Fuzheng Kang-Ai decoction increases the sensitivity of Gefitinib via inducing Non-Small Cell Lung Cancer ferroptosis through AMPK/SLC7A11 pathway

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Research Article

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Abstract

Background:

Fuzheng Kang-Ai decoction (FZKA), which consists of twelve Chinese herbal medicines (CHM), has been extensively used in the treatment of Non-Small Cell Lung Cancer (NSCLC) patients. It was reported in our preceding study that the progression-free survival (PFS) could be prolonged and the toxic effect was reduced after the combination of FZKA and Gefitinib (GFTN) comparing to GFTN alone. Recent studies have shown that FZKA could promote ferroptosis in NSCLC cells. Hence, we assumed that FZKA might strengthen the effect of GFTN by regulating cell ferroptosis in NSCLC.

Results:

First of all, according to our results, FZKA increased the inhibition effect of NSCLC by GFTN *in vitro*. Next, we found that FZKA increased the sensitivity of GFTN on cell ferroptosis by testing lipid peroxidation and intracellular-free iron. Interestingly, the effect of FZKA on increasing sensitivity of GFTN in NSCLC cells could be reversed by blocking ferroptosis. Additionally, the results demonstrated that the expression of AMPKα was increased and SLC7A11 reduced in the combined treatment group, at both mRNA and protein levels. Notably, the inhibition of AMPKα reversed the effect of FZKA on increasing GFTN sensitivity. Finally, *in vivo*experiments validated that FZKA strengthened the effect of GFTN by inducing NSCLC cell ferroptosis.

Conclusions:

Based on our findings, we conclude that FZKA increases the sensitivity of GFTN via inducing NSCLC ferroptosis through AMPK/SLC7A11 pathway, providing the solid evidences to the treatment of FZKA together with GFTN in NSCLC patients.

Introduction

Globally, one of the most common malignant tumors is lung cancer, and it is the leading cause of cancer death [1]. Among males and females, lung cancer is considered to be the leading cause of death in 2022 [2]. About 85% of lung cancers are non-small cell lung cancers, which can be classified histologically into adenocarcinoma, squamous cell carcinoma, and large cell carcinoma [3]. TKIs have significantly improved tumor response rates and survival benefits in advanced NSCLC. As a first-generation epidermal growth factor receptor-tyrosine kinase inhibitor (EGFR-TKI), GFTN was approved as the first-line treatment for patients with NSCLC who have sensitizing mutations in EGFR (exon 19 deletion or L858R point mutation). However, the widespread benefits of GFTN were limited by acquired resistance in patients [4].

FZKA, a Chinese herbal compound consists of twelve CHM, is generally used in the treatment of NSCLC. It was reported in our preceding study that the combination of FZKA and GFTN could prolong progression-free survival (PFS) and decrease the side effects in comparison with GFTN alone [5]. Additionally, FZKA

could improve the disease control rate (DCR) and median survival time (MST) in patients with NSCLC [6, 7]. In terms of mechanism, our study indicated that FZKA inhibits lung cancer cell growth via the AMPKα/IGFBP1/FOXO3a and STAT3/Bcl-2/Caspase-3 pathways, separately [8, 9]. Furthermore, the effect of EGFR-TKI may be raised by FZKA considering inhibition of lung cancer cells. We found that the combination of FZKA and erlotinib resulted in greater inhibition on NSCLC cell growth via DNMT1/SP1/MET pathway [10]. As shown in another study, FZKA increased the effect of GFTN-induced cell apoptosis in lung cancer cells by mitochondrial activity [11]. In the recent past, we discovered that FZKA promoted ferroptosis in NSCLC [12]. Hence, we hypothesized that FZKA could enhance GFTN's effect by regulating cell ferroptosis in NSCLC.

Ferroptosis, a newly defined form of programmed cell death (PCD), is distinguished from necrosis, apoptosis, autophagy, necroptosis, and pyroptosis [13], resulting in cell death from the accumulation of iron and oxidative stress [14]. System Xc^- is a cysteine/glutamate transporter consisting of a heavy chain (4F2, gene name SLC3A2) and SLC7A11 [15]. AMPK, a heterotrimeric enzyme with catalytic subunit (α1 or α2) and regulatory subunits, plays a central role in multiple physiological and pathological cellular processes, especially the regulation of energy metabolism and oxidative stress [16]. AMPK has been proved to play a significant role in FZKA's anti-cancer effect [8]. Rising evidence indicates an intimate connection between AMPK and ferroptosis [17–19]. For the first time, we demonstrated the mechanism of FZKA in regulating ferroptosis through AMPK/ SLC7A11 pathway as well as examined the efficacy of FZKA in combination with GFTN for the treatment of NSCLC in this study.

Materials and methods

Fuzheng Kang-Ai decoction (FZKA)

A number of NSCLC patients have been treated with FZKA in Guangdong Provincial Hospital of Traditional Chinese Medicine (TCM) in previous years. 12 components in total were obtained from Guangdong Kangmei Pharmaceutical Company Ltd (Guangdong, China). FZKA consists of the following components: *Pseudostellaria heterophylla* (Miq.) Pax 30g, *Atractylodes macrocephala* Koidz.30g, *Astragalus mongholicus* Bunge 30g, *Scleromitrion diffusum* (Willd.) R.J.Wang 30g, *Solanum nigrum* L. 30g, *Salvia chinensis* Benth. 30g, *Cremastra appendiculata* (D.Don) Makino 30g, *Coix lacryma-jobi var. ma-yuen* (Rom.Caill.) Stapf 30g, *Akebia quinata* (Thunb. ex Houtt.) Decne. 30g, *Rubus parvifolius* L. 30g, *Curcuma phaeocaulis* Valeton 15g, *Glycyrrhiza uralensis* Fisch. ex DC. 10g. Vitro experiments were performed with granules melted in RPMI-1640 medium to a final concentration of 20 mg/mL, centrifugated at 15,000 rpm for 10 minutes; as a result, the overlying section was filtered using a 0.22 m filter and the pH value in the cultured cells was conformed to 7.2–7.4 after addition of FZKA. FZKA was administered intragastrically to animals for *in vivo* experiments.

Chemicals and cell culture

We purchased the Cell Counting Kit (CCK-8) from Shanghai Yisheng Biotechnology Co., Ltd. (Shanghai, China). Thermo Fisher Scientific (Waltham, MA) offered BODIPY™ 581/591 C11. GFTN, liproxstatin-1 and Compound C were obtained from Selleck Chemicals (Huston, TX, USA). Antibodies against GAPDH (ab9485) was purchased from Abcam (Cambridge, UK); Antibodies against phosphorylated (p)-AMPKα (Thr172) (2535S), AMPKα (5831S), SLC7A11 (12691S), horseradish peroxidase (HRP) –conjugated goat anti-rabbit antibody (7074S), were obtained from Cell Signalling Technology (Danvers, MA); FerroOrange was obtained from Dojindo Molecular Technologies Company (Kumamoto, Japan). The NSCLC cell lines A549, H1650, and PC9GR were obtained from Guangzhou Cellcook Biotechnology Co. (Guangzhou, China). The cells were grown in the humidified 5% CO2 and 95% air atmosphere in RPMI-1640 medium (Life Technologies, Carlsbad, CA, USA) containing 10% FBS (Gibco, USA) and 0.5% penicillin-streptomycin sulfate (Invitrogen Life Technologies, Carlsbad, CA, USA).

High performance liquid chromatography (HPLC)

An initial analysis of batch-to-batch consistency was conducted using HPLC, as earlier described [10].Concisely, samples were placed into the HPLC system (250×4.6 mm, 5 μm, ACE, Scotland). Deionized water was used as the mobile phase alongside formic acid with a concentration of 0.1% (A) and acetonitrile with a concentration of 0.1% (B). The gradient elution program included the following steps: 5% B at 0 to 5 minutes, 5–20% B at 5 to 10 minutes, 20–40% B at 10 to 15 minutes, 40–95% B at 15 to 40 minutes, and 95–100% B at 40 to 45 minutes. The flow rate was set to 1.0 mL/min, and 280 nm wavelength was detected. We used a volume of 10μL and a temperature of 30°C for the injection. There is a high degree of consistency in the efficacy of different batches of FZKA [11].

Cell proliferation assay

CCK-8 assay was used to measure cell proliferation. In 96-well plates, cells were seeded and then with different treatments for 24h. It was then added to the CCK-8 reaction solution and incubated for one hour. Afterwards, the optical density (OD) values at 450nm were measured in order to assess cell viability.

EdU proliferation assay

For measuring cell proliferation, 5-ethynyl-2'-deoxyuridine (EdU) proliferation assays were also performed. In 96-well plates, cells were plated and treated with FZKA or GFTN for 24 hours. After exposure to 50μM EdU (RiboBio, Guangzhou, China), the cells were fixed for 30 minutes in PBS containing 4% paraformaldehyde (PA-PBS). Using 0.5% TritonX-100 for permeabilization, cells are dyed with Apollo reaction reagent for 30 minutes. Following this, the DNA contents were stained for thirty minutes with Hoechst 33342. Fluorescence microscopy was used to take the photographs.

Lipid peroxidation measurement

The cells were cultured in 6-well plates. Upon 24 hours of culture, cells were treated with FZKA, GFTN or liprostatin-1 in different groups, and then stained in 10 μM C11-BODIPY for 0.5 h. It was observed that the fluorescence emission peak shifted from 590 to 510 nm following the oxidation of the poly-unsaturated

butadienyl portion of C11-BODIPY. After washing twice with PBS, samples were measured by flow cytometry (Ex: 488 nm, Em: 510 nm), and results were analyzed using NovoExpress.

Detection of cellular Fe^{2+} ions generation

FerroOrange (1 μM, a probe for intracellular Fe^{2+} ions, Ex: 543 nm, Em: 580 nm) which could test Fe^{2+} ions in cells was added to the cells after FZKA and GFTN in different group, and the cells were incubated in a 37ºC incubator for 30 minutes. Afterwards, cells were collected using trypsin. After washing twice with PBS, fluorescence of cells was taken by flow cytometry.

Western blot analysis

As previously reported, western blotting was carried out according to the protocol. In short, the cells were collected, washed, lysed, and their protein concentration was measured using the Thermo BCA protein assay kit. Each sample was processed using SDS-PAGE to separate the proteins. In this process, protein was transferred from gel to membrane. After rinsing the membrane, primary antibodies were incubated on it. It was then washed and incubated with secondary antibodies against rabbit IgG for one hour. It was visualized using the ECL solution (Millipore, Darmstadt, Germany), and scanned by the ChemiDoc XRS + Chemiluminescence imaging system (Bio-Rad, Hercules, CA, USA). Image J was used to analyze all the results.

Quantitative real-time (qRT) PCR

Trizol (Invitrogen, CA, USA) was used to extract the total RNA. RNAs were converted to cDNA by using the Transcriptor first strand cDNA synthesis kit (Roche, Basel, Switzerland). qRT-PCR was detected by FS essential DNA green master (Roche, Basel, Switzerland). Data were analyzed with $2 - \Delta\Delta Ct$ for relative changes in the expression of genes.

Animal studies

1.0×10^6 A549 cells were injected hypodermically into the flanks of BALB/c nude mice (aged 6–8 weeks, weighing 18–20 grams, females; Vital River, Beijing, China). The Ethics Committee of the Guangdong Provincial Hospital of Chinese Medicine approved the animal experiments (2020079). Once the mass of the tumor became palpable, the mice were divided into four groups for treatment: control, FZKA (31 g/kg), GFTN (10 mg/kg), and combination of FZKA and GFTN at random. FZKA was administered by gavage, while GFTN was administered intraperitoneally. The tumors were measured with digital calipers every four days. The formula was used: Volume = $(\text{Length}\times\text{Width}^2)/2$. Mice were sacrificed at the end of the experiments, and tumors were collected for further analyses.

Statistical analysis

The SPSS statistical software was used to perform the statistical analysis. Data analysis was conducted using Student's t-test when there were only two groups, and one-way ANOVA was used to assess

differences between the groups. The data were presented as mean ± SD. It was considered significant if the *P* values < 0.05.

Results

FZKA enhanced the inhibition effect of Gefitinib on NSCLC cell growth in vitro

In our previous study, the inhibitory effect of FZKA and GFTN on NSCLC cell growth were observed in A549 [11]. According to this study, we further explored the effect of combining FZKA with GFTN in extra NSCLC H1650 and PC9GR cell types, both of which are GFTN insensitive cell lines, by CCK-8 assay. According to the results, the combination group showed significant inhibition effect on the cell viability comparing to the FZKA or GFTN group, separately (Fig. 1A). Again, the EdU proliferation assay was also performed and the results reconfirmed the combination effect of FZKA and GFTN in inhibiting NSCLC cell proliferation, compared to FZKA or GFTN alone (Fig. 1B). Together, our *in vitro* data showed that FZKA could enhance the inhibition effect of GFTN on NSCLC cell.

FZKA enhanced the sensitivity of Gefitinib on NSCLC cell ferroptosis.

To further explore the enhancement of GFTN induced NSCLC cell death after FZKA treatment, it was observed that lipid peroxidation and intracellular-free iron, two central characteristics of ferroptosis, were increased [20]. A549 and PC9GR cells were treated with FZKA (1.5mg/ml) and GFTN (10 μM) for 24 h and examined via flow cytometry. A lipid peroxidation probe, C11-BODIPY, was used in mammalian cells [21]. FerroOrange measured the levels of intracellular labile Fe in live cells [22]. Detection of lipid peroxidation showed that the mean fluorescence intensity of FZKA and GFTN group had a significant increase in comparation with the GFTN alone group. Similar conclusions were also obtained in the detection of the Fe^{2+} fluorescence intensity inside the living cells. Thus, FZKA increased the sensitivity of GFTN both on lipid peroxidation (Fig. 2A) and intracellular iron (Fig. 2B) in A549 and PC9GR cells. These results proved that FZKA could enhance the sensitivity of GFTN on NSCLC cell ferroptosis.

Blocking ferroptosis reversed the effect of FZKA on increasing the sensitivity of Gefitinib in NSCLC cells.

To clarify the role of ferroptosis in FZKA enhancing the effect of GFTN in NSCLC cells, we applied the ferroptosis inhibitor liproxstatin-1 to block ferroptosis.

Liproxstatin-1 could suppress ferroptosis in human cells and prevented GPX4-inhibition-induced ferroptosis[23]. Figure 3A illustrated that FZKA enhanced the sensitivity of lipid peroxidation on GFTN, and it was approximately reversed after treatment with liproxstatin-1. Moreover, blocking ferroptosis could reverse the inhibition effect in the combination group (FZKA and GFTN) on NSCLC cell lines, as shown in Fig. 3B. These findings denoted that ferroptosis plays an essential role in FZKA enhancing sensitivity of GFTN in NSCLC cells.

AMPKα, SLC7A11 were involved in FZKA enhancing the sensitivity of Gefitinib on NSCLC cell ferroptosis.

To investigate the molecular mechanism by which FZKA acts in enhancing the sensitivity of NSCLC cells to GFTN, our study aims to explore the potential molecular targets in it. AMPK, functions as a central regulator of energy metabolism, stimulating the production of ATP and reducing the depletion of ATP [24]. There is a close link between AMPK and ferroptosis in a number of studies. As the catalytic subunit of system Xc-, SLC7A11 is crucial in ferroptosis [25]. Our former studies showed that FZKA inhibited the expression of SLC7A11[12] and upregulated the expression of AMPK[8] in a dose-dependent way. Both in protein levels and mRNA levels, the combined treatment group demonstrated a rise in AMPK expression and a reduction in SLC7A11 expression. (Fig. 4A-4B).

Inhibiting AMPKα rescued the effect of FZKA on increasing the sensitivity of Gefitinib.

To further validate the critical role of AMPKα in the process, we suppressed AMPKα in NSCLC cells by AMPKα inhibitor. Dorsomorphin (Compound C) is an effective and a reversibly selective AMPK inhibitor. Then, combining compound C with GFTN and FZKA for the treatment of NSCLC cells, the results reflected an increased SLC7A11 protein level via Western Blot (Fig. 5A) and a decreased lipid peroxidation by flow cytometry (Fig. 5B). In addition, the inhibition of cell viability by the combination group was also partially reversed when AMPKα was inhibited (Fig. 5C). Therefore, the above results indicated that FZKA enhancing the sensitivity of GFTN on NSCLC cell ferroptosis might be mediated through AMPK/SLC7A11 pathway.

FZKA enhanced the sensitivity of Gefitinib on NSCLC cell ferroptosis through AMPK/SLC7A11 pathway in vivo

To confirm that FZKA increased the sensitivity of GFTN on NSCLC cell ferroptosis *in vivo*, we established the BALB/c nude mouse xenografts model. As shown in Fig. 6A, the combination of FZKA and GFTN had a greater inhibitory effect on NSCLC tumor growth *in vivo* in comparison to FZKA alone or GFTN alone. There was a significant decrease in volume and weight of tumors in the combination group. Then we extracted the protein from the tumor tissues and identified the protein expression of AMPKα and SLC7A11 by Western Blot. The results were consistent with external data showed upregulated expression of AMPKα. Additionally, SLC7A11 protein expression was significantly downregulated in the combination group compared to FZKA alone and GFTN alone (Fig. 6B). Overall, FZKA strengthened the sensitivity of GFTN on cell ferroptosis through AMPK/SLC7A11 pathway.

Discussion

Previously, we discovered that FZKA could induce ferroptosis in NSCLC [12]. Accordingly, we hypothesized that FZKA could strengthen the effect of GFTN in NSCLC by modulating cell ferroptosis. It has been indicated that ferroptosis, a new programmed cell death characterized by two major biochemical features of iron accumulation and lipid peroxidation [26], and differs from apoptosis,

autophagy, necroptosis, and other forms of cell death in mediating iron-dependent lipid peroxides accumulation [27]. According to our results, NSCLC cells had characteristics of ferroptosis after FZKA intervention, which showed that FZKA alone or the combination of FZKA and GFTN significantly promoted the lipid peroxidation and intracellular-free iron in NSCLC cells. Meanwhile, we discovered that blocking ferroptosis by ferroptosis inhibitors could reverse the effect of FZKA on increasing the sensitivity of GFTN in NSCLC cells, suggesting that ferroptosis plays a pivotal role in the procedure.

Increasing evidence proves that lipid peroxidation plays a significant role in ferroptosis. A heterodimeric cystine/glutamate antiporter, system Xc^- consists of two components, SLC7A11 and SLC3A2. xCT is often overexpressed in plenty of cancers [28]. Inhibiting system Xc^- could result in lipid peroxidation and ferroptosis [29]. Besides, the inhibition of SLC7A11 expression could reduce system Xc^- activity and bring about ferroptosis [30, 31]. AMPK is a vital switch in energy metabolism, and that it has a significant impact on the physiology and pathology of chronic diseases, including cancer [32]. Furthermore, it has been found that FZKA could increase AMPKα expression and the most additional effect on the expression of AMPKα was observed in the combination of FZKA and GFTN. A report accordance exactly with our study indicated that AMPK phosphorylation inhibited the activity of SLC7A11, which downregulate the cysteine-glutamate antiporter system Xc^- thus eventually lead to ferroptosis [17, 33]. However, another study found that energy-stress-induced activation of AMPK significantly inhibited ferroptosis [19]. Additionally, other findings indicated that the imbalance of AMPK could lead to ferroptosis [34]. In ferroptosis, the role of AMPK has not been fully elucidated yet, and it is still remains to be explored in the future.

TCM, as an adjuvant treatment for cancer patients, has a long history in China. FZKA could increase the efficacy of GFTN and reduce drug toxicity, which has been widely used in clinic. The results of our study indicated that FZKA could raise the growth inhibition effect of GFTN in NSCLC cells by inducing ferroptosis in both *in vitro* and *in vivo* experiments. According to our findings, FZKA enhanced the sensitivity of GFTN via inducing NSCLC ferroptosis through AMPK/SLC7A11 pathway. Thus, it might be beneficial to apply FZKA to improve the efficacy of GFTN against cancer by inducing ferroptosis.

Conclusion

Our study aimed to explore the combination effect of FZKA and GFTN on NSCLC cell ferroptosis *in vitro* and *in vivo*. Based on the results, it was determined that FZKA increased the sensitivity of GFTN via inducing NSCLC ferroptosis through AMPK/SLC7A11 pathway. While preliminary, the finding provides the novel evidences to the treatment of FZKA together with GFTN in NSCLC patients.

Abbreviations

FZKA: Fuzheng Kang'ai decoction; NSCLC: Non-Small Cell Lung Cancer; GFTN: Gefitinib; EdU: 5-ethynyl-2'-deoxyuridine; qRT: Quantitative real-time; AMPK: AMP-activated protein kinase; SLC7A11: light-chain subunit xCT; TKIs: Tyrosine kinase inhibitors; EGFR-TKI: Epidermal growth factor receptor-tyrosine kinase

inhibitor; CHM: Chinese herbal medicine; PFS: progression-free survival; DCR: disease control rate; MST: median survival time; PCD: programmed cell death; SLC3A2: heavy-chain subunit CD98; TCM: Traditional Chinese Medicine; CCK-8: Cell counting kit-8; HRP: horseradish peroxidase; OD: optical density.

Declarations

Ethics approval and consent to participate

The ethics committee of the Guangdong Provincial Hospital of Chinese Medicine (2020079) has validated all animal experiments according to the Guide for Care and Use of Laboratory Animals issued by the National Institutes of Health.

Availability of data and materials

Data that was used and/or analyzed during the present study may be obtained from the corresponding author upon reasonable demand.

Competing interests

There is no conflict of interest between the authors.

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CRediT author statement

Yueyang Zhao (zyy6181@126.com), Mengfei Xu (13522215040@163.com) and ShuJing Wang(wshujez@163.com) performed most of the experiments and wrote the manuscript draft. Nanxing Zhong (znx1999@foxmail.com), Shenghong Qiu(hong1936@126.com), Qing Tang (tangqingyanjiu@163.com), Qichun Zhou (szyzqcky@126.com), Yanqing Pang(luofengpyq@163.com) and Huanmei Lin(lhm13760794295@126.com) performed some of the experiments. Xiaobing Yang (yangxiaobing2002@126.com) provided corrected the draft. Sumei Wang (wangsumei198708@163.com) and Wanyin Wu (wwanyin@163.com) supervised the experimentators and were responsible for the

project design and manuscript editing. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Figures

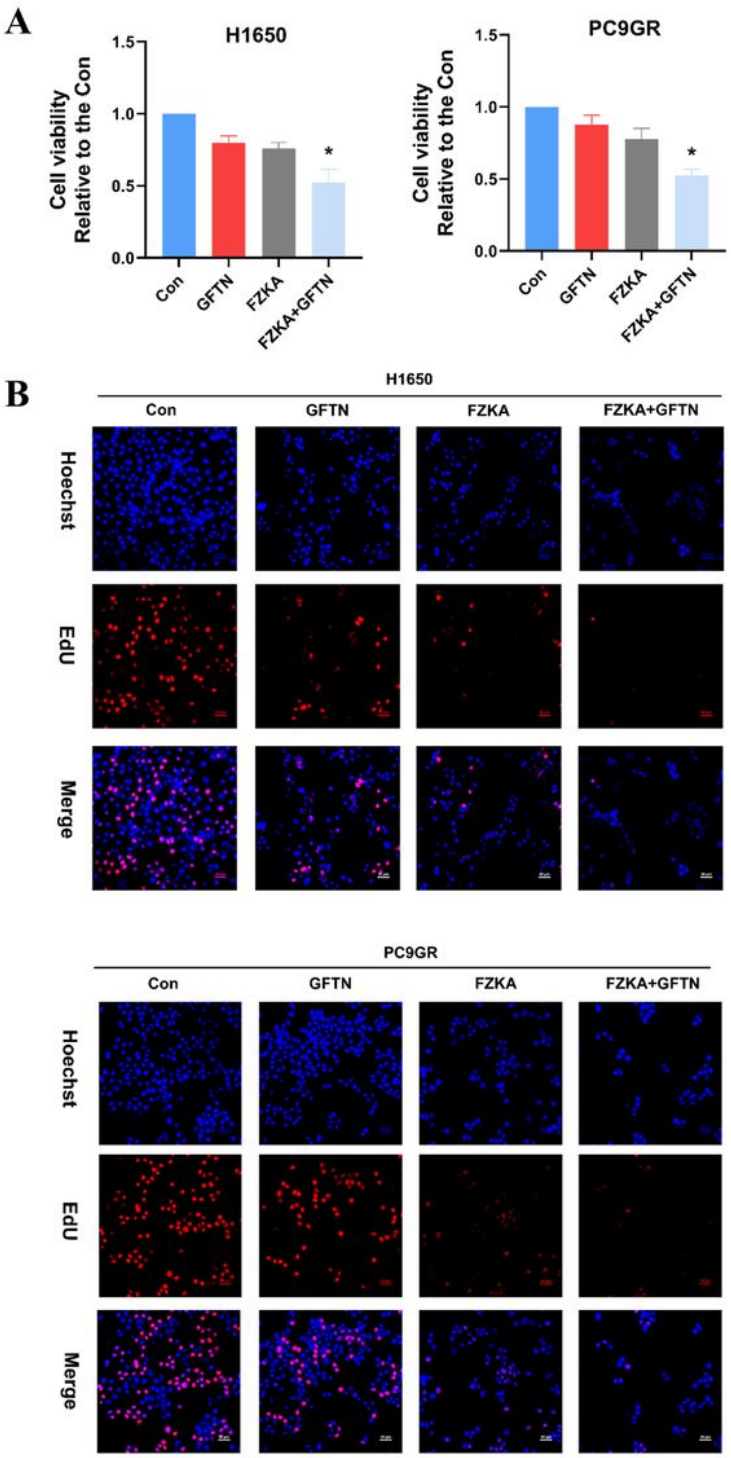


Figure 1

FZKA sensitized the inhibition effect of Gefitinib in NSCLC cells *in vitro*

A, H1650 and PC9GR cells were treated with FZKA (1.5 mg/mL) and GFTN (10 μM) for 24 h. The cells were collected and processed for CCK-8 assay as described in the Materials and Methods section,

*p<0.05. B, H1650 and PC9GR cells were treated with FZKA (1.5 mg/mL) and GFTN (10μM) for 24 h, followed by EdU proliferation assay.

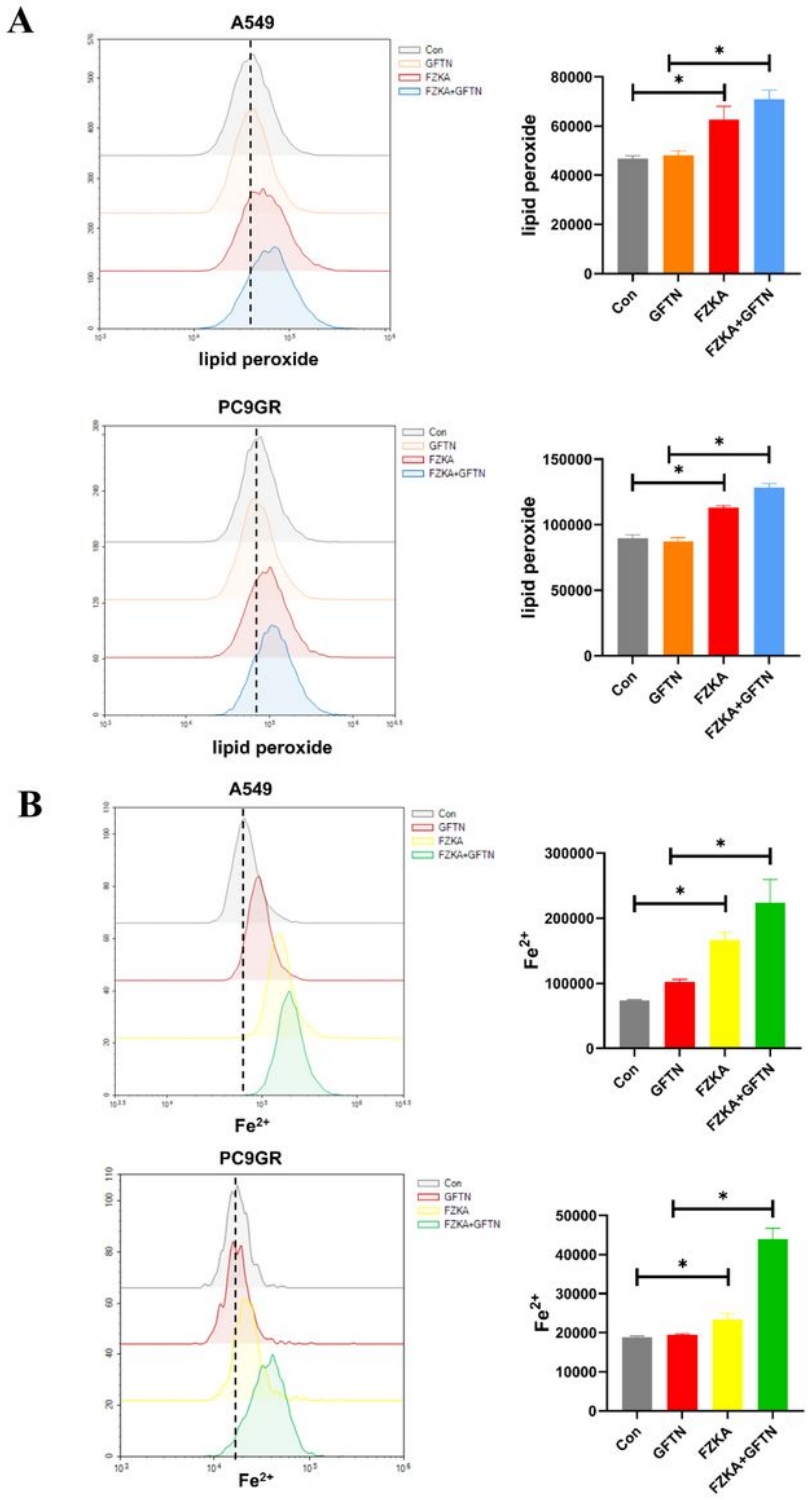


Figure 2

FZKA enhanced the effect of Gefitinib through cell ferroptosis by FCM.

A&B, Cultured A549 and PC9GR cells were treated with FZKA (1.5mg/ml) and GFTN (10 μM) for 24 h. Cells were stained with C11-BODIPY(10 μM) or FerroOrange (1 μM) for 30 min, the level of lipid peroxidation and Fe^{2+} Ions was detected by flow cytometry, * p<0.05.

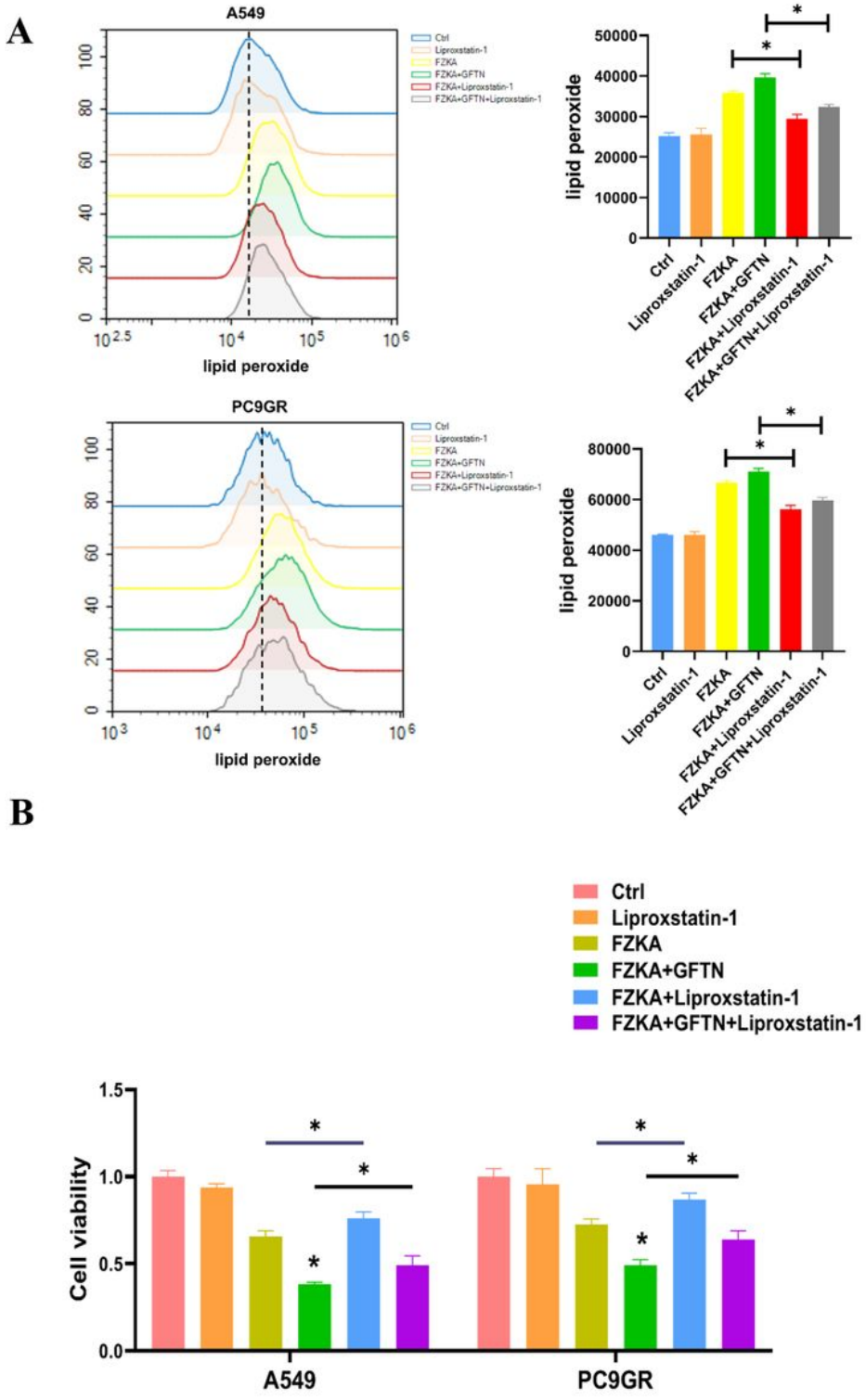
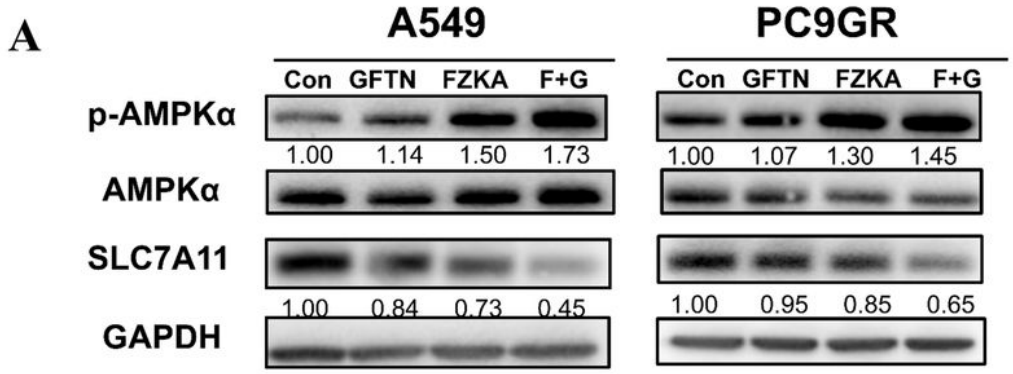


Figure 3

Ferroptosis inhibitors liproxstatin-1 reversed the effect of FZKA on increasing the sensitivity of Gefitinib in NSCLC cells.

A, A549 and PC9GR cells were treated as FZKA (1.5mg/mL) and GFTN (10 μM) with liproxstatin-1 (200 nM) for 24h., and stained with BODIPY™ 581/ 591 C11 (10 μM) for 30 min. The level of lipid peroxidation was detected by flow cytometry. Each point represents the mean ± SEM, n = 3, *p<0.05. B, Cultured NSCLC cells were seeded in 96 well plate and treated as A. Cell viability was detected by CCK-8 assay, *p<0.05.



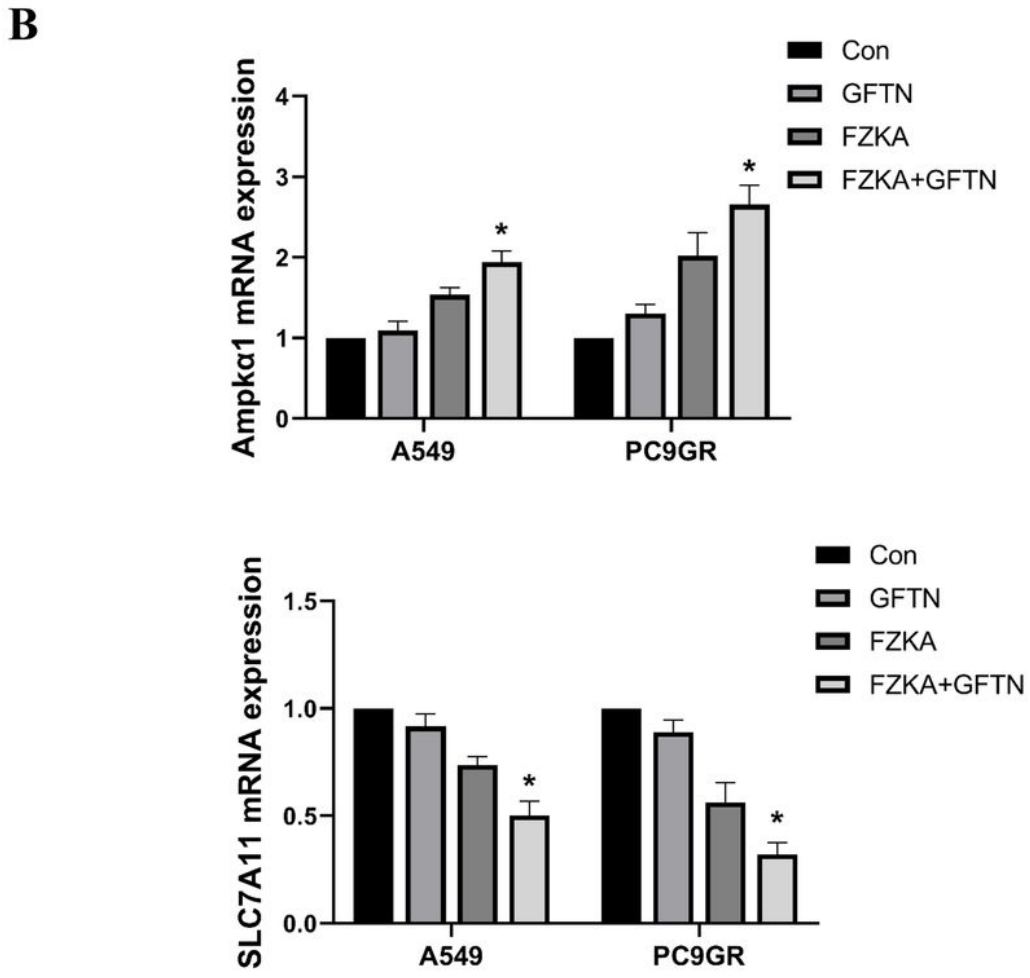


Figure 4

FZKA downregulated the SLC7A11 and increased the AMPKα expression in NSCLC cells at both protein and mRNA levels.

A, The protein expression levels of SLC7A11 and AMPKα were detected by Western blot. B, The mRNA expression of SLC7A11 and AMPKα were detected by qPCR. Each point represents the mean ± SEM, n = 3. * $p < 0.05$.

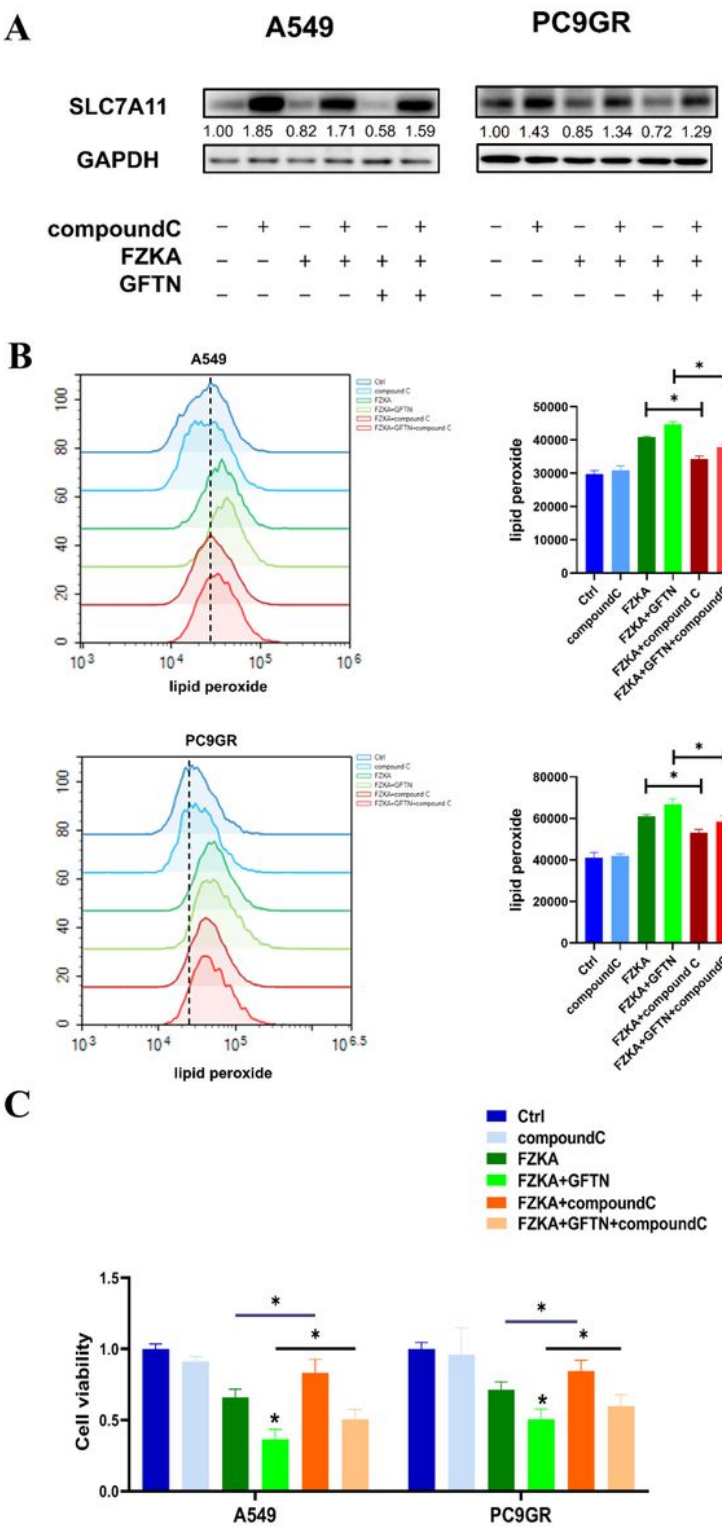


Figure 5

Inhibiting AMPKα rescued the effect of FZKA on increasing the sensitivity of Gefitinib.

A, Suppressed A549 and PC9GR cells by AMPKα inhibitor, then treated with or without FZKA and GFTN for 24 h. The expression of protein was detected by western blot. B, lipid peroxidation assay was performed in A549 cell after treatment with FZKA or/and AMPKα inhibitor. C, Cells were transfected and

treated with FZKA, and CCK-8 assay were then conducted. Each point represents the mean ± SEM, n = 3. *p<0.05.

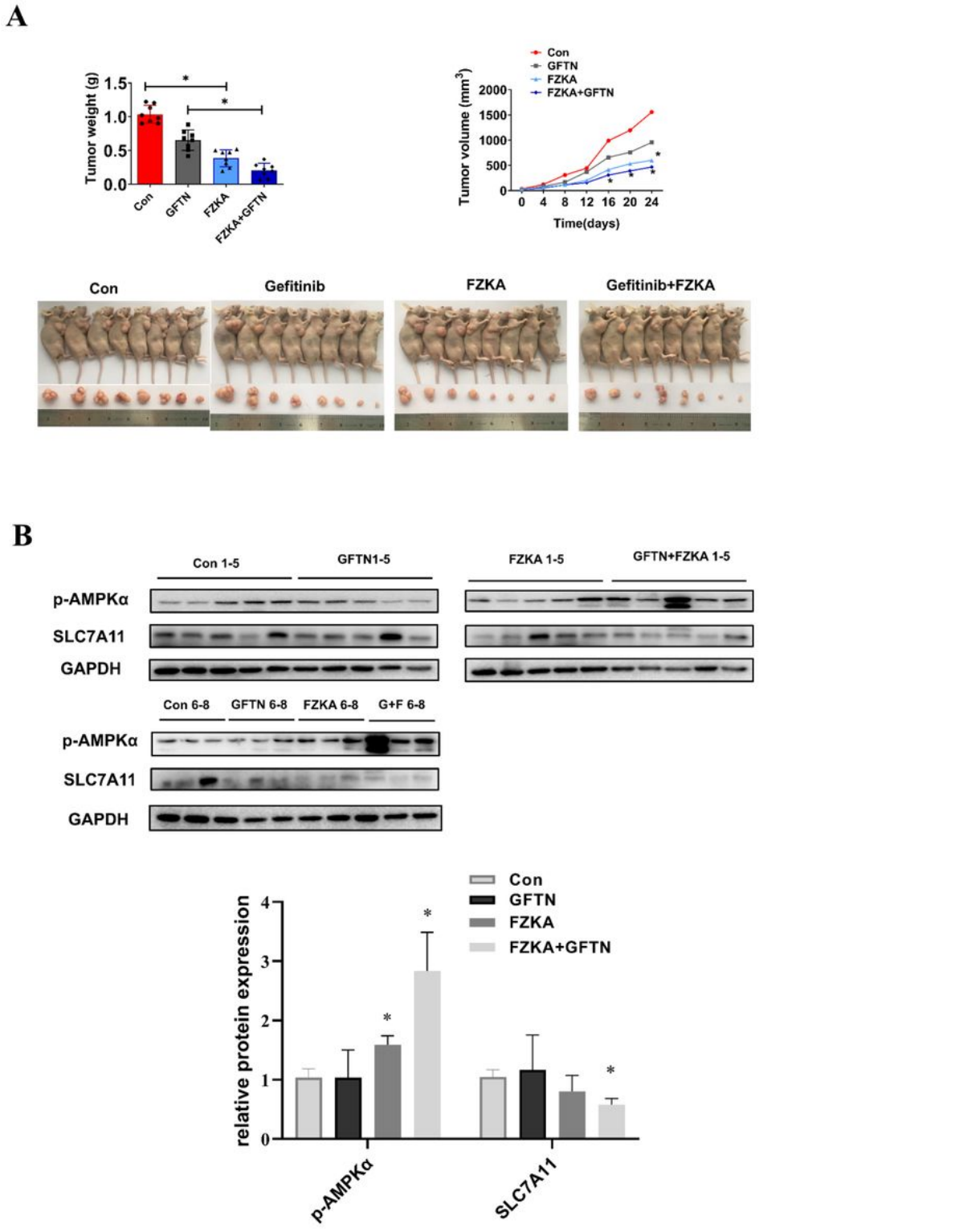


Figure 6

FZKA enhanced the sensitivity of Gefitinib on NSCLC cell ferroptosis *in vivo*.

A, Mice tumor photograph and tumor weight was showed. Tumor volume in each group was showed. Data represents Mean ± SEM, n = 8. *p<0.05. B, Western blot analyses of SLC7A11 and AMPKα expression from tumor tissues. Data represents Mean ± SEM, n = 8. *p<0.05.