Original Article

Quinalizarin, a specific CK2 inhibitor, reduces cell viability and suppresses migration and accelerates apoptosis in different human lung cancer cell lines

Zhou Y[#], Li K[1#], Zhang S, Li Q, Li Z, Zhou F, Dong X, Liu L, Wu G, Meng R

Cancer Center, [1]Department of Pharmacy, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430023, China

[#]Yu Zhou and Ke Li contribute equally to this work

Correspondence to: Dr. Rui Meng, E-mail: mengruivip@163.com

Abstract

BACKGROUND: Protein kinase CK2 is widely expressed in eukaryotic cells, and plays an important role in cell proliferation, migration, apoptosis, etc. The aim of the current study is to explore how Quinalizarin, a specific CK2 inhibitor, affects the cell proliferation, migration, and apoptosis of different pathological and genetic types of human lung cancer cell lines. **MATERIALS AND METHODS:** MTT assays were performed to evaluate the cell viability after being treated by Quinalizarin. Transwell migration assays were used to assess whether Quinalizarin could suppress cell migration. Flow cytometry was employed to test the apoptosis rate of different cells. **RESULTS:** After being treated by Quinalizarin, the viability of different pathological types of lung cancer cells (H446, H460, A549) were significantly suppressed in a time and dose-dependent manner. More interestingly, in a serial of human lung adenocarcinoma cell lines with different epidermal growth factor receptor (EGFR) mutation status, Quinalizarin was shown to have a much better ability to reduce the viability of cells with EGFR sensitive mutation than those with resistance mutations. Meanwhile, we also found that the cell migration of different pathological types of lung cancer cells (H446, H460, A549) was significantly decreased by Quinalizarin dose-dependently. In addition, the apoptosis rates in those cells were proved to be increased after exposed to Quinalizarin. **CONCLUSIONS:** Quinalizarin, the specific CK2 inhibitor, could reduce cell viability with emphasis on adenocarcinoma cells harboring EGFR sensitive mutation, suppresses migration, and accelerates apoptosis in different human lung cancer cell lines.

Key Words: Epidermal growth factor receptor, lung cancer, protein kinase CK2, Quinalizarin

Introduction

According to the results of a new survey in 2014 by the American Cancer Society, lung cancer is still the second most in the estimated new cases while the leading cause of cancer-related death.[1] Meanwhile, lung cancer has also taken the first place of cancer incidence and mortality in China based on the statistics of Chinese National Cancer Institute in 2014. Thus, we are currently in urgent need for more effective treatment modalities of lung cancer.

Protein kinase CK2 is a messenger-independent serine/threonine kinase, which is proved to be widely expressed in eukaryotic cells.[2] Studies have indicated that CK2 was over-expressed in various human cancers including lung cancers.[3] Several studies even shown that the activity of CK2 was up to 2–3 folds in lung cancer cells compared with the normal lung tissues. Recently, a Kaplan–Meier plotter analysis revealed that the overexpression of CK2β leaded to the overall lower survival rates in lung cancer.[4] In addition, CK2 was convinced of involving in the malignant proliferation, apoptosis resistance, and other malignant behaviors of different cancer cells.[5,6] More and more ongoing preclinical studies and clinical trials have confirmed the potential of targeting protein kinase CK2 for various cancer therapies.

Down-regulation of CK2 activity by means of chemical inhibition,[7] as well as antisense oligodeoxynucleotide,[6] RNA interference (RNAi),[8] or overexpression of exogenous kinase-inactive CK2 can induce apoptosis in cancer cells.[9] Chemical inhibitors can penetrate the cells easily and presumably are able to inhibit all CK2 in cells. CK2 inhibitors of different classes of chemical compounds have been investigated as ATP-competitive inhibitors of CK2.[10] One of the ATP-competitive inhibitors, Quinalizarin has already been acknowledged as a potent, selective, and cell-permeable inhibitor of protein kinase CK2, and been recognized as more efficiently than the commonly used CK2 inhibitors 4,5,6,7-tetrabromo-1H-benzotriazole and 2-dimethylamino-4,5,6,7-tetrabromo-1H-benzimidazole on suppressing endogenous CK2 and inducing apoptosis.[11]

In this study, we try to determine the role of Quinalizarin in reducing cell viability, suppressing migration, and promoting apoptosis in different human lung cancer cell lines, and to evaluate its potential use in the treatment of lung cancer.

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Materials and Methods

Cell lines

The small cell lung cancer cell line NCI-H446, human large cell carcinoma NCI-H460, as well as human lung adenocarcinoma A549, and PC9, NCI-H1975, NCI-H1650 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and were used within 3 months after resuscitation. The culturing was done according to protocols prescribed by ATCC.

Reagents

Quinalizarin was purchased from Merck.

Cell viability assays

For each cell lines, cells were harvested at logarithmic phase, then seeded onto a 96-well plate at a density of 6000 cells per well with 100 μL of culture medium. Cells were cultured at 37°C with 5% CO_2 overnight, then several concentrations (12.5, 25, 50.0 μmol/L or 6.25, 12.5, 25.0, 50.0, and 100.0 μmol/L) of Quinalizarin were added to each well with 200 μL of culture medium, and incubation

was continued for a further 24 h, 48 h, or 72 h. After incubation, 20 μL MTT solution was added into each well. Moreover 4 h later, 150 μL of dimethyl sulfoxide was used to dissolve the crystals. Plates were placed on a low-speed shaker for 10 min to dissolve fully crystals. The optical density (OD) of each well was read at 560 nm using an ELISA reader. Values were calculated as follows: Absorbance fraction = (OD value of experimental well − OD value of blank control well)/(OD value of no drug control well − OD value of blank control well), cell viability = (OD value of experimental well − OD value of blank control well)/(OD value of no drug control well − OD value of blank control well) × 100% and each experiment for one cell lines was repeated three times.

Transwell migration assays

Transwell chambers (BD, Franklin Lakes, NJ, USA) were set up with 8-mm pore size filters to assess migration ability. Cells were harvested at the logarithmic phase and seeded onto the chamber's upper compartment with 100 μL of serum-free medium cell suspensions (3×10^5/ml) with several concentrations (0, 12.5, 25.0, 50.0 μmol/L) of Quinalizarin, and each group had 3 holes. At the same time, 500 μL medium with 10% serum was added into the lower chamber. After 24 h incubation at 37°C and 5% CO_2 culture, the migrated cells would locate on the lower surface of the Transwell filters. Then, these cells were fixed for 30 min in methanol and stained with 0.1% crystal violet for another 30 min. In each of 3 randomly selected sights under the microscope magnifying 200, the stained cells were observed and counted.

Apoptosis assays

Detection of apoptosis using Annex V-EGFP/PI staining and flow cytometry, cells in the logarithmic growth phase were plated onto 12-well plates. After suspended for 24 h for attachment, cells were washed with Phosphate buffered saline 2–3 times, and drugs were added, in which the concentrations were 6.25, 12.5, 25.0, 50.0, and 100.0 μmol/L. After being treated by 48 h later, the cells were cultured with trypsin enzyme digesting technique, and the apoptotic cells were appraised using an Annexin V-FITC-PI Kit (Beyotime Institute of Biotechnology) as product specification described. After stained, the cells were then detected by flow cytometry (Cytomics FC 50 MPL, Beckman Coulter). The results were analyzed by mean values of three independent experiments.

Statistical analysis

All statistical analyses and statistical diagrams were generated with Prism 5 software (GraphPad Software, San Diego, CA, USA).

Results

Quinalizarin suppresses cell growth of different pathological types of human lung cancer cell lines

MTT assays were performed to evaluate the cell viability after being treated by Quinalizarin. Figure 1 shows that Quinalizarin suppresses cell growth of different pathological types of human lung cancer cell lines. As shown in Figure 1a, the cell viability of small cell lung cancer cell H446 was inhibited by Quinalizarin time-dependently between 24 h and 72 h and dose-dependently with the concentration of 12.5, 25.0, and 50.0 μmol/L ($P < 0.0001$, $P < 0.0001$). Meanwhile, from Figure 1b, we get the result that Quinalizarin can suppress cell viability in a same time and dose-dependent manner in H460, which is known as human large cell carcinoma cell lines ($P < 0.0001$, $P < 0.0001$). In the currently most common pathological type of nonsmall cell lung cancer-human lung adenocarcinoma, such as

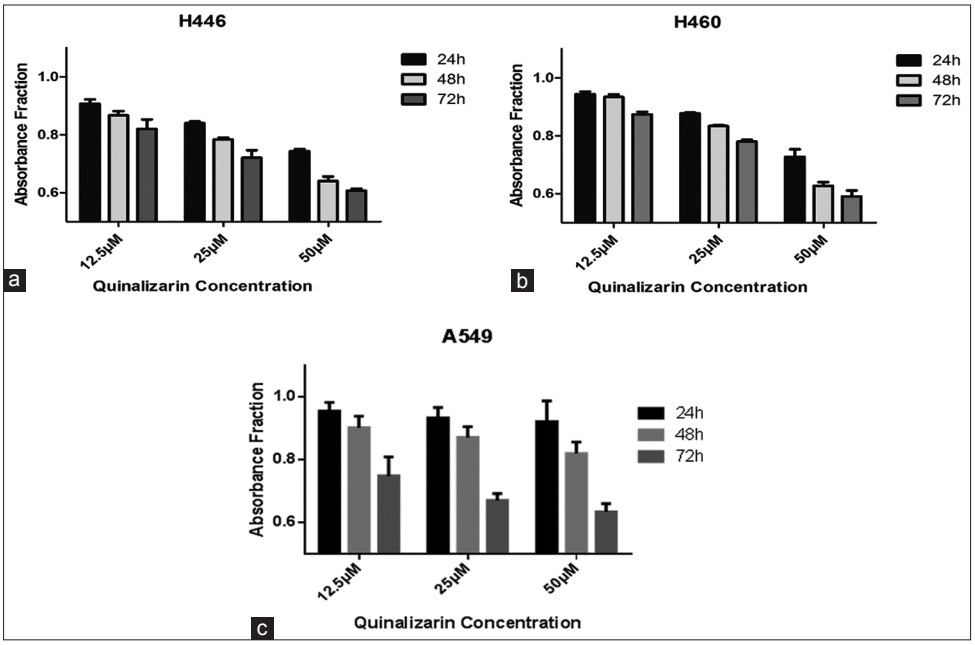

Figure 1: Quinalizarin suppresses cell proliferation in different pathological types of human lung cancer cell lines. (a) The inhibition effect of Quinalizarin was time and dose-dependent between 24 h and 72 h with the concentration of 12.5, 25.0, and 50.0 μmol/L ($P < 0.0001$, $P < 0.0001$) in small cell lung cancer cell lines H446. (b) Quinalizarin also shows evidently time and dose dependence in H460, which is known as human large cell carcinoma cell lines ($P < 0.0001$, $P < 0.0001$). (c) In human lung adenocarcinoma cell lines A549, Quinalizarin also shows time and dose dependent ($P < 0.0001$, $P < 0.0001$) when analyzed with two-way ANOVA

A549, the inhibition effect of Quinalizarin is evidently shown in Figure 1c in a time and dose-dependent manner ($P < 0.0001$, $P = 0.0002$), when analyzed with two-way ANOVA.

Quinalizarin reduces the cell viability in human lung adenocarcinoma cell lines with different epidermal growth factor receptor status

From the above experiments, we found that Quinalizarin could effectively inhibit the proliferation of different pathological types of lung cancer cell lines H446, H460, and A549. As is known, more than 80% of lung cancer were lung adenocarcinoma. Then we try to evaluate if Quinalizarin could also reduce the cell viability in serial of human lung adenocarcinoma cell lines with different epidermal growth factor receptor (EGFR) status. We select 4 kinds of cell lines A549 (EGFR wild type), PC9 (EGFR E716-A750del), H1650 (EGFR E716-A750del and PTEN lost), and H1975 (EGFR L858R+T790M). PC9 is sensitive to EGFR tyrosine kinase inhibitors (EGFR-TKIs), A549, H1650, and H1975 are known as EGFR-TKIs resistance cell lines. As Figure 2 shows, Quinalizarin could reduce cell viability in all the selected cell lines, and have a much better ability to reduce the viability of cells with EGFR sensitive mutation than those with resistance mutations.

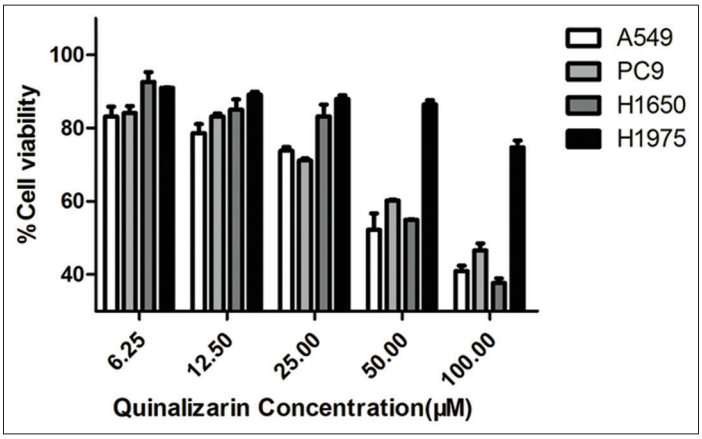

Figure 2: Quinalizarin reduces cell viability in various human lung adenocarcinoma cell lines with different epidermal growth factor receptor status when treated with different concentration (6.25, 12.5, 25.0, 50.0, and 100.0 μmol/L) for 48 h. Cell viability among different cell lines at the same dose of Quinalizarin shows a statistically significant difference ($P < 0.0001$). Among the various doses 6.25, 12.5, 25.0, 50.0, and 100.0 μmol/L, the cell viability for each one cell line was also statistically significant ($P < 0.0001$)

Quinalizarin inhibits migration in various human lung cancer cell lines

Transwell migration assays were employed to assess whether Quinalizarin could suppress migration in different kinds of lung cancer cell lines. Results showing that the cell migration ability was significantly decreased after being treated by Quinalizarin for 24h. Figure 3a-c shows respectively in different lung cancer cell lines A549, H446, and H460 that migration of these cells is significantly suppressed by Quinalizarin in a dose-dependent manner. Especially in A549 and H460, Quinalizarin represents a potent drug to suppress migration.

Quinalizarin promotes apoptosis in kinds of human lung adenocarcinoma cell lines

Since we determined that the Quinalizarin could reduce the cell viability of human lung adenocarcinoma cell lines with different EGFR status and do more effectively in cells

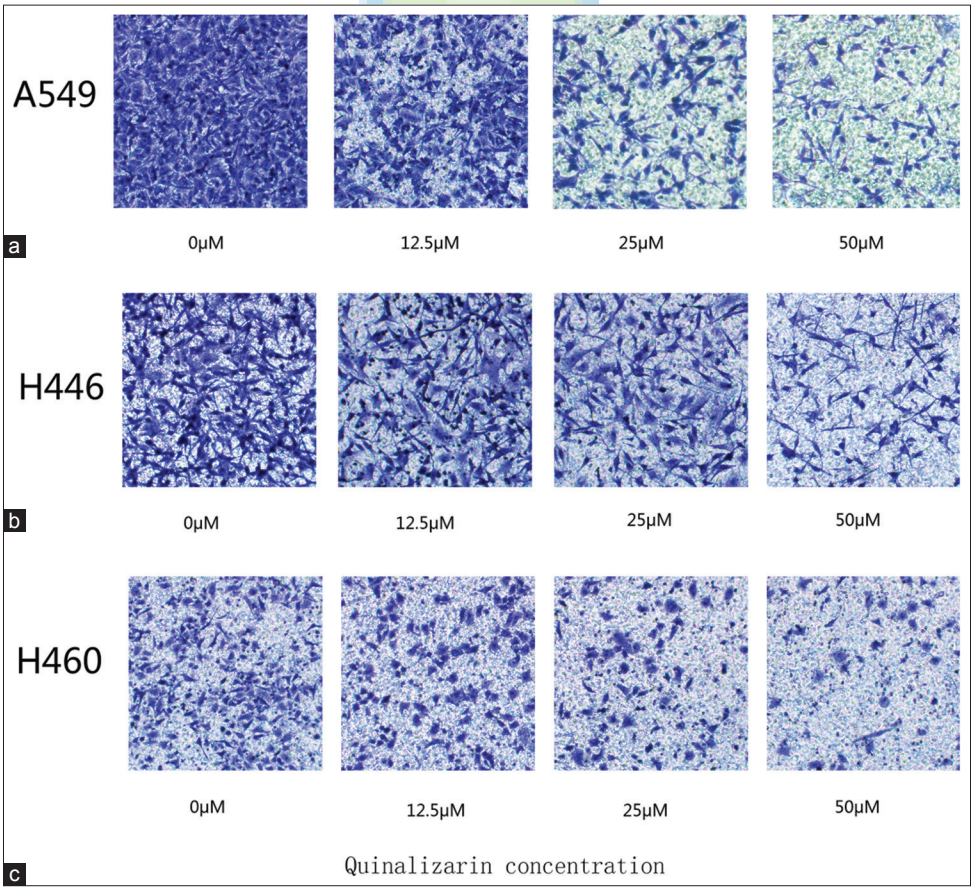

Figure 3: (a-c) Quinalizarin inhibited migration in various lung cancer cell lines. Migration was also evaluated using Transwell assays

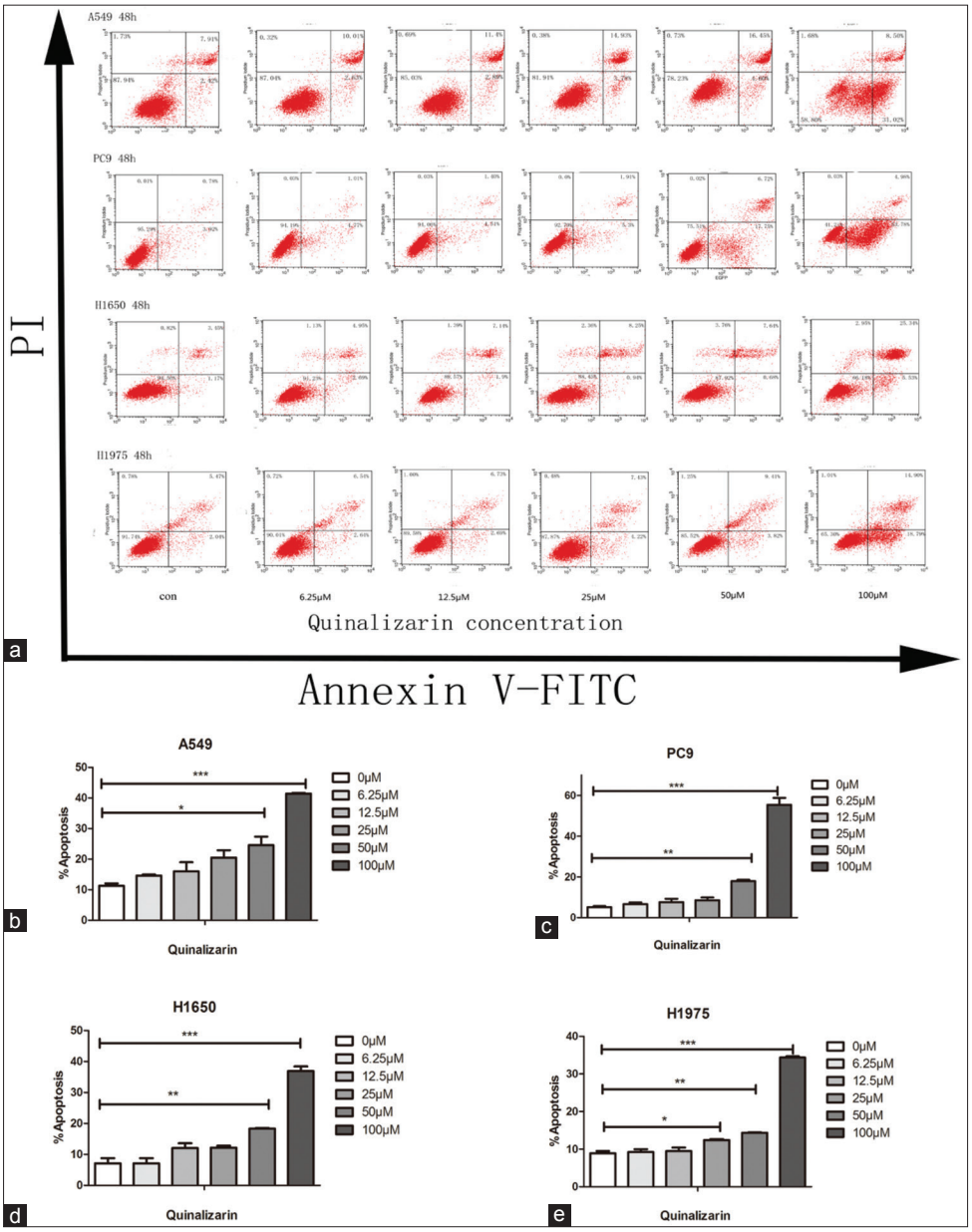

Figure 4: Apoptosis induced by Quinalizarin. (a) Apoptosis detected by flow cytometry in various kinds of human lung adenocarcinoma cell lines. (b-e) Quantitative analysis of apoptotic cells in pulmonary carcinosis A549 cell, PC9, H1650, and H1975. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ versus control

with EGFR sensitive mutation, we next wonder that if Quinalizarin would play a similar role on cell apoptosis of these cells. Flow cytometry was used for apoptosis assays after the cells being treated by Quinalizarin. As shown in Figure 4a, Quinalizarin could promote apoptosis in different kinds of human lung adenocarcinoma cell lines. Figure 4a-e shows, respectively, the analysis of apoptotic cells in A549, PC9, H1650, and H1975. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control.

Discussion

Lung cancer is the leading cause of cancer death world-wide, and the existing treatment modalities for lung cancer is limited.[12] There is an urgent call for new promising therapeutic targets for solving the problem.[13] Protein kinase CK2 has been proved to be widely expressed in eukaryotic cells, and many studies have indicated that CK2 was over-expressed in various human cancers, especially in lung cancer, the activity of CK2 was up to 2–3 folds.[14] As known, Quinalizarin is proved to be a potent, selective, and cell-permeable inhibitor of protein kinase CK2.[11] Quinalizarin may play an important role on the treatment of lung cancer, especially, targeting CK2 may be a way to overcome EGFR-TKIs resistance that has long time confused us.

CK2 is associated with many diseases and plays an important role in the course of tumor progression.[15,16] CK2 inhibitors are shown to have antineoplastic activity in various cancers.[17-20] In 2001, Landesman-Bollag *et al*. found that the over-expression of CK2 in the breast tissue lead to the breast cancer of mice, and their further experiments confirmed that CK2 inhibitors may be beneficial for the treatment of the breast cancer.[21] Then, a series of tests about CK2 are implemented in many other kinds of cancer. Importantly, several experiments about lung cancer also show the similar results by using CK2 inhibitors. Latest, So *et al*., find that a CK2 inhibitor, CX4945, could promote apoptosis in nonsmall cell lung

cancer cells by the down-regulation of AKT/mammalian target of rapamycin (mTOR) pathway.[22] Ku MJ and Lee SY also report that the inhibition of CK2 by CX4945 could suppress the migration and invasion of A549 human lung cancer cells.[18] More importantly, Hung *et al*. find that CK2 inhibitor, hematein could suppress the lung cancer tumor growth even in a murine xenograft model.[23] Quinalizarin, which is known as one of ATP-competitive inhibitors of CK2, has the similar effects with CX4945 on the protein kinase CK2.[24] Dose Quinalizarin have the similar effects on lung cancer? In the experiment, we get the conclusion that Quinalizarin could also reduce cell viability and suppress migration in lung cancer cell lines H460, H446, and A549. Moreover the effects are more obvious in the lung adenocarcinoma cancer cell lines A549.

As we know, EGFR-TKIs is very effective and widely used for the treatment of lung cancer, but the secondary drug resistance of EGFR-TKIs is ubiquitous and Masty.[25,26] Some scientists have established the positive effects of CK2 inhibitors on overcoming the EGFR-TKIs resistance. Bliesath *et al*. report that the inhibition of both EGFR and CK2 would show stronger antitumor effects by depressing the PI3K-Akt-mTOR signaling.[27] Hence *et al*., also find that the CK2 inhibitor may enhance the efficacy of EGFR-TKI on lung cancer cells, which is EGFR-mutant and resistance by T790M.[28] Some other experts even proved CK2 inhibitor could reverse the multidrug resistance in the treatment of lung cancer to some extent.[29,30] In our experiments, we found that Quinalizarin exerted a significant inhibition effects on cell viability and migration of different lung cancer cells, with an emphasis on lung adenocarcinoma. Furthermore, we determined that the Quinalizarin could also suppress the lung adenocarcinoma with different EGFR genotypes, especially in those cells harboring a sensitive EGFR mutation. In addition, we also demonstrate that Quinalizarin may accelerate apoptosis in these lung cancer cell lines with different EGFR genotypes, which represents a great support for the rational of combining EGFR-TKI and CK2 inhibitors to treat lung cancers much more effectively, as is already shown by other preclinical trials.[27,28]

Conclusion

Taken together, our experiments proved that Quinalizarin, a specific CK2 inhibitor, could effectively reduce cell viability and migration, as well as accelerate apoptosis in different pathological and genetic types of human lung cancer cell lines, which may considered as a new promising approach to treat lung cancer.

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