the use of graphene oxide to form complexes with other drugs. Zhang²⁷ covalently combined Folic Acid (FA) with carboxyl groups on Graphene Oxide to obtain FA-GO (Folic acid-graphene oxide), and found that FA-GO could be stably dispersed in D-Hanks (containing phenol red) buffer. And it can target and bind to MCF-7 (human breast cancer cells). Miao²⁸ modified graphene oxide with CHA (Cladribine + Homoharringtonine + Cytarabine), and utilized the targeting property of HA to synthesize the targeted drug vector CHA-GO to load doxorubicin hydrochloride. Song²⁹ loaded doxorubicin hydrochloride into the HA-GO complex, enabling faster and more sustained release in the tumor microenvironment and thereby enhancing the anti-tumor effect. Cheng³⁰ used pegG-magnetic graphene oxide (PEG-MGO) as a drug carrier to load arsenic trioxide (ATO) and adenophora japonicum extract -O-glucobinol (SOG) in a synergistic anti-human hepatocellular carcinoma cell line (HepG2). The results showed that the cytotoxicity of ATO and SOG was greater than that of single-drug-loaded nanoparticles. And it has a distinct synergy effect.

In conclusion, a few recent articles have investigated pure graphene oxide (GO). Most studies focus on composite materials, leaving research on GO underexplored. This study shows that graphene oxide inhibits cancer cell metastasis by suppressing Snail mRNA translation. However, the mechanisms by which GO impedes the translation of Snail mRNA have not been thoroughly elucidated. Future research efforts by our group will aim to conduct more comprehensive investigations.

Conclusion

In this study, we reveal for the first time the mechanism by which GO inhibits EMT in tumor cells. The proposed mechanism is as follows: GO enters the cytoplasm via endocytosis or directly through the cell membrane, subsequently

inhibiting Snail mRNA translation. This action reduces Snail protein levels, increases E-cadherin expression, and inhibits EMT in A549 cells, ultimately leading to decreased cancer cell migration. Additionally, this study is the first to conduct a comparative analysis between tumor cells (A549, H226) and normal bronchial epithelial cells (BEAS-2B). Under specific conditions, GO shows no cytotoxicity toward normal cells. This finding holds significant implications for the broader application of GO in the development of anti-tumor drugs. Undoubtedly, further research is necessary to elucidate the mechanism by which GO inhibits Snail mRNA translation.

Ethics Approval and Informed Consent

The Institutional Review Board (IRB) of Wuhan University of Science and Technology has approved the use of the cell line in this study.

Consent for Publication

This article contains no individual person's data, images, or videos in any form. All data presented are derived from in vitro cell culture experiments.

Funding

This work was funded by the Scientific Research Program of Hubei Provincial Department of Education (Q20211113).

Disclosure

There is no conflict of interest.

References

- Gao Y, Zhang JF, Li QC, et al. The clinicopathological and prognostic features of Chinese and Japanese inpatients with lung cancer. *Oncotarget*. 2016;7(41):67425–67434. doi:10.18632/oncotarget.11850
- Zhang ZY, Jiang AN, Yang W, et al. Percutaneous radiofrequency ablation is an effective method for local control of liver metastases from lung cancer. *Front Oncol*. 2022;12:877273. doi:10.3389/fonc.2022.877273
- Jiang Q, Wei Z, Liu P, et al. Global trends and research hotspots in perioperative management of lung cancer: a bibliometric analysis from 2004 to 2024. *Front Immunol*. 2024;15:1500686. doi:10.3389/fimmu.2024.1500686
- Zeng Z, Li Y, Zhou H, et al. System-wide identification of novel de-ubiquitination targets for USP10 in gastric cancer metastasis through multi-omics screening. *BMC Cancer*. 2024;24(1):773. doi:10.1186/s12885-024-12549-3
- Dong P, Konno Y, Watari H, Hosaka M, Noguchi M, Sakuragi N. The impact of microRNA-mediated PI3K/AKT signaling on epithelial-mesenchymal transition and cancer stemness in endometrial cancer. *J Transl Med*. 2014;12:231. doi:10.1186/s12967-014-0231-0
- Jiang W, Wang PY, Zhou Q, et al. Tri©DB: an integrated platform of knowledgebase and reporting system for cancer precision medicine. *J Transl Med*. 2023;21(1):885. doi:10.1186/s12967-023-04773-5
- Geim AK, Novoselov KS. The rise of graphene. *Nat Mater*. 2007;6(3):183–191. doi:10.1038/nmat1849
- Liu H, Li T, Liu Y, Qin G, Wang X, Chen T. Glucose-reduced graphene oxide with excellent biocompatibility and photothermal efficiency as well as drug loading. *Nanoscale Res Lett*. 2016;11(1):211. doi:10.1186/s11671-016-1423-8
- Chang Q, Huang J, Ding Y, Tang H. Catalytic oxidation of phenol and 2,4-Dichlorophenol by using horseradish peroxidase immobilized on graphene Oxide/Fe₃O₄. *Molecules*. 2016;21(8):1044. doi:10.3390/molecules21081044
- Tian X, Yang Z, Duan G, et al. Graphene oxide nanosheets retard cellular migration via disruption of actin cytoskeleton. *Small*. 2017;13(3):1602133. doi:10.1002/smll.201602133
- Zhao M, Liu Y, Qu H. Expression of epithelial-mesenchymal transition-related genes increases with copy number in multiple cancer types. *Oncotarget*. 2016;7(17):24688–24699. doi:10.18632/oncotarget.8371
- Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. doi:10.3322/caac.21834
- Pei Q, Luo Y, Chen Y, Li J, Xie D, Ye T. Artificial intelligence in clinical applications for lung cancer: diagnosis, treatment and prognosis. *Clin Chem Lab Med*. 2022;60(12):1974–1983. doi:10.1515/cclm-2022-0291
- London D, Patel DN, Donahue B, et al. The incidence and predictors of new brain metastases in patients with non-small cell lung cancer following discontinuation of systemic therapy. *J Neurosurg*. 2022;137(2):544–554. doi:10.3171/2021.9.Jns212150
- Ma G, Du S, Li X, et al. pH-responsive neutrophil membrane camouflage Ga–Mn bimetallic nanodecoy triggers apoptosis-immunity-metastasis suppression for tumor therapy. *Biomaterials*. 2026;327:123794. doi:10.1016/j.biomaterials.2025.123794
- Huang L, Qiu S, Liu Z, et al. Proximity hybridization induced DNA assembly for label-free surface-enhanced Raman spectroscopic detection of carcinoembryonic antigen. *Anal Chim Acta*. 2022;1191:339314. doi:10.1016/j.aca.2021.339314
- Sheng L, Zhuang S. New insights into the role and mechanism of partial epithelial-mesenchymal transition in kidney fibrosis. *Front Physiol*. 2020;11:569322. doi:10.3389/fphys.2020.569322
- Semba S, Kodama Y, Ohnuma K, et al. Direct cancer-stromal interaction increases fibroblast proliferation and enhances invasive properties of scirrhous-type gastric carcinoma cells. *Br J Cancer*. 2009;101(8):1365–1373. doi:10.1038/sj.bjc.6605309

19. Han G, Wang Y, Liu T, et al. Salvianolic acid B acts against non-small cell lung cancer A549 cells via inactivation of the MAPK and Smad2/3 signaling pathways. *Mol Med Rep.* **2022**;25(5):184. doi:10.3892/mmr.2022.12700
20. Kang Y, Liu J, Wu J, et al. Graphene oxide and reduced graphene oxide induced neural pheochromocytoma-derived PC12 cell lines apoptosis and cell cycle alterations via the ERK signaling pathways. *Int J Nanomed.* **2017**;12:5501–5510. doi:10.2147/ijn.S141032
21. Krętowski R, Cechowska-Pasko M. The Reduced Graphene Oxide (rGO) induces apoptosis, autophagy and cell cycle arrest in breast cancer cells. *Int J Mol Sci.* **2022**;23(16):9285. doi:10.3390/ijms23169285
22. Kodous AS, Atta MM, Abdel-Hamid GR, Ashry HA. Anti-metastatic cancer activity of ultrasonic synthesized reduced graphene oxide/copper composites. *Chem Papers.* **2022**;76(1):373–384. doi:10.1007/s11696-021-01866-7
23. Liao Y, Wang W, Huang X, Sun Y, Tian S, Cai P. Reduced graphene oxide triggered epithelial-mesenchymal transition in A549 cells. *Sci Rep.* **2018**;8(1):15188. doi:10.1038/s41598-018-33414-x
24. Xu L, Du X, Zhou Y, et al. Polyaspartic Acid-Stabilized CaCO₃-containing in situ hydrogel for protection and treatment of gastric ulcer. *Mol Pharm.* **2023**;20(4):2105–2118. doi:10.1021/acs.molpharmaceut.2c01062
25. Zhang H, Zhou P, Jiang Y, et al. Sustained-release esketamine based nanoparticle-hydrogel delivery system for neuropathic pain management. *Int J Nanomed.* **2023**;18:1131–1143. doi:10.2147/ijn.S400798
26. Wang H, Tang Q, Lu Y, et al. Berberine-loaded MSC-derived sEVs encapsulated in injectable GelMA hydrogel for spinal cord injury repair. *Int J Pharm.* **2023**;643:123283. doi:10.1016/j.ijpharm.2023.123283
27. Zhang L, Xia J, Zhao Q, Liu L, Zhang Z. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. *Small.* **2010**;6(4):537–544. doi:10.1002/smll.200901680
28. Miao W, Shim G, Kang CM, et al. Cholesteryl hyaluronic acid-coated, reduced graphene oxide nanosheets for anti-cancer drug delivery. *Biomaterials.* **2013**;34(37):9638–9647. doi:10.1016/j.biomaterials.2013.08.058
29. Song E, Han W, Li C, et al. Hyaluronic acid-decorated graphene oxide nanohybrids as nanocarriers for targeted and pH-responsive anticancer drug delivery. *ACS Appl Mater Interfaces.* **2014**;6(15):11882–11890. doi:10.1021/am502423r
30. Cheng J, Hong K, Sun J, et al. PEGylated magnetic nanographene oxide for targeted delivery of arsenic trioxide and sec-o-glucosylhamaudol in tumor treatment with improved dual-drugs synergistic effect. *AAPS Open.* **2023**;9(1):12.

International Journal of Nanomedicine

Publish your work in this journal

The International Journal of Nanomedicine is an international, peer-reviewed journal focusing on the application of nanotechnology in diagnostics, therapeutics, and drug delivery systems throughout the biomedical field. This journal is indexed on PubMed Central, MedLine, CAS, SciSearch®, Current Contents®/Clinical Medicine, Journal Citation Reports/Science Edition, EMBase, Scopus and the Elsevier Bibliographic databases. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/international-journal-of-nanomedicine-journal>

Dovepress
Taylor & Francis Group