

A monoclonal antibody against basic fibroblast growth factor attenuates cisplatin resistance in lung cancer by suppressing the epithelial-mesenchymal transition

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Abstract

Objectives: To investigate the underlying mechanisms of how the basic fibroblast growth factor monoclonal antibody (bFGFmAb) attenuates cisplatin (DDP) resistance in lung cancer using A549 cells and cisplatin-resistant A549 cells (A549/DDP). **Methods:** Cancer cell proliferation, cell viability, and 50% inhibitory concentration (IC50) of cisplatin were assessed. Transwell assays were utilized to evaluate the invasion activity of tumor cells in response to treatment. Epithelial-to-mesenchymal transition markers and drug resistance proteins were analysed using Western blots. **Results:** We demonstrate that the bFGFmAb inhibits the proliferation and invasion of both A549 and A549/DDP cells. The bFGFmAb increases cisplatin sensitivity of both A549 and A549/DDP cells as evidenced by an increase in the IC50 of cisplatin in A549 and A549/DDP cells. Furthermore, bFGFmAb significantly increases the expression of E-cadherin, whilst decreasing the expression of N-cadherin and bFGF in both cell lines, thereby showing inhibition of epithelial-to-mesenchymal transition. In addition, we demonstrate that bFGFmAb significantly reduces the expression of the lung resistance protein. **Conclusions:** Our data suggests that the humanized bFGFmAb is a promising agent to attenuate cisplatin resistance in NSCLC. The underlying mechanism for this effect of bFGFmAb may be associated with the inhibition of epithelial-to-mesenchymal transition and reduced expression of lung resistance protein.

Keywords

Non-small cell lung cancer, cisplatin-resistance, basic fibroblast growth factor monoclonal antibody, epithelial-mesenchymal transition, chemotherapy

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Introduction

Lung cancer is the most prevalent cancer type and the leading cause of cancer-related deaths worldwide, responsible for 19.4% of total cancer deaths.^{1,2} Among the different histological types of lung cancer, non-small cell lung cancer (NSCLC) accounts for approximately 85% of lung cancer.^{3,4} Despite great advancement in cancer treatment, platinum-based chemotherapy, like cisplatin, remains the first-line therapy for advanced NSCLC patients that lack targetable mutations.⁵ For patients with high expression of programmed death ligand 1 (PDL-1), 50% or greater, pembrolizumab, an immune checkpoint inhibitor (ICI), has recently replaced cytotoxic chemotherapy as the first-line treatment of choice. However, ICI treatment is not suitable for patients that lack PDL-1 expression,⁵ therefore the cisplatin-based doublet regimen remains the foundation of treatment for the majority of patients with advanced NSCLC.⁶ Intrinsic and acquired drug resistance remains problematic. To enhance the efficacy and to minimize the development of drug resistance, the chemotherapeutic management of NSCLC patients usually involves combining the use of platinum-derived salts (such as cisplatin) with a second or third generation drug such as gemcitabine, pemetrexed, or taxanes.⁷ One of the most challenging issues in the treatment of advanced NSCLC patients is acquired drug resistance, leading to morbidity and mortality of NSCLC patients, despite advancement in chemotherapy and targeted therapy.⁷⁻⁹ Thus, strategies to counter drug resistance and to increase therapeutic efficacy are urgently needed.

The molecular mechanisms underlying cisplatin resistance in cancer treatment are not fully understood. The main mechanisms are believed to be associated with a number of factors including induction of anti-apoptotic signals, the active efflux of cisplatin from the cell, tolerance to DNA lesion, epigenetic regulation by microRNAs.^{10,11} Epithelial-to-mesenchymal transition (EMT), a process involving in the transformation of static epithelial cells into mobile mesenchymal cells, is also considered to be a significant factor in chemoresistance related to the use cisplatin.¹² In recent years, increasing studies have shown that EMT plays a key role in the drug resistance of lung cancer. EMT reduced the sensitivity of lung cancer cells to antitumor drugs and produced drug resistance through the regulation of multiple signal pathways. Report by Shintani et al. demonstrated that EMT resulted in increased malignant potential and reduced sensitivity to cisplatin and paclitaxel in NSCLC cells.¹³ Resistance of NSCLC to EGFR TKIs is accompanied by the activation of EMT and downstream PI3K/Akt and MAPK/ERK signaling pathways, suggesting that reversing EMT is expected to reverse the acquired resistance of EGFR TKIs.¹⁴ Fuchs et al. also demonstrated EMT mediate sensitivity to epidermal growth factor

receptor inhibition in human hepatoma cells.¹⁵ Since bFGF is one of the important factors inducing EMT,¹⁶ and bFGF protein is highly expressed in patients with malignant tumors.^{17,18} Therefore, targeting bFGF involving in EMT process may become a new strategy for tumor treatment. However, research examining EMT change following bFGF blocking in resistant lung cancer has not been conducted.

The basic fibroblast growth factor (bFGF) belongs to the family of heparin-binding growth factors, which mediates a series of cellular responses in embryonic development, haematopoiesis, wound healing, tumor growth, and tumor angiogenesis.¹⁹ It utilizes heparan sulfate proteoglycans (HSPGs) as binding partners to bind to specific tyrosine kinase transmembrane receptors, fibroblast growth factor receptors (FGFRs), to form a ternary bFGF-FGFR-HSPG complex.^{20,21} Increased expression of bFGF has been frequently observed in several types of human malignancies, such as hepatocellular carcinoma, and melanoma.¹⁹⁻²¹ The blockade of the bFGF pathway has been shown to impede tumor progression and bFGF-targeting peptides and vaccinations have demonstrated inhibition of angiogenesis and tumor growth in melanoma xenografts.²²⁻²⁴ Previously, our group demonstrated that a bFGFmAb combined with chemotherapy²⁵ or/and radiotherapy^{26,27} inhibited the growth of transplanted cancers by reducing bFGF expression, resulting in a decrease in tumor angiogenesis and an increase in cell apoptosis. Moreover, this bFGFmAb played a critical role in the inhibition of MCF-7/ADM (doxorubicin-resistant MCF-7 breast cancer cells) proliferation. Furthermore, this bFGFmAb reversed doxorubicin resistance in breast cancer cells by reducing the drug efflux through downregulation of P-glycoprotein.²⁸ All these studies demonstrate that bFGFmAb plays a role in inhibiting the development of resistance to chemotherapeutic drugs. However, no studies have been performed to identify the underlying mechanism of bFGFmAb as an agent to prevent chemotherapy resistance.

In the present study, utilizing the NSCLC cell line A549 and cisplatin-resistant cell line A549/DDP, we investigated the effects of an in-house developed humanized bFGFmAb on cisplatin resistance and their underlying mechanisms involving the suppression of EMT and reduction in lung-resistant protein (LRP) expression. Findings from this study may provide new treatment targets and novel insights to overcome chemotherapy drug resistance.

Materials and methods

The study described here was to investigate the antitumor property and associated molecular mechanism of a humanized bFGFmAb that was developed from our

laboratory. The details of how the study was performed were described in the following seven sections.

Cell lines and cell culture

The A549 cell line (CRM-CCL-185) was obtained from the Guangdong Provincial Key Laboratory of Molecular Immunology and Antibody Engineering (GPKLMIAE) at Jinan University, and the cisplatin-resistant A549/DDP cell line, a derivative of A549 cells (CRM-CCL-185), was purchased from Yinzijing Biological Pharmaceutical Technology Co., Ltd (Beijing, China). Cells were routinely cultured in RPMI 1640 medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and incubated at 37°C in a humidified incubator (Thermo, Japan) with 5% CO₂. Only cells in the exponential growth phase were used for all experiments. The bFGF used in this study was purchased from PeproTech Co., Ltd (New Jersey, USA). The bFGFmAb was obtained from the GPKLMIAE at Jinan University. The purity of the bFGFmAb stock solution was determined by the sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) method. Titers and concentrations of purified bFGFmAb stock solutions were assayed using indirect enzyme-linked immunosorbent assay (ELISA) tests and the bicinchoninic acid (BCA) standard assay kits (Pierce, USA), respectively. Finally, the purified bFGFmAb stock solution was stored at -20°C for further experiments. Experimental groups were treated with bFGF or/and bFGFmAb or/and DDP, while only phosphate buffer (PBS) was added to the control group.

Cell proliferation and toxicity tests

The cell-counting kit-8 (CCK-8) colorimetric assays (Dojindo Laboratories, Japan) were performed to determine the effects of bFGF and bFGFmAb on lung cancer cells according to the manufacturer's protocol. The experiments were divided into four groups (A549+bFGF, A549+bFGFmAb, A549/DDP+bFGF, and A549/DDP+bFGFmAb). The bFGF concentration was adjusted to 10, 20, 40, 80, and 160 ng/mL, whereas the concentration of the bFGFmAb was set as 3.125, 6.25, 12.5, 25, 50, and 100 µg/mL. A549 and A549/DDP cells were first seeded into 96-well plates (3×10^4 cells/well) and treated with different concentrations of bFGF or bFGFmAb. Cells were cultured 37°C with 5% CO₂ for 48 h, the medium removed and replaced with 90 mL of RPMI 1640 medium and 10 mL of CCK-8 solution. Following incubation for 2 h, the absorbance was measured at a wavelength of 450 nm using a microplate reader (Thermo, Japan). Each sample was plated in triplicate wells, and the experiment was repeated at least three times. Finally, the proliferation and inhibition rates of tumor cells were calculated.

Cisplatin resistance test

CCK-8 colorimetric assays were used to examine the effects of bFGF and bFGFmAb on cisplatin sensitivity of NSCLC cells. The experiments were divided into six groups including A549+PBS+DDP, A549+bFGF+DDP, A549+bFGFmAb +DDP, A549/DDP+PBS+DDP, A549/DDP+bFGF+DDP, and A549/DDP+bFGFmAb +DDP. The concentration of bFGF and bFGFmAb were 80 ng/mL and 100µg/mL, respectively, while cisplatin concentrations were set to a different gradient (0.25, 0.5, 1, 2, 4, and 8 µg/mL). The CCK-8 assays were performed according to the manufacturer's protocol. Firstly, tumor cells (3×10^4 cells/well) were seeded into 96-well plates treated with bFGF or bFGFmAb or PBS and DDP. Secondly, after culturing for 48 h, the cultured medium was removed and replaced with 90 mL RPMI 1640 medium and 10 mL CCK-8 solution. Thirdly, following incubation for 2 h, the absorbance was measured at a wavelength of 450 nm. Each sample was plated in triplicate wells, and experiments were repeated at least three times. Finally, the IC₅₀ of cisplatin in each group was calculated.

Assessment of the cell morphology

The NSCLC cell lines, A549 and A549/DDP, were seeded into six-well plates (2.5×10^5 /well). After being cultured in RPMI 1640 medium supplemented with 10% FBS for 24 h, the cells were treated with bFGF (80 ng/mL) or/and bFGFmAb (100µg/mL) or PBS by directly adding them into the cell culture medium. Twenty-four hours later, the cells were photographed under a microscope (Olympus, Japan).

Transwell invasion assay

Transwell assays were used to evaluate the invasiveness of tumor cells (A549 and A549/DDP) in response to treatments (bFGF and bFGFmAb). The assays were performed according to the manufacturer's protocol. Logarithmic growth phase cells (1×10^5) were seeded into the upper compartment of a Transwell chamber (354480; BD Biosciences) with 0.1mL of serum-free medium, followed by addition of bFGF (80 ng/mL) and/or bFGFmAb (100 µg/mL) or PBS. Complete RPMI 1640 medium (0.6 mL) containing 10% FBS was added to the lower chamber as the chemical attractant. After incubation for 12 h, non-invasive cells were swabbed from the upper chamber, the cells attached to the lower filter surface were fixed with methanol then stained with 0.1% crystal violet. Finally, the number of cells was calculated under a light microscope (Olympus, Japan) in five randomly selected views (original magnification 200×).

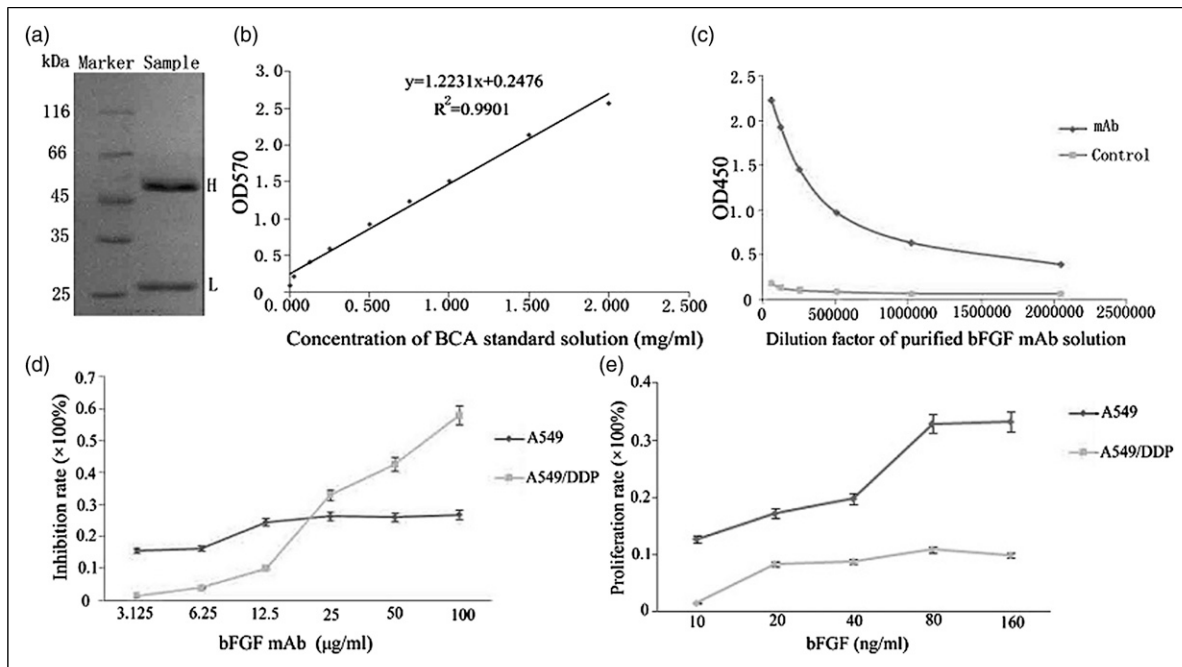


Figure 1. The bFGFmAb and its characteristics. (a) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified bFGFmAb (H, heavy chain; L, light chain). (b) Concentration of the purified bFGFmAb stock solution determined by bicinchoninic acid (BCA) standard assays. (c) Titer of the purified bFGFmAb stock solution tested by indirect ELISA. (d) The bFGFmAb inhibits the proliferation of A549 and A549/DDP cells. (e) Treatment with bFGF increases the proliferation in A549 and A549/DDP cells. bFGF, basic fibroblast growth factor; DDP, cisplatin; ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; OD, optical density.

Western blot analysis

Tumor cells (A549, or A549/DDP) were seeded into six-well plates (2.5×10^5 /well). After 24 h culture, the cells were treated with bFGFmAb 100 μ g/mL or PBS by adding them directly into the culture medium. On the next day, the cells were lysed with RIPA lysis buffer for 30 min at 4°C and centrifuged at 12,000 g for 15 min. A Pierce BCA Protein Assay Kit (Thermo Fisher Scientific) was used to determine protein concentrations following the manufacturer's instructions. The protein was separated by 10% SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). After blocking with 5% blotto (skimmed milk) in Tris-buffered saline and Tween 20 for 1 h, the primary antibodies rabbit monoclonal anti-bFGF (CST, USA; 1:1000 dilution), mouse monoclonal anti-E-cadherin (CST, 1:1000 dilution), rabbit polyclonal anti-N-cadherin (Abcam, UK; 1:1000 dilution), rabbit polyclonal anti-LRP (Santa Cruz, USA, 1:200 dilution), and mouse monoclonal anti- β -actin (CST, USA, 1:1000 dilution) were added and incubated at 4°C overnight. After incubation with the secondary antibody goat anti-rabbit IgG-HRP (GE Healthcare, 1:1000 dilution), the bound antibodies were detected using the ECL Plus Western Blotting Detection system (Life Technologies, USA). Finally, the intensities of the bands were analyzed using the BandsScan Software.

Statistical analysis

Each experiment was independently repeated at least three times. All statistical analyses were performed using SPSS 25.0 (IBM, USA). Descriptive data are presented as the mean \pm standard deviation. The two-tailed Student's t-test was used to perform comparisons between experimental groups. A p -value < 0.05 was considered to indicate statistical significance.

Results

Characteristics of the bFGFmAb

As shown in Figure 1(a), only two bFGFmAb chains are presented with no additional nonspecific chains, confirming the high purity of the bFGFmAb used. The upper band represents the heavy chain of the antibody with a molecular weight of about 50 kDa, and the lower band shows the light chain with a molecular weight of about 26 kDa. To determine the antibody concentration, a BCA standard curve with the optical density value at 570 nm as the y-value and the BCA concentration as the x-value was generated. The resulting formula was $y = 1.2231x + 0.2476$ ($R^2 = 0.9901$). Thus, the concentration of the bFGFmAb was determined as 15 mg/mL (Figure 1(b)). The

bFGFmAb titer assessed using indirect ELISA was approximately 1:500,000 (Figure 1(c)).

bFGFmAb inhibits the proliferation of NSCLC cells

To study the influence of bFGF and bFGFmAb on the proliferation of NSCLC cells, we performed CCK-8 assays using the A549 and A549/DDP cell lines. As shown in Figure 1(d), with the increase of bFGFmAb, the inhibition rate of both cells increases gradually. When the bFGFmAb concentration was higher than 12.5 $\mu\text{g/mL}$, the inhibition rate of A549/DDP cells became more efficient, which reached the maximum value (58%) at 100 $\mu\text{g/mL}$. In contrast, bFGF promotes tumor cell proliferation (Figure 1(e)). When bFGF concentration $>40\text{ng/mL}$, the increase of proliferation rate in A549 cells was the most significant.

Table 1. Effects of bFGF and bFGFmAb on cisplatin resistance in non-small cell lung cancer.

Group	IC50 ($\mu\text{g/mL}$)
A549	3.04 ± 1.2
A549 + bFGF	$4.31 \pm 2.0^*$
A549 + bFGFmAb	$2.10 \pm 0.8^*$
A549/DDP	$6.15 \pm 1.7^*$
A549/DDP + bFGF	6.28 ± 1.2
A549/DDP + bFGFmAb	$3.78 \pm 0.5^*$

Data are presented as the mean \pm standard deviation. Treatment with bFGFmAb led to increased sensitivities of cisplatin in both A549 and A549/DDP cells when compared with the nontreated group ($p < 0.05$). * $p < 0.05$. A549, lung adenocarcinoma cell; A549/DDP, cisplatin resistance cell; bFGF, basic fibroblast growth factor; DDP, cisplatin; IC50, 50% inhibitory concentration; mAb, monoclonal antibody.

However, there was little change in the proliferation of the A549/DDP cells, A549/DDP cells were not sensitive to exogenous bFGF.

bFGFmAb reverses cisplatin resistance in NSCLC cells

CCK-8 assays were used to investigate the effect of bFGF and bFGFmAb on NSCLC cisplatin resistance in A549 cells and A549/DDP cell lines. The results are summarized in Table 1. We observed that A549/DDP cells exhibit more resistance to cisplatin compared to A549 cells ($p < 0.05$). Administration of bFGF increases the resistance to cisplatin in both cell lines, especially in A549 cells ($p < 0.05$). By contrast, bFGFmAb decreases the cisplatin resistance in both examined cell lines. This is most apparent in A549/DDP cells; the average IC50 value of 6.15 $\mu\text{g/mL}$ significantly decreased to 3.78 $\mu\text{g/mL}$ in the presence of the bFGFmAb ($p < 0.05$). These results suggest that bFGF significantly increases the cisplatin resistance in NSCLC, whereas the bFGFmAb was able to reverse this.

bFGFmAb inhibits morphological changes associated with EMT

To explore the association between bFGFmAb treatment and EMT in lung cancer cells, the effects of the bFGFmAb on morphological changes in A549 and A549/DDP cells were observed. After treatment with bFGF, both A549 and A549/DDP cells show morphological features of an EMT phenotype (Figure 2). As shown in Figure 2, A549 cells morphology change from a long fusiform and spindle

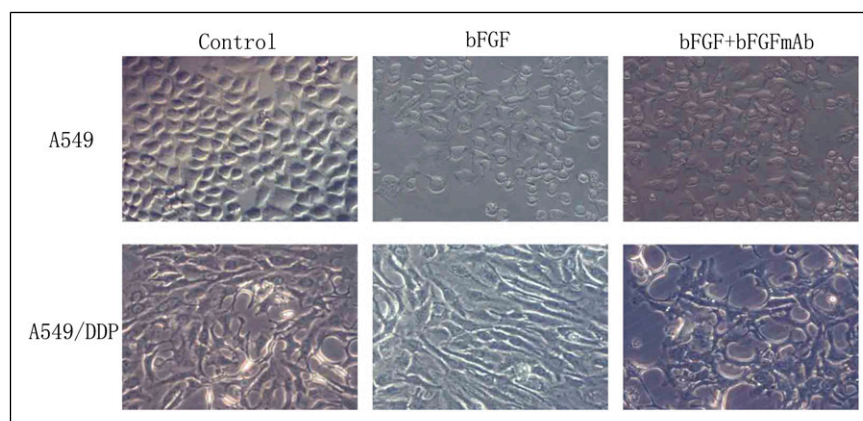


Figure 2. Effects of the bFGFmAb on the morphology of non-small cell lung cancer cells. EMT cell morphology is evident in both A549 and A549/DDP cells after treatment with bFGF. A549 cells become a long fusiform and spindle shaped phenotype, while A549/DDP cells transform to a long, thin and more irregular shape. Treatment of the cells with bFGFmAb attenuates/reverses the bFGF-induced EMT transformation. bFGF, basic fibroblast growth factor; DDP, cisplatin; EMT, epithelial-to-mesenchymal transition; mAb, monoclonal antibody.

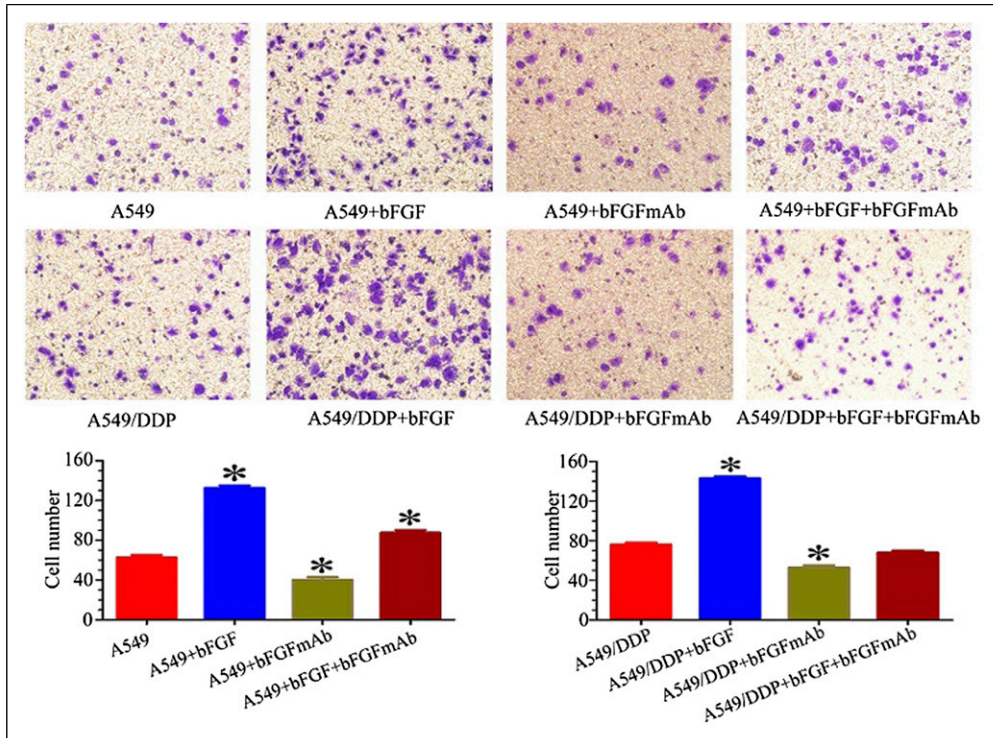


Figure 3. bFGFmAb suppresses cell invasiveness in both A549 and A549/DDP cells. Cell invasion was detected using transwell assays. Treatment of the cells with bFGF significantly enhances cell invasion in both A549 and A549/DDP cells, whereas the bFGFmAb exhibits the opposite effect by reducing the number of invading cells. Their effects on cell invasion are cancelled out each other when both bFGF and bFGFmAb are added immutaneously to the cultured cells. * $p < 0.05$. bFGF, basic fibroblast growth factor; DDP, cisplatin; mAb, monoclonal antibody.

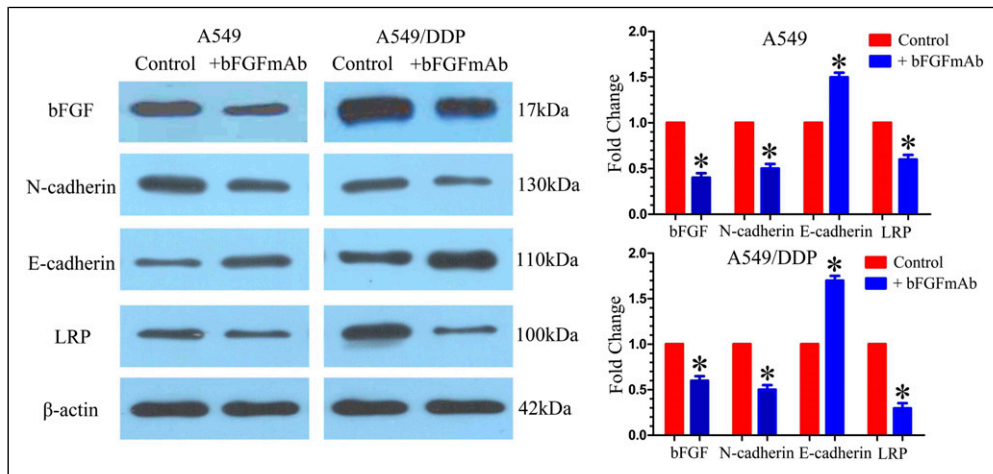


Figure 4. The bFGFmAb regulates markers for EMT and suppresses LRP expression. Treatment with bFGFmAb leads a significantly decrease in expression of bFGF, N-cadherin, and LRP, and a significantly increases in the expression of E-cadherin in both A549 and A549/DDP cells. N-cadherin and E-cadherin are EMT markers. * $p < 0.05$. bFGF, basic fibroblast growth factor; DDP, cisplatin; EMT, epithelial-to-mesenchymal transition; LRP, lung resistance protein; mAb, monoclonal antibody.

shaped phenotype, whereby the pseudopods become slender and numerous. A549/DDP cells transform to a long, thin and more irregular shape, in addition, there is an increase in the number of pseudopods and dendrites

present. Moreover, intercellular connections are less frequently observed. In the presence of the bFGFmAb, these morphological changes consistent with EMT are inhibited or reversed indicating that bFGF plays a key role

in EMT, and bFGFmAb effectively blocks the process. This suggests that the bFGFmAb may have therapeutic potential.

bFGFmAb inhibits the invasiveness of lung cancer cells

Transwell assays were utilized to examine the effects of bFGF and bFGFmAb on lung cancer cell invasion. As shown in Figure 3, bFGF treatment significantly enhances cell invasion, whereas the bFGFmAb exhibits the opposite effect by reducing the number of invading cells ($p < 0.05$). When both bFGF and bFGFmAb were simultaneously added to the cultured cells, their effects on cell invasion cancelled each other out. This suggests that bFGF plays an important role in cancer cell invasion leading to a deterioration in lung cancer, and bFGFmAb is a potential candidate drug that may slow down the proliferation and metastasis processes in NSCLC.

bFGFmAb inhibits EMT and decreases drug resistance markers

To determine whether the bFGFmAb plays a role in EMT and drug resistance and to clarify whether it activates the corresponding signaling proteins, western blotting was performed to measure the levels of bFGF, N-cadherin, E-cadherin, and LRP in the A549 and A549/DDP cell models (Figure 4). As shown in Figure 4, after bFGFmAb treatment, the expression of E-cadherin significantly increases while bFGF, N-cadherin, and LRP expression levels significantly decrease in both A549 and A549/DDP cells (all $p < 0.05$). These results suggest that the bFGFmAb can inhibit EMT and reduce drug resistance in NSCLC.

Discussion

Resistance to chemotherapy is a major problem in chemotherapy leading to failure of long-term effective treatment, and is the most challenging issue in the treatment of advanced NSCLC patients. Here we report a novel strategy using bFGFmAb to effectively reverse cisplatin resistance in lung cancer cells. Utilising an in-house humanized bFGFmAb and human NSCLC cell lines A549 and the A549/DDP, we demonstrate that bFGFmAb effectively inhibits the proliferation and invasion of both NSCLC cancer cells and cisplatin resistance cancer cells. Here we demonstrate the specific bFGF antibody significantly enhances cisplatin sensitivity (IC₅₀) in both A549 and A549/DDP cells. Further, we demonstrate that EMT suppression and LRP downregulation appear to be the mechanism of action for bFGFmAb as anti-cisplatin resistance agent. These findings pave the way for a new antibody-based treatment strategy targeting chemotherapy resistance.

The platinum-based regimen is one of the most important therapeutic methods for NSCLC patients. However, inherent and acquired resistance of tumor cells to cisplatin has led to the failure of treatment in patients with NSCLC. Thus, new strategies to enhance the sensitivity of NSCLC cells to current therapeutic drugs are required.

The bFGF (FGF-2) is an oncogenic factor, an important secreted cytokine and encodes heparin-binding proteins with growth, proliferation, differentiation, and angiogenic activity.²⁹ Deregulation of bFGF/FGFR signaling in cancer cells is correlated with pathogenesis, lymph node metastasis, and prognosis.³⁰ Moreover, fibroblasts can mediate resistance to treatment via bFGF secretion in advanced stages of cancer.³¹ It has been reported that bFGF is one of the epigenetic mechanisms underlying multidrug resistance of tumors.³² Indeed, bFGF confers chemoresistance by inducing apoptosis in small cell lung cancer and breast cancer.^{33–35} Thus, targeting bFGF/FGFR signaling may provide an opportunity to improve the efficacy of chemotherapy. It has been reported that various molecules can inhibit bFGF (ligand) activity, binding, or expression in endothelial and tumor cells.³⁶ For instance, FP-1039, a type of bFGF ligand trap, inhibits bFGF-mediated cell proliferation and angiogenesis in lung and endometrial cancer models.³⁷ Also, small molecules, such as sm27, pentosan, and pentraxin-3 inhibit bFGF binding to FGFRs.³⁸

The bFGFmAb used in the current study is a humanized monoclonal antibody developed and produced by our group.²⁶ Previously, we produced a murine bFGFmAb, that effectively suppresses tumor growth through the inhibition of angiogenesis and the induction of apoptosis in melanoma.³⁹ Furthermore, we have demonstrated that bFGFmAb combined with gimeracil and oteracil potassium (S-1) exerts a synergistic inhibitory effect on the growth and angiogenesis of Lewis-transplanted lung cancers.²⁵ Moreover, combination of bFGFmAb and radiotherapy synergistically inhibits proliferation of B16-transplanted melanoma tumors and hepatocellular carcinoma.²⁷ The bFGFmAb also inhibits the proliferation and reverses multidrug resistance in MCF-7/ADM breast cancer cells via downregulation of P-glycoprotein.²⁸ In the present study, the humanized bFGFmAb demonstrates an enhancement of cisplatin sensitivity in both A549 cells and A549/DDP cells (cisplatin-resistant lung cancer). This antibody decreases the invasiveness of A549 and A549/DDP cells, suggesting that the bFGFmAb can efficiently suppress invasion in cisplatin-resistant NSCLC. Thus, these findings support that bFGFmAb has potential as an effective drug to increase cisplatin sensitivity in cisplatin-resistant NSCLC cells.

EMT is a process involved in cancer cell migration, invasion, and metastatic dissemination. During EMT, epithelial traits, including E-cadherin, plakoglobin, and cytotokeratins, as well as the dismantling of adherent junctions

and desmosomes, are lost, whereas mesenchymal characteristics, such as N-cadherin, vimentin, and fibronectin are acquired.^{40,41} This phenomenon is considered to be an important event during malignant tumor metastasis and drug resistant development.^{42–44} Our results clearly show that the humanized antibody bFGFmAb possesses potent EMT suppressing activity as evidenced by the suppression of key molecules and pathways involved in EMT, such as N-cadherin, and the increase in E-cadherin levels in bFGFmAb -treated A549 cells and A549/DDP cells (Figure 4). We also demonstrate that, compared to the control group, the expression of LRP significantly decreases ($p < 0.05$) after bFGFmAb treatment. These data suggest that EMT suppression and LRP downregulation are involved in the mechanism of action, leading to reduced cisplatin resistance following bFGFmAb treatment. To the best of our knowledge, this is the first report showing that a bFGFmAb attenuates cisplatin resistance in lung cancer through suppression of EMT and downregulation of LRP, representing a novel mechanism of overcoming drug resistance. Our data derived from in vitro experiments compliments and supports previous reports where bFGF is an important target for drug resistant in NCSLC.

Limitations of our study included that the data was from in vitro experiments only. Further, in vivo study certainly is needed for moving to translational stage. Additionally, limited number of cell lines, A549 cells and cisplatin-resistant A549 cells, was used in the experiments, limiting the generalization of the findings. Furthermore, the study is limited to NCSLC cell lines and no other cancer type is involved. Therefore, interpretation of the findings should be done with cautions. Although promising, further in vivo study, translational work and pharmacokinetic and toxicological assessments are necessary before moving to clinical trials. We believe that the limitations are outweighed by the notable strengths and a promising future outlook of potential new treatment strategy.

Conclusion

In conclusion, building on our previous work on humanized bFGFmAb, in this study, we demonstrated that bFGFmAb significantly inhibits the proliferation and invasion of both NSCLC cells and cisplatin-resistant NSCLC cells. The sensitivity, as measured by IC₅₀, of the resistance of the cells to the therapeutic drug cisplatin was successfully reversed by bFGFmAb treatment. The underlying mechanism appears to involve the suppression of EMT and reduction in LRP expression. Our data supports that the bFGFmAb may serve as a potential targeted adjunct therapy for NSCLC patients who are resistant to cisplatin.

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Author contributions

Xu M and Lin Y conceived and designed the study. Hu P and So K carried out the majority of the experiments, drafted and edited the manuscript. Chen H and Lin Q completed part of experiment and made all the figures. All authors analyzed and interpreted the data. All authors had final approval of the submitted version.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Appendix

Abbreviations

A549/DDP, cisplatin-resistant A549 cell line
 BCA, bicinchoninic acid
 bFGF, basic fibroblast growth factor
 CCK-8, cell counting kit-8
 DDP, cisplatin
 ELISA, enzyme-linked immunosorbent assay
 EMT, epithelial-to-mesenchymal transition
 FBS, fetal bovine serum
 FGFR, fibroblast growth factor receptor
 HSPG, heparan sulfate proteoglycan
 IC50, 50% inhibitory concentration
 LRP, lung resistance protein
 mAb, monoclonal antibody
 NSCLC, non-small cell lung cancer

PBS, phosphate-buffered saline
 BCA, bicinchoninic acid
 bFGF, basic fibroblast growth factor
 CCK-8, cell counting kit-8
 DDP, cisplatin
 ELISA, enzyme-linked immunosorbent assay
 EMT, epithelial-to-mesenchymal transition
 FBS, fetal bovine serum
 FGFR, fibroblast growth factor receptor
 HSPG, heparan sulfate proteoglycan
 IC50, 50% inhibitory concentration
 LRP, lung resistance protein
 mAb, monoclonal antibody
 NSCLC, non-small cell lung cancer
 PBS, phosphate-buffered saline
 SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.