T4 Reduces Cisplatin Resistance by Inhibiting AEG-1 Gene Expression in Lung Cancer Cells

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Abstract

Background

Lung cancer is one of the deadliest diseases in the world. Most lung cancer patients are resistant to chemotherapy drugs. In our study, we investigated whether T4 can reduce the resistance of lung cancer cells to chemotherapeutic drugs through the action of AEG-1.

Materials and Methods

1.A549 and A549/DDP cells were respectively transfected with overexpressing AEG-1 and knockdown AEG-1 plasmid. A549 and A549/DDP cells were added 0、25、50、100、200nM T4 respectively. 200nM T4 was selected for following experiments. A549/DDP cells were divided into A549/DDP empty group, T4 group, T4+AEG-1 overexpressing group. CCK8 assay was used to detect the proliferation of cells in each group. RT-qPCR and Western blotting were used to detect the expression of AEG-1 and MDR-1.

Results

As expected, the expression of AEG-1 in A549 and A549/DDP cells is positively correlated with cisplatin resistance. When AEG-1 protein was overexpressed in A549 cells, the lethal effect of cisplatin on A549 cells was attenuated (all $P<0.05$). After AEG-1 protein was knocked down in A549/DDP cells, cisplatin was applied to the A549/DDP cells. The lethal effect was significantly increased compared to that in the control cells (all $P<0.05$). The expression of AEG-1 protein gradually decreased with increasing concentration of T4 in A549 and A549/DDP cells; The resistance to cisplatin was reduced after the addition of T4 to A549/DDP cells ($P<0.05$), and this effect was enhanced after transfection with the AEG-1 plasmid.

Conclusion

In summary, T4 is important for increasing the sensitively of lung cancer cells to cisplatin. AEG-1 may be a key protein involved in this effect and may have an important impact on the survival rate of chemotherapy in patients with lung cancer in the future.

Background

Lung cancer is the most malignant tumour and has the highest morbidity and mortality rates in the world. Among malignant tumours, it continues to rank first in mortality rate in the United States as well as in China. Moreover, The incidence of lung cancer has increased by 465% during the past three decades, and the 5-year survival rate of lung cancer is very low, generally less than 15% [1]. The main reason for this phenomenon is that most patients are diagnosed in the advanced stage at which point they have lost the chance of surgery. More than 50% non-small-cell lung cancer (NSCLC) patients and most small-cell lung cancer (SCLC) patients have not the chance of targeted therapy, so chemotherapy is the primary choice for most lung cancer patients. Correspondingly, medical professionals prescribe chemotherapy, usually

third-generation drugs and platinum drugs, for lung cancer patients to improve their survival, disease control and quality of life [2]. However, clinical data shows that chemotherapy for lung cancer, especially for non-small-cell lung cancer, is not effective or is only effective in the initial treatment, but it is difficult to achieve the desired effect with repeated treatment. The main reason is the resistance of tumours to chemotherapy drugs[3]. Moreover, cancer patients often need to receive large doses of anticancer drugs; correspondingly, these doses can cause more harmful side effects. Therefore, the most attractive way to improve the treatment effect in non-small-cell lung cancer is to effectively reverse drug resistance[4].

Drug resistance, especially multidrug resistance (MDR), is a major cause of clinical failure in cancer therapy. MDR refers to the cross resistance of tumor cells to a variety of anti-tumor drugs with different structures and mechanisms, which can be tolerated the lethal dose of a variety of anti-tumor drugs and greatly reduce the anti-tumor effect. Drug resistance developments for most drugs in the clinic via inhibition of drug transport proteins, such as p-glycoprotein (P-gp), which mediates tumour MDR[5].Effectively reducing the expression of the MDR1 gene can increase the sensitivity of chemotherapy patients to chemotherapy drugs, especially cisplatin, and this has become a hot topic in recent years. The mechanism of MDR is complex, including multi genes and factors. The development of a ‘pan-cancer’ therapy may be possible by targeting an oncogene which is ubiquitously overexpressed in almost all types of cancer and has a regulatory role in the multistep processes of carcinogenesis. [6]

A novel gene that has been identified is astrocyte upregulated gene 1 (AEG-1). AEG-1 is a new oncogene discovered in recent years. The AEG-1 gene consists of 12 exons/11 introns. This gene is located on human chromosome 8 (8q22) and is closely related to the occurrence of various malignant tumour, and further studies have shown that it is a key factor promoting the development of tumours. [7]An increasing number of studies have shown that the abnormal expression of the AEG-1-encoded protein can regulate the carcinogenic transformation of cells through various signalling pathways, promote the proliferation and anti-apoptosis ability of tumour cells, facilitate the invasion and metastasis of tumour cells, and accelerate tumours. Neovascularization mediates the autophagy activity and chemoresistance of tumour cells.[8]As a multifunctional oncogene, AEG-1 has been confirmed to be overexpressed in a variety of human cancer cells, including breast cancer, glioma, prostate cancer, salivary gland cancer, rectal cancer, cervical cancer and other human cancers. Elevated AEG-1 leads to enhanced phenotypic characteristics of malignant invasion of tumour cells, including increased proliferative capacity, invasion of surrounding tissues, migration, induction of neovascularization, and improvement of tumour resistance. [9] Studies have shown that a major cause of chemoresistance is the overexpression of AEG-1. Inhibition of AEG-1 by siRNA not only reduces the metastasis and invasion of human glioma cells [10]but also reduces the expression of the MDR1 gene, thereby decreasing the resistance of tumours to chemotherapy drugs. [11] Due to the multiple functions of AEG-1 in drug resistance, AEG-1 is a viable target as an anticancer agent for a wide range of cancer types.[9]Therefore, the identification of strategies for effective inhibition of AEG-1, which should reduce the expression of MDR1 protein and increase the sensitivity of tumours to chemotherapy drugs, is essential.[12]However, AEG-1, an interesting gene, has rarely been reported in non-small-cell lung cancer cells.

In recent years, traditional Chinese medicine treatment has been widely used in clinical practice. Medicinal plants are therapeutic drugs rich in biological compounds that provide raw materials for nearly 75% of prescription drugs in the world. [13]Among them, the natural extract of Tripterygium has been found to have strong immunosuppressive and anti-inflammatory functions and has been widely used in the treatment of autoimmune-related diseases such as rheumatic diseases in China. The extract of the natural plant *Tripterygium wilfordii* has a strong immunosuppressive effect and anti-inflammatory activity; it has been widely used in the treatment of rheumatic diseases, kidney disease and other autoimmune-related diseases in China. However, the serious toxicity and side effects of the crude extract of Tripterygium limit its clinical application. [14]Triptolide, a diterpene triperoxide extracted from the Tripterygium wilfordii, has been shown to inhibit the proliferation of cancer cells in vitro and reduce the growth and metastases of tumors in vivo. But triptolide has great influence on gastrointestinal tract, skin and mucosa, reproduction, bone marrow hematopoietic system and kidney; and it has a significant impact on the female reproductive system. Tripchlorolide (T4) is an attenuated monomer extracted from Tripterygium or by hydroxy acylation of lead compound Triptolide with chlorination. After structural modification, not only is the toxicity of T4 greatly reduced, but its pharmacological activity is also greatly improved. T4 has strong anti-inflammatory, anti-tumor, immunosuppressive and anti-fertility activities. Its toxicity was significantly less than triptolide, and its chemotherapeutic index was greater than triptolide. In our previous study, we found that T4 can increase the sensitivity of A549/DDP cells to cisplatin by decreasing the expression of MDR1. [15] Therefore, in this experiment, we decided to investigate the following: (1) whether AEG-1 affects the drug resistance of lung cancer cells, and (2) whether T4 can affect the expression of AEG-1 protein in lung cancer cells, and (3)whether T4 reduces cisplatin resistance by inhibiting AEG-1 gene expression in lung cancer cells. We clearly determined that AEG-1 protein is closely related to the drug resistance of lung cancer cells and found that T4 can influence the expression of AEG-1 protein in lung cancer cells. Finally, we further clarified that T4 can reduce the expression of the MDR1 protein and increase the sensitivity of tumour cells to chemotherapy drugs by influencing the expression of the AEG-1 protein. Due to the multiple functions of AEG-1/MTDH in drug resistance, AEG-1/MTDH is a viable target as an anticancer agent for a wide range of cancer types.

Methods

Materials and reagents

Tripchlorolide (T4) was purchased from Amresco (Amresco, CA, USA).CCK-8 kit was purchased from Dojin-do (Dojin- do, Japan), and dimethyl sulfoxide (DMSO)、protease inhibitor cocktail and TEMED were purchased from Sigma (St. Louis, MO, USA). Protein quantitative BCA kit and RIPA lysate were purchased from Beyotime.30% acrylamide was purchased from Xiamen Lu Long Company and Ammonium persulfate (AP) from Amresco Company; Pre-stained Protein Ladder and RNA reverse transcription kit were purchased from Thermo Scientific. Western Blot chemiluminescence assay kit was purchased from KPL. High glucose medium, 1640 medium, penicillin and streptomycin were purchased from Hyclone and fetal bovine serum from GEMINI.

Cell culture

A549 and A549/DDP lung cancer cells (cisplatin-resistant lung cancer cell lines) were obtained from the Cell Line Bank, Chinese Academy of Sciences. A549 cells were cultured in DMEM-high glucose supplemented with10% fetal bovine serum (FBS) and 100 μg/mL penicillin/streptomycin in a humidified incubator under 5% CO_2 at 37℃. A549/DDP cells were cultured in RPMI-1640 supplemented with 10% fetal bovine serum and 100 μg/mL penicillin/streptomycin in a humidified incubator under 5% CO_2 at 37℃. The cell culture media were replaced with fresh media every two days.

Drug treatments

A549 and A549/DDP lung cancer cells were seeded in 6-well plates at a density of 4 ×10^5 cells per dish and randomly divided into the following five groups: i) the control group, with no drug treatment; ii-v)T4 group, exposed to T4 at a series of concentration of 25, 50, 100 and 200nM.The cells were collected after they were treated with/without T4 for 24 h. The optimum T4 concentration is selected. Each experiment was performed in triplicate.

Silencing and overexpression

Cells were seeded into 6-well plates. 24 h later, the culture medium was replaced with serum-free medium. Plasmid complementary DNA AEG-1 cDNA (pcDNA-AEG-1) was obtained by introducing AEG-1 cDNA sequence into the pcDNA3.1 expression vector. The shRNA and siRNA targeting AEG-1 and its controls were synthesized by Genepharma Company (Shanghai, China). Oligonucleotide and plasmid transfection was performed by using Lipofectamine 2000 Reagent (Invitrogen).

CCK-8 Assay

Approximately 1×10^5 cells were plated into a 96-well plate and cultured for 24 h. A549 cells or A549/DDP cells were digested in logarithmic growth phase and treated with cell suspension without FBS (excluding the effect of FBS on cell proliferation). 200 L cell suspensions were taken from 5 ×10 4 /mL each and inoculated in 96-well plates. The cells were cultured for 24 h and covered with the bottom of the well.T4 was dissolved in DMSO and the experimental group, control group and blank group were set. The experimental group was divided into 6 subgroups. Serum free T4 medium containing 0, 25, 50, 100 and 200nM was added. The cells of the control group were cultured in serum-free medium and only DMSO was added. The blank group only had medium and DMSO and no cells. After twenty-four hours, CCK8 was added to each well, and the plate was re-incubated at 37℃ for 1-4 h. The absorbance value was analyzed at 595 nm with a microplate reader. The cell viability rate was calculated according to the following formula: viability rate=A595(experimental group)/A595(control group)×100%. Viability rate=A595/DDP(experimental group)/A595/DDP(control group)×100%. Each experiment was performed in triplicate.

Western Blotting

The total protein extracted from cells was isolated using RIPA lysis buffer with 1 mM PMSF and kept on ice for 10 min followed by 15 min of centrifugation at 12 000 rpm and 4℃, and the protein con-centration was measured using the BCA protein assay kit. 40 ng of protein was separated by sodium dodecyl-sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride membrane. After blocking with 5% nonfat milk for 2 h, the membranes were incubated with the primary antibody against AEG-1(1 : 1000), MDR-1(1 : 1000) and GAPDH(1 : 2000), overnight at 4℃, and followed by subsequent incubation with the respective secondary antibodies for 2 h at room temperature. After washing the blots three times with TBST, they were visualized using electrochemiluminescence plus reagent. Finally, the intensities of these blots were quantified using Image Lab 3.0 software(Bio-Rad).

Quantitative real-time PCR

The total RNA of A549 and A549/DDP cells were extracted using the TRIzol reagent. 1000 ng of total RNA was reverse transcribed to synthesize cDNA. To quantify real-time PCR (qPCR), a total of 10μl of the reaction volume was used, including 5μl of 2×SYBR Master Mix, 0.25μl of each primer and 4.5μl of diluted cDNA. The parameters of RT-PCR were: 10 min at 95℃, followed by 40 cycles of 15 s at 95℃ and 1 min at 60℃. The reaction was performed using a CFX96 Real-Time PCR System (Bio-Rad). The cycle threshold (C_t) values were obtained and normalized to the level of GAPDH. The level of relative mRNA of each target gene was calculated using the $2^{-\triangle\triangle Ct}$ method. The primer sequences are as follows: AEG-1 forward：5'-TGTGTGTCCGTCTACAGATGTG-3' and reverse, 5'-TCGGCAGGAAGTGTGATTGG-3'. MDR-1 forward：5'-TTGCCAACCATAGATGAAGG-3',and reverse, 5'-CACCACTGGAGCATTGACTAC-3'. GAPDH forward,5'-GTG CCA GCC TCG TCT CA TAG-3', and reverse,5'-CTT TGT CAC AAG AGA AGG CAG-3'.Each reaction was run in triplicate. All experiments had efficiencies between 95% and 105%, and the primers displayed normal melt curves.

Statistical analysis

The data are expressed as the mean SD (standard deviation) of triplicates. The statistical significance of the differences throughout this study was assessed by one-way ANOVA. All analyses were conducted using SPSS 13.0 software. P-values <0.05 were considered statistically significant.

Results

The protein expression of AEG-1 in A549 and A549/DDP cells is positively correlated with cisplatin resistance.

In this study, A549/DDP cells resistant to cisplatin were constructed using A549 cells in lung cancer cell lines and transfected into A549 cells by exogenously overexpressing AEG-1 plasmid and knockdown AEG-1 plasmid. In the A549 and A549/DDP cell lines, the MDR-1 qPCR level increased with the overexpression of AEG-1 protein in A549 cells (Figure 1.A); when AEG-1 was knocked down in A549/DDP cells, the qPCR level of MDR-1 decreased (Figure 1.B). Based on this result, we harvested the A549 and A549/DDP cell lines after transfection of the plasmid and observed the overexpression and knockout effects by Western

blotting (Figure 1.Cand Figure 1.D), and the results showed that the plasmid was successfully transfected. The resulting cell lines has significant overexpression and knockdown of AEG-1 effects compared with those in the control cell lines. In addition, it was found that the expression of MDR-1 protein was significantly increased with the overexpression of AEG-1 protein and that the expression of MDR-1 protein was decreased with the knockdown of AEG-1 protein expression (Figure 1.C.D.E and F). This result suggests that AEG-1 protein is closely related to the expression of MDR-1 protein, which also indicates that the change of AEG-1 protein expression maybe is related to the drug resistance of lung cancer cells. Therefore, we further observed the effect of AEG-1 protein on the drug resistance of lung cancer cells by CCK-8 assay. The results showed that when AEG-1 protein was overexpressed in A549 cells, the lethal effect of cisplatin on A549 cells was attenuated; after AEG-1 protein was knocked down in A549/DDP cells, cisplatin was applied to the A549/DDP cells. The lethal effect was significantly increased compared to that in the control cells (Figure 1.G and H), indicating that the expression of AEG-1 protein is closely related to the drug resistance of lung cancer cells. Therefore, we found that the expression of AGE-1 protein is positively correlated with the expression of the drug resistance gene MDR-1 in lung cancer cells, which also indicates that the change in AEG-1 protein expression and the resistance of lung cancer cells to cisplatin is positively correlated.

T4 can affect the expression of AEG-1 protein in A549 and A549/DDP cells.

In our previous studies, we found that T4 is related to the drug resistance of lung cancer cells. Therefore, in this study, we wanted to further explore the potential mechanisms of T4 in lung cancer cell resistance. Based on the results of Figure 1, we further explored the correlation between T4 and AEG-1 protein expression. We first added different concentrations of T4 drugs to A549 and A549/DDP cells and detected the transcription level of the corresponding AEG-1 gene. The transcript level of AEG-1 in A549 and A549/DDP cells decreased with increasing T4 drug concentration (Figure 2.A and B). Therefore, we collected the protein of A549 and A549/DDP cells after treating them with different concentrations of T4 drugs and detected the expression of T4 and AEG-1 protein by Western blotting. The expression of AEG-1 protein gradually decreased with increasing concentration of T4 (Figure 2. C, D, E and F); thus, T4 can dose-dependent regulate the expression of AEG-1 protein in A549 and A549/DDP cells.

T4 can affect the drug resistance of lung cancer cells by affecting the expression of AEG-1 protein.

In our previous study, AEG-1 was closely related to A549 resistance to cisplatin; our previous studies also showed a correlation between T4 and A549 resistance to cisplatin. In further studies, it was clarified that T4 drugs can regulate the protein expression of AEG-1 in A549 cells. This indicates that the effects of T4 on the resistance of A549 cells to cisplatin may be further realized by affecting the expression of AEG-1 protein. Based on this, we carried out further research. We first treated A549/DDP cells with T4, and the protein of the corresponding cell strain was collected. Plasmid expressing AEG-1 was transfected into another similarly treated cell strain, and the protein of the corresponding cell strain was collected. Through Western blotting, we found that when T4 was added to A549/DDP, the expression of AEG-1 protein was significantly decreased and that the expression of MDR-1 was also decreased. After the AEG-

1 plasmid was transfected into the same treated cells, the expression level of MDR-1 was found to increase (Figure 3. A, B and C), which indicates that T4 regulates the expression of MDR-1 by affecting the expression of AEG-1. To further confirm this conjecture, we validated this hypothesis with the CCK-8 assay, which revealed that the resistance to cisplatin was reduced after the addition of T4 to A549/DDP cells, and this effect was attenuated after transfection with the AEG-1 plasmid (Figure 3. D). This indicates that T4 can affect the resistance of lung cancer cells to cisplatin by affecting the expression of AEG-1 protein.

Discussion

AEG-1 was first reported in 2002 as a neuropathology-associated gene induced in human fetal astrocytes following human immunodeficiency virus-1(HIV-1) infection or treatment with recombinant HIV-1 envelope glycoprotein (gp120), which has emerged as a potentially crucial mediator of malignant tumors, and a key converging point of a complex network of oncogenic signalling path-ways. [10,16] One of the important hallmarks of aggressive cancers is chemoresistance. Studies have suggested that AEG-1 contributes to a broad spectrum of resistance to various chemotherapeutics, including 5-fluorouracil, doxorubicin, paclitaxel, cisplatin and 4-hydroxycyclophosphamide. [5,10,17-18]AEG-1 has been reported to be related to the resistance of tumours to chemotherapy drugs, with an increasing number of studies published on this matter in recent years. AEG-1 increases multidrug-resistance gene 1 (MDR1) protein expression, which facilitates the association between MDR1 mRNA and polysomes, leading to increased translation, the inhibition of ubiquitination and the resultant proteasome-mediated degradation of the MDR1 protein. [5] Studies have shown that elevated AEG-1 expression can increase the resistance of tumours to chemotherapeutic drugs. For example, AEG-1 can increase the expression of the MDR1 gene in tumours such as liver cancer and breast cancer. AEG-1 can increase the MDR1 mRNA translocation to polysaccharide nucleosome so as to promote the translation of MDR1 protein [19]. Lung cancer is a malignant tumour that is extremely resistant to chemotherapy drugs. Based on our previous research, we found that triptolide, a kind of attenuated monomer extracted from Tripterygium, increases the sensitivity of lung cancer cells to cisplatin, thereby increasing the lung cancer cell death rate; [15]in addition, the literature has reported that AEG-1 is associated with tumour resistance. Therefore, in our study, we investigated the effect of triptolide on the expression of AEG-1 in lung cancer cells and the effect of AEG-1 expression on the sensitivity of chemotherapeutic drugs and further verified whether triptolide can inhibit the drug resistance of lung cancer cells by inhibiting the expression of AEG-1, providing a new direction for the treatment of lung cancer patients.

Cisplatin is a powerful anticancer drug. Currently, cisplatin is widely used in the treatment of various human cancers. It has significant clinical effects on various solid tumours, such as bladder cancer and head and neck cancer, ovarian cancer and testicular cancer. [19] Due to its strong antitumor activity, cisplatin is also the key drug for chemotherapy of non-small-cell lung cancer in the clinical treatment of lung cancer. However, drug resistance often leads to the failure of chemotherapy for lung cancer. Therefore, we selected A549/DDP cells as the research object in this study. In this study, we first explored

the relationship between AEG-1 and drug resistance in the lung adenocarcinoma cell line A549 and the cisplatin-resistant lung adenocarcinoma cell line A549/DDP. We first used plasmids to construct cell lines that overexpressed and knocked down AEG-1 and then observed changes in the survival rate of cells treated with cisplatin. qPCR and WB expression analysis of MDR1 was used to observe the relationship between AEG-1 and drug resistance in lung cancer cells. The experimental results showed that the gene expression of AEG-1 was positively correlated with the resistance of the cell line to cisplatin.

However, our previous studies have shown that triptolide can increase the sensitivity of A549/DDP cells to cisplatin. Combined with our results, the gene expression of AEG-1 was demonstrated to be positively correlated with the resistance of cell lines to cisplatin. Therefore, we speculate that triptolide can affect the sensitivity of cells to cisplatin by affecting the gene expression of AEG-1. In this study, we first explored the changes in the expression of AEG-1 in response to triptolide. The results indicate that triptolide inhibits the expression of the AEG-1 gene, and as supported by previous findings, overexpression of the AEG-1 gene increases the expression of the MDR1 gene in lung cancer cells. Therefore, in this study, we explored the overexpression and knockdown of AEG-1 under the action of triptolide to determine whether it affects the expression of MDR1. The results showed that triptolide can reduce the expression of MDR1 by inhibiting the expression of AEG-1, thereby increasing the sensitivity of lung cancer cells to cisplatin. Combined with our previous studies, we found that T4 can induce autophagy by inhibiting the PI3K/AKT/mTOR pathway, causing tumour cell death. AEG-1 has also been reported in many studies related to autophagy and apoptosis in tumour cells and has a close relationship with the PI3K/AKT/mTOR pathway. Therefore, further exploration of pathways in tumour cells will be performed in the future to clarify the regulation of AEG-1 protein expression by T4.

Conclusion

In summary, our current research indicates that triptolide is important for increasing the sensitivity of lung cancer cells to cisplatin. AEG-1 may be a key protein involved in this effect and may have an important impact on the survival rate of chemotherapy in patients with lung cancer in the future. AEG-1 will present as an ideal target for the development of the next generation of effective cancer therapeutics.

Abbreviations

NSCLC: non-small-cell lung cancer; SCLC :small-cell lung cancer ; MDR :multidrug resistance; P-gp :p-glycoprotein; AEG-1:astrocyte upregulated gene 1; T4:Tripchlorolide; MTDH: Metadherin; DMSO :dimethyl sulfoxide; CCK-8:Cell Counting Kit-8; TEMED:N,N,N',N'-Tetramethylethylenediamine; AP: Ammonium persulfate; DMEM: Dulbecco's modified eagle medium; FBS: fetal bovine serum; RPMI: Roswell Park Memorial Institute; SDS-PAGE :sodium dodecyl-sulfate-polyacrylamide gel electrophoresis; GAPDH:glyceraldehyde-3-phosphate dehydrogenase; PCR :polymerase chain reaction; SD: standard deviation; HIV-1:human immunodeficiency virus-1; WB: western blotting.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

None.

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Author contributions

Li-min Chen conceived the study, participated in its design and coordination, supervised the experiments. Tian-jiao Song□Xiao-hong Lin□Ping-ting Huang Yu-qing Chen performed most of the experiments and drafted the manuscript. Tian-jiao Song and Li-min Chen took part in qRT-PCR and Western blotting analysis. Xiao-hong Lin and Ping-ting Huang contributed to statistical analysis and polish the manuscript. All authors read and approved the manuscript.

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Figures

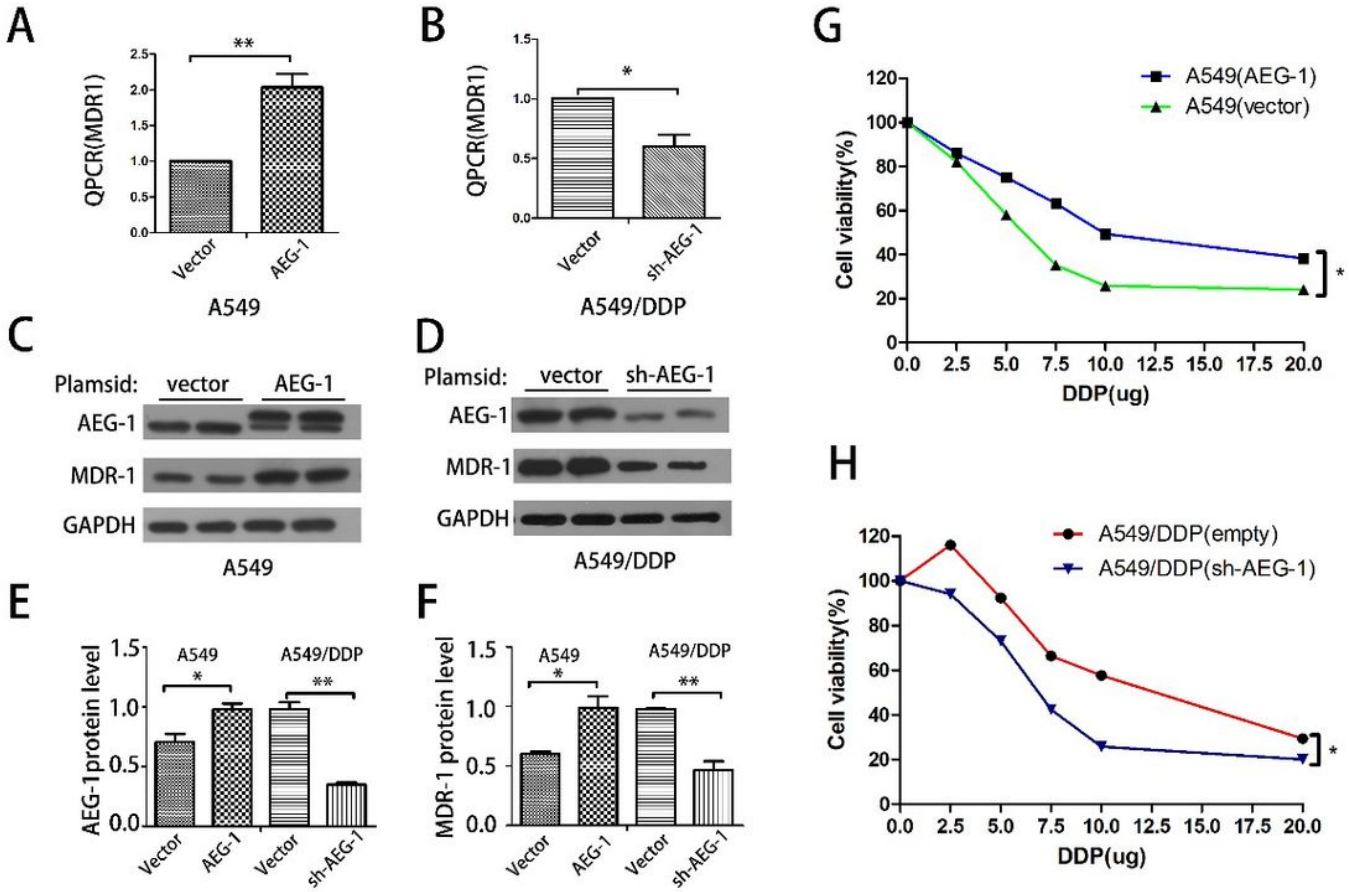

Figure 1

A and B. Overexpression of AEG-1 in A549 cells and knockdown of AEG-1 in A549/DDP cells revealed that the transcription level of MDR-1 increased and decreased, respectively, compared to that in control cells. C. The protein expression level of MDR-1 was increased after overexpression of AEG-1 in A549 cells. D. The protein expression level of MDR-1 was decreased after knockdown of AEG-1 in A549/DDP cells. E. Verification of expression levels after overexpression of AEG-1 and knockdown of AEG-1 in A549 and A549/DDP cells. F. AEG-1 protein expression level was positively correlated with MDR-1 protein expression level in A549 and A549/DDP cells. G. Overexpression of AEG-1 in A549 cells attenuates the lethal effect of cisplatin on cells. H. The knockdown of AEG-1 in A549/DDP cells increases the lethal effect of cisplatin on cells.

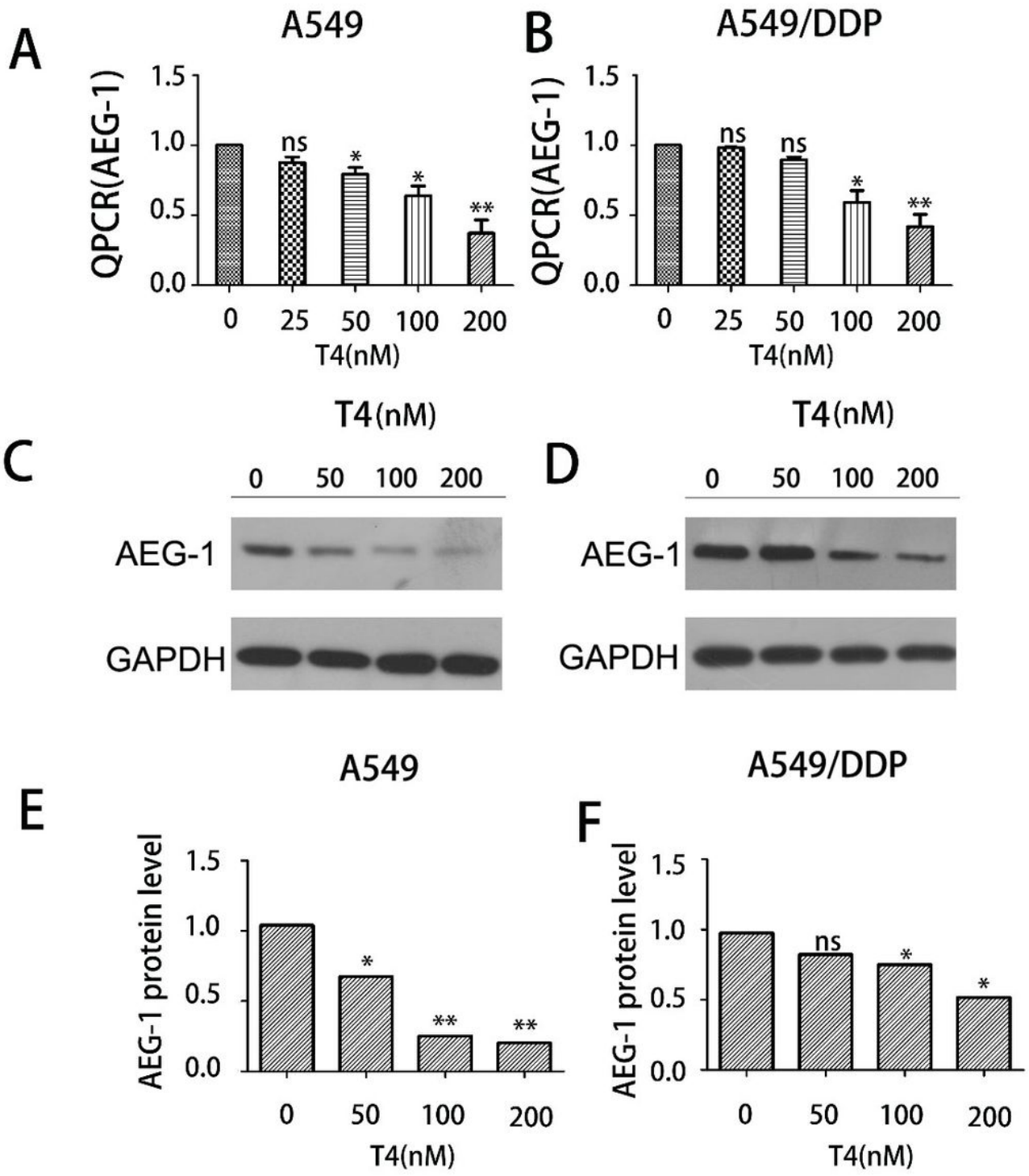

Figure 2

A and B. As the concentration of T4 increased in A549 and A549/DDP cells, the transcription level of AEG-1 gradually decreased. C, D, E and F. As the concentration of T4 increased in A549 and A549/DDP cells, the level of MDR1 protein gradually decreased.

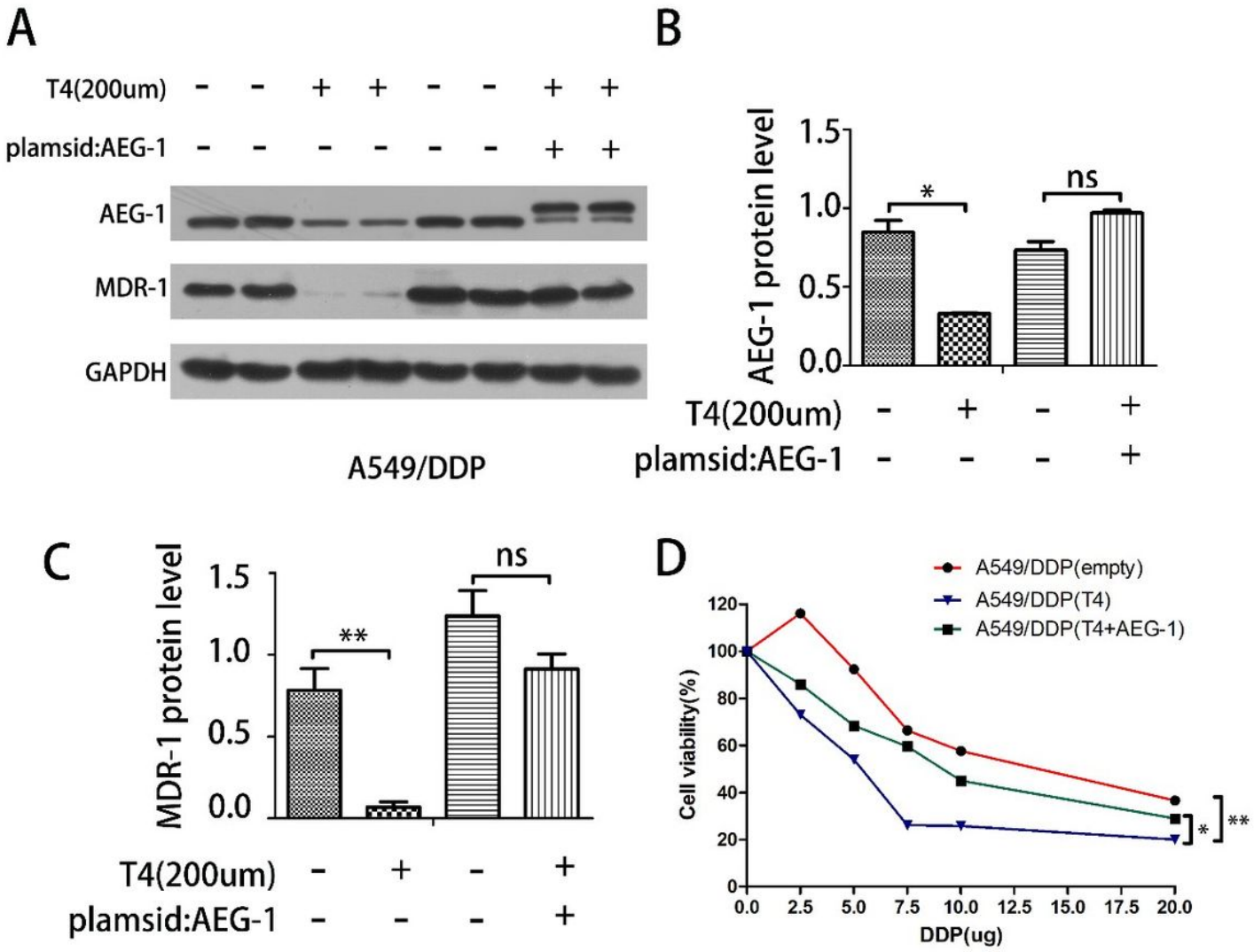

Figure 3

A. The expression levels of AEG-1 and MDR-1 protein decreased after transfection of T4 in A549/DDP cells. The effect of T4 on MDR-1 protein expression was reversed after overexpression of AEG-1. B. Changes in AEG-1 protein expression levels were quantified after transfection of AEG-1 overexpression plasmid and/or treatment with T4 and A549/DDP cells. C. Quantification of changes in MDR-1 protein expression levels in A549/DDP cells after overexpression of AEG-1 protein and/or treatment with T4. D. CCK-8 experiments demonstrated that the addition of T4 to A549/DDP cells increased the lethal effect of cisplatin on A549/DDP cells, and this lethal effect was attenuated after re-expression of AEG-1.