Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2020, **18**(7): 483-490
doi: 10.1016/S1875-5364(20)30058-3
Chinese Journal of Natural Medicines

•Special topic•

In vitro antitumor effect of cucurbitacin E on human lung cancer cell line and its molecular mechanism

JING Si-Yuan[1], WU Zi-Dan[1], ZHANG Tie-Hua[1], ZHANG Jie[1*], WEI Zheng-Yi[2*]

[1] *College of Food Science and Engineering, Jilin University, Changchun 130062, China;*
[2] *Institute of Agricultural Biotechnology, Jilin Academy of Agricultural Sciences, Changchun 130033, China*

Available online 20 Jul., 2020

[ABSTRACT] Cucurbitacin E (CuE) is previously reported to exhibit antitumor effect by several means. In this study, CuE acted as a tyrosine kinase inhibitor interfering with the epidermal growth factor receptor/mitogen-activated protein kinase (EGFR/MAPK) signaling pathway and subsequently induced apoptosis and cell cycle arrest in non-small-cell lung cancer (NSCLC) cell line A549. The apoptosis regulators, cleaved Caspases-3 and Caspases-9, were observed to be increased with the treatment of CuE. The activated transcription factor STAT3 and the apoptosis inhibitor protein survivin were also observed to be reduced. The cell cycle regulators, CyclinA2, cylinB1, CyclinD1 and CyclinE, were also investigated and the results suggested that the cell cycle was arrested at G1/G0 phase. Treatment of CuE also altered the existence status of most of the participants in the EGFR/MAPK signaling. Phosphorylation of EGFR enhanced significantly, leading to the alteration of members downstream, either total amount or phosphorylation level, notably, MEK1/2 and ERK1/2. Moreover, the results of molecular simulation brought an insight on the interaction mechanism between CuE and EGFR. In summary, CuE exhibited anti-proliferative effect against A549 cells by targeting the EGFR/MAPK signaling pathway.

[KEY WORDS] Cucurbitacin E; Antitumor effect; Lung cancer cell; Apoptosis; Cell cycle arrest
[CLC Number] R965 **[Document code]** A **[Article ID]** 2095-6975(2020)07-0483-08

Introduction

In recent years, more and more attention has been paid to plant-derived compounds for the prevention and treatment of various diseases. For example, saponins, as natural products of plant-derived, have been widely used in dietary modification of metabolic syndrome [1]. Cucurbitacins, as a set of bioactive plant source compounds, are tetracyclic triterpenes found in cucurbitaceous plants originally. Unlike most other tetracyclic triterpenes, they are highly unsaturated and containing numerous hydroxy-, keto- and acetoxy-groups. Cucurbitacins have shown cytotoxic properties and are currently being studied for their pharmacological and potential biological activities [2]. Many of them have been developed as potential drugs to treat inflammatory and cancer [3, 4]. For example, cucurbitacin C has antitumor activity *in vivo* and *in vitro*, inhibiting the proliferation and cloning potential of multiple cancer cells in a dose-dependent manner [5]. There are many varieties of cucurbitacins, and researches on the anticancer activity of them have been focused on the four varieties B, D, E and I [6]. Among them, cucurbitacin E (CuE) with strong antioxidant and anticancer activity derives from plants of the genus cucurbitaceae melon (*Cucumis melo* L.) stem. As CuE is the first cucurbitacin synthesized [7] and abundantly distributed in plants, it can be extracted in large quantities in many traditional Chinese medicinal plants. In the matter of fact, CuE is the a major cucurbitacin in Chinese medicine Guadi (fruit base of *Cucumis melo* L.) [8] and occupies the largest portion of cucurbitacin in water melon (*C. lanatus*), a popular fruit [9], thus there were abundant original material for its production. Given CuE is reported to have lower half inhibitory concentration in many researches [10-12], it is an attractive compound for deeper investigation. For instance, one study *in vitro* has demonstrated that CuE therapy can inhibit the expression of YAP and its downstream signaling genes in non-small cell lung cancer, and inhibit brain metastasis of non-small cell lung cancer in mouse model experiments [13]. CuE was reported to inhibit tumor angiogenesis through VEGFR2 mediated Jak2/STAT3 signaling pathway [14]. It also can activate AMPK and block mTOR depending on signaling pathway to improve hepatic fibrosis [15]. CuE

[Received on] 12-Dec.-2019
[Research funding] This work was supported by the National Natural Science Foundation of China (Nos. 31871717, 31972160, and 31601534), the Science and Technology Development Project Foundation of Jilin Province (Nos. 20200201204JC and 20160441003SC), and the National Key Research and Development Program of China (Nos. 2018YFD0300201 and 2016YFD0300103).
[*]**Corresponding author]** E-mail: zhangjjlu@163.com (ZHANG Jie); weizy80@163.com (WEI Zheng-Yi)
These authors have no conflict of interest to declare.

co-treatment can enhance doxorubicin concentration in the M5076 ovarian sarcoma cells.

Epidermal growth factor receptor (EGFR) is a transmembrane protein and a receptor tyrosine kinase. Mutations that lead to EGFR over-expression are associated with the development of various tumors [16]. Therefore, EGFR is a hot molecular target in cancer therapy [17-19]. Several cucurbitacins have been reported to exert their antitumor activities via EGFR/mitogen-activated protein kinase (EGFR/MAPK) signaling pathway [20]. Studies presented thus far support the idea that the EGFR target is a therapeutic target for many cucurbitacins varieties as potential anticancer drugs. For instance, cucurbitacin IIa can interfere the EGFR/MAPK signaling pathway and lead to inhibition the proliferation of A549 cell [21]. Cucurbitacin B (CuB) induces lysosomal degradation of EGFR and inhibits the CIP2A/PP2A/Akt signaling axis in patients with gefitinib-resistant NSCLC [21]. CuB was also reported to induce cell cycle inhibition and apoptosis in colorectal cancer cells by inhibiting the STAT3 and EGFR signaling [22]. Treatments with CuB inhibited the expression of EGFR and its downstream signaling pathways to suppress the proliferation of pancreatic cancer cells by combining of inducing cell cycle arrest [23]. In breast cancer cell, cucurbitacin I can inhibit the motility and Rac1 activation by targeting to EGFR [24]. Although CuE has been reported to be capable of inhibiting variety of cancer cells in various mechanism [13-15, 25-30], up to date, available mechanism involving EGFR or the EGFR/MAPK pathway is limited in the treatment of two leukemia cell lines [30]. As lung cancer is concerned, several studies with CuE treatments are available [13, 14, 31, 32], but EGFR or EGFR/MAPK pathway involved mechanism is still unrevealed.

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer, and as a typical cell line of NSCLC, A549 is a famous platform in anticancer research for revealing mechanisms and therapeutic drugs exploration for NSCLC. This study was designed to reveal the potential mechanism in the inhibition of CuE to NSCLC cell line A549. In this study, apoptosis and cell cycle in A549 cells were investigated after treatments of CuE. The regulators of apoptosis and cell cycle as well as the members of possible upstream EGFR/MAPK signaling pathway were examined. In addition, the binding mode and binding stability of CuE (Fig. 1) with EGFR were explored by combination of molecular docking and molecular dynamics (MD) simulation.

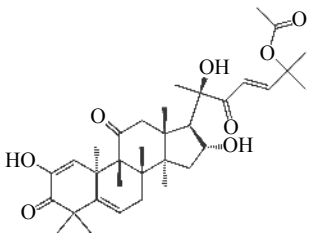


Fig. 1 Structure of cucurbitacin E

Materials and Methods

Materials

Human non-small cell lung cancer (A549) was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Dulbecco's modified eagle's medium (DMEM), dimethyl sulfoxide (DMSO), 0.25% trypsin solution (with EDTA) and Penicillin-Streptomycin Liquid were obtained from Solarbio Life Science Ltd. (Beijing, China). Fetal bovine serum (FBS) was obtained from HyClone (Logan, UT, USA). A549 cells were cultured in DMEM with 1% of Penicillin-Streptomycin Liquid and 10% FBS in 10 cm petri dishes at 37 °C with 5% CO_2 in incubator.

Primary antibodies for EGFR, p-EGFR(Y1068), RAS, BRAF, p-BRAF (T401), Raf1, p-Raf1(S338), MEK1/2, p-MEK1/2(S218/S222), ERK1/2, p-ERK1/2(T202/Y204), Caspase-3, Caspase-9, STAT3, p-STAT3(Y705), Survivin, CyclinA2, CyclinB1, CyclinD1, cyclinE1 and GADPH were obtained from Abcam Co. (Cambridge, UK). Goat anti-rabbit HPR-conjugated secondary antibody was obtained from Sino Biological Inc. (Beijing, China) and ECL Immunoblotting Detection Reagents were obtained from Clinx Science Instrument Co., Ltd. (Shanghai, China). CuE was obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). The eBioscience™ Annexin V-Fluorescein isothiocyanate (FITC) Apoptosis Detection Kit and propidiumiodide (PI) were purchased form Thermo Fisher Scientific (San Jose, CA, USA). All the chemical reagents used in the cell culture experiments are cell culture grade.

Apoptosis and cell cycle analysis

According to the previously reported toxicity of CuE to A549 cells [26], three different concentrations of CuE were adopted for cell treatments. Cells were cultured in 6-well plate in 2 mL of DMEM medium with 10% FBS (2.5×10^5cells per well) for approximately 14 h, then the medium was discarded and 2 mL of new medium was added to each well and subsequently cultured for 1 h before 2 μL of different concentration of CuE stock solutions (0, 0.25, 1.0 and 2.5 mmol·L^{-1}) was added. The cells were cultured for 24 h and harvested for apoptosis and cell cycle analysis. Annexin V-FITC and PI were added to the cells and cells were cultured in the dark at temperature for 15 min. After incubation, analyzed with the guava easyCyte™ flow cytometer (EMD Millipore, Inc., Germany).

Western blotting

Cells with a density of 5×10^6/10 mL DMEM medium were cultured in a 10 cm culture dish for 14 h, and then fresh DMEM medium was replaced. After 1 h of culture, 10 μL of DMSO with or without cucurbitacin E was added to make the final concentration of cucurbitacin E was 0, 0.25, 1.0 and 2.5 μmol·L^{-1}. The cells were cultured for 4, 8 and 12 h respectively and collected for total protein extraction. The protein concentration was quantitated by BCA method and adjusted to be equivalent to performed SDS-PAGE and the samples were loaded 25 μg per well. Proteins were then transferred to a PVDF membrane and blocked with 5% skim milk or 5% bovine serum albumin. The membrane was subsequently in-

cubated with rabbit primary antibodies. After the end of incubation, the membrane was washed with TBST buffer and incubated with HRP-labelled goat anti-rabbit secondary antibodies. After incubation, the film was washed and ECL luminescent solution was added. The bands were observed with the protein gel imager. GADPH was taken as the internal reference. The gray density of bands was measured by ImageJ software referenced to GAPDH.

Molecular simulation

Firstly, for the docking prediction, the 3D structure of EGFR-erlotinib complex is available (1M17) in the Protein Data Bank [33]. The water molecules and erlotinib were removed by Chimera 1.11 and then the hydrogen atoms were added by AutoDockTools 1.5.6. The structure of CuE was built and optimized by using GaussView and Gaussian 09W respectively. Following the validation of the docking protocol, both of CuE and EGFR were loaded to AutoDockTools 1.5.6 for a total of 10 docking runs. Afterwards the docking conformation with the lowest binding free energy was used to perform the subsequent MD calculation. Briefly, the CuE-EGFR complex was solvated in a cubic box with simple point charge (SPC) explicit water solvent. The counterion was distributed randomly to maintain charge neutrality. The whole system was equilibrated with position restraints on both of the protein and ligand at the constant temperature and pressure conditions after its energy minimization. Afterwards the position restraints were removed and the system was subjected to a 20 ns MD simulation. The root mean squared deviation (RMSD) values of EGFR were calculated with respect to its crystal structure. Then the RMSD values of CuE were also calculated against its best docking pose. Both of the aforementioned RMSD values were used to assess the binding stability between EGFR and CuE.

Results and Discussion

Cucurbitacin E induced apoptosis in A549 cells

Apoptosis induction was reported in other lung cancer cells by treatments of CuE [31, 34]. The apoptosis induction in A549 cells by CuE was observed in this study. As shown in Figs. 2A and 2B, the numbers of normal cells were obviously decreased by treating with CuE at all concentrations tested, which were very significantly different to the carrier control ($P < 0.001$). On the contrary, the numbers of apoptosis cells increased in all treatments: the viable apoptotic cells were very significantly different between carrier and CuE treatments ($P < 0.001$), and so did the numbers of late apoptotic cells ($P < 0.001$, 0.01 or 0.05), though the difference of number of necrosis cells were not significant. Interestingly, it seemed that the high concentration of CuE did not promote the viable apoptotic cells forward to the late phase but on contrary, inhibited the process (Fig. 2B). These observations confirmed the complex effects of CuE to different lung cancer cells [31, 34].

The apoptosis regulators, caspase-9 and casepase-3 were tested by western blotting (Fig. 2C). The results suggested that in the treatments of lower and moderate concentrations, the cleaved caspase-9 was observed to be increased time-dependently and but in high concentration treatments, the increase reversed at 12 h-test-point. On the other hand, in the early treatments, the accumulation of cleaved caspase-9 was dose-independent and nearly constant, but in the moderate treatment time, the accumulation increased was dose-dependently. The increase of accumulation of cleaved caspase-9 was also observed to be dose-dependent in 12 h treatments at low and moderate concentration, but this increase descended at high concentration. The finding that regulation of apoptosis depending on caspases in A549 cells is accordant with the previous report in other lung cancer cells [31].

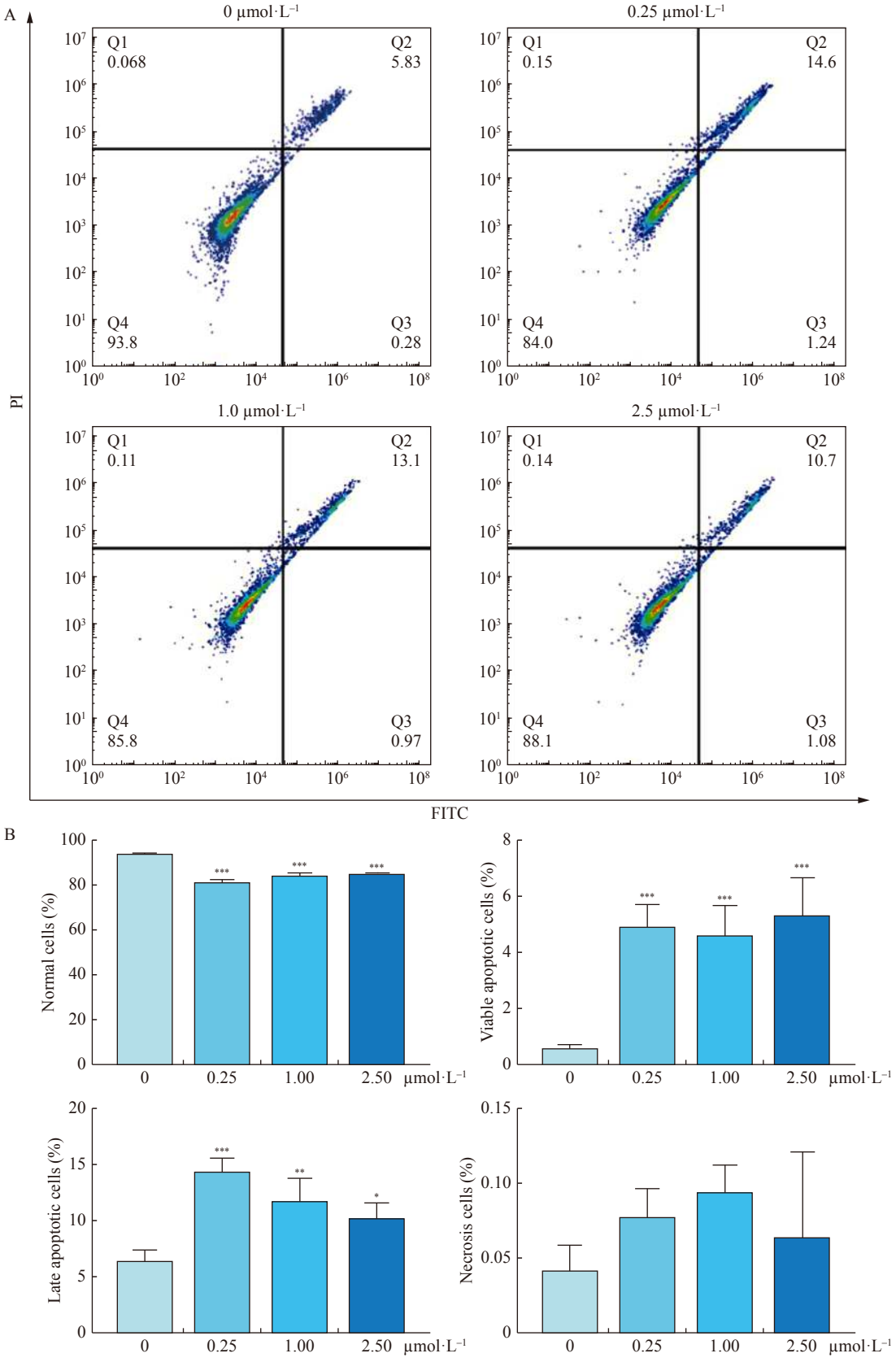
STAT3, a transcriptional factor and the product of the oncogene *STAT3* [35], was also tested by Western blotting (Fig. 2, Fig. S1). Its total accumulation was relatively constant in all the treatments with only a slight decrease in the 12 h treatments. However, the phosphorylated STAT3 was decreased time-dependently at all treatments and and dose-dependently in the 4 h and 8 h treatments. In 12 h treatments, the phosphorylation was increased compared to the carrier treatment and the increment was higher at lower concentration. Previous studies revealed that CuE modulated apoptosis by various pathway [27, 28, 31, 34], but in the studies related to lung cancer, the STAT3 dependent pathway was absent. Our results suggested that CuE induced apoptosis *via* STAT3 dependent pathway. The accumulation of survivin, an inhibitor of apoptosis protein [36], was also investigated. The results suggested that, the treatment of CuE resulted in the degradation of survivin in all treatments, but the residuary amounts were higher in the higher concentration and longer treatments (Fig. 2C, Fig. S1). This may help to understand why high concentration of CuE inhibited the apoptosis process in Fig. 2B.

Cucurbitacin E arrested the cell cycle at G1/G0 phase in A549 cells

CuE have been demonstrated to be capable of modulating the expression of cell cycle regulators in multiple cell lines [26], but cell cycle in A549 was not analyzed. In this study, the treatments of CuE were also observed to be capable of inducing cell cycle arrest in A549 cells (Figs. 3A and 3B). After the treating with CuE, the numbers of cells in G1/G0 phase increased sharply ($P < 0.001$) in all treatments. On the contrary, the numbers of G2/M cells were decreased significantly ($P < 0.001$, 0.01. or 0.05). Likewise, the number of cells in S phase decreased in low (0.25 μmol·L^{-1}) and moderate (1.0 μmol·L^{-1}) concentration treatments and the difference were very significant ($P < 0.001$), but the same trend could not be observed in high concentration (2.5 μmol·L^{-1}) treatment. The present finding that CuE arrest cell cycle at G1/G0 phase is totally different to the previous studies which arrested at G2/M phase in other cells [26, 28, 29].

Four cell cycle regulators, cyclinA2, cyclinB1, cyclin E1 and cyclinD1 were investigated by Western blotting (Fig. 3C, Fig. S2). The results suggested that the accumulation of cyclinA2, cyclinB1 and cyclin E1 were decreased and were both time- and dose-dependent. The accumulation of CyclinE1

was decreased in the all treatments, though only was time-dependent. The decrement of cyclinB1 was the most significant compared to others. It is reported that the decrease of cyclinA2 and cyclinD1 in A549 cells resulted in G1/G0 phase ar-



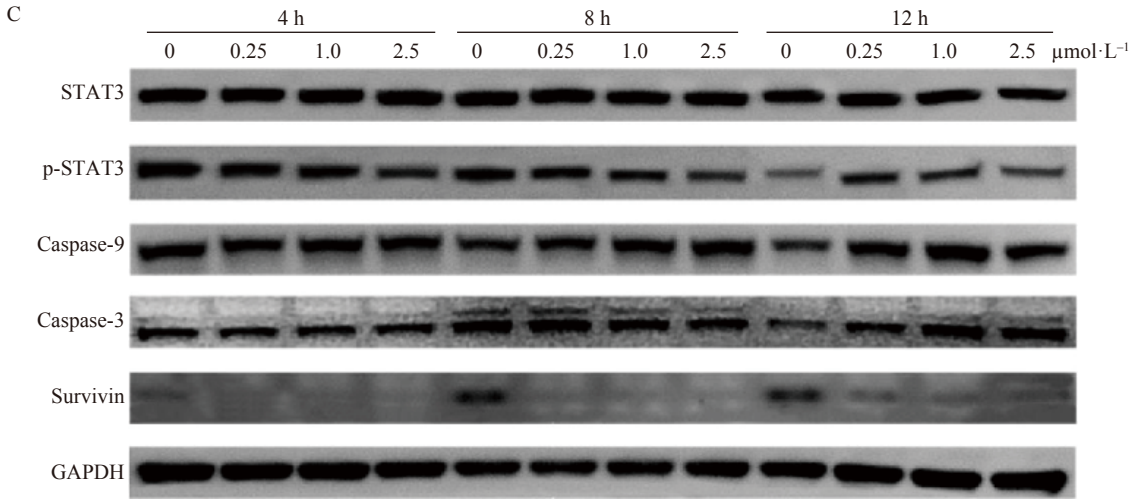


Fig. 2 Apoptosis analysis after treatments of cucurbitacin E. Apoptosis analysis were performed using cells treated below: 2.5 × 10^5 cells per well precultured for 14 h in 2 mL medium on 6-well plates treated with 0.25, 1.0, 2.5 μmol·L^{-1} of CuE for 24 h. DMSO (0.1%) was used as carrier controls. (A) Flow cytometry charts; (B) Statistical charts. All the data are from 3 independent repeats per treatment and presented as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* carrier control groups; (C) Western blotting analysis on cells treated with CuE at the concentration of 0, 0.25, 1.0 and 2.5 μmol·L^{-1} respectively for 4, 8 and 12 h for the apoptosis regulators and oncogene products indicated. DMSO (0.1%) was used as carrier controls

rest [37], which is accordant to the current results. Therefore, these results combined with the large decrement of cyclinB1 resulted in the cell cycle arrest at G1/G0 phase. In this study, decrease of the cyclinA2 and cyclinB1 by CuE was first observed in A549 cells. On the other hand, decrease of the cyclinB1 is contrary to previous studies in breast cells [29].

Cucurbitacin E affected to the EGFR/MAPK pathway

EGFR/MAPK pathway is an essential pathway in cells that plays a key role in multiple biological and pathological process in cells. It is important in the process of development and migration of numerous malignant tumors including non-small cell lung cancer (NSCLC) [16]. Thereby, the member of EGFR/MAPK pathway, namely, EGFR, RAS, Raf1/BRAF, MEK1/2 and ERK1/2 were investigated by Western blotting with the aim to further illustrate the above results of apoptosis and cell cycle arrest. The results are presented in Fig. 4 and Fig. S3. As shown in the figure, the accumulation of total RAS, Raf1, MEK1/2 and ERK1/2 were relatively constant in all treatments. However, the total amount of EGFR increased slightly in a dose-dependent manner, but time-independent. As for total BRAF, it remained constantly at low early treatments, and decreased dose-dependently in later treatments, the decreases were time-dependent in all treatments. The phosphorylated EGFR was increased, but the increments in long-time treatment were smaller than short-time treatments. In short-time treatments, the accumulation burst at low concentration of CuE and decreased afterward as the concentration increased. But in long-time treatments, the accumulation was increasingly as the dose increased, which was accordant to the previous investigation in leukemia cell lines [30]. The phosphorylated BRAF was relative constant despite of long-time treatment with high CuE concentration. The phosphorylation of Raf1 was decreased in short-time treatment and the decrement was dose-dependently increased. In moderate treatment time, the phosphorylation of Raf1 was increased with the increase of CuE and the same results were observed in the long-time treatment with low and moderate concentration of CuE. However, the phosphorylated Raf1 turned down in at high concentration of CuE in long-time treatments. The accumulation of phosphorylated MEK1/2 was observed to be decreased both dose- and time-dependent. On contrary, phosphorylated ERK1/2 increased both and dose- and time-dependently.

Activation and inhibition of members in EGFR/MAPK pathway basically result in alteration of biological processes related to the pathway. MEK inhibition was reported to be capable of inducing apoptosis [38]. The present results that phosphorylation MEK1/2 was downregulated by treatment of CuE maybe one of the reasons to the induction of apoptosis. The activation of ERK leads to genes induction, i.e., *caspase-9* and *cyclin B1* which can regulate cell growth and survival [32]. A549 cells treated with CuB and one of its derivatives (DACE) showed increased phosphorylation of ERK, cleavage of casapase-3/9 and degradation of cylinB1 and therefore resulted apoptosis and cell cycle arrested [39, 40]. In this work, the phosphorylation of ERK was upregulated, and this could be one of reasons causing the caspase-9 and cynlcinB1 were observed increased and decreased respectively above.

Binding mode and binding stability of EGFR with CuE

Herein, the binding mode and binding stability of EGFR with CuE were explored by combination of molecular docking and MD simulation. As shown in Fig. 5A, CuE is located at the ATP-binding site of EGFR tyrosine kinase domain and is stabilized by the hydrogen-bonding interactions with neighboring amino acid residues, including Leu694, Met769, Arg817, and Asp831. Among which, Leu694 and Met769 have been reported to play a pivotal role in maintaining the

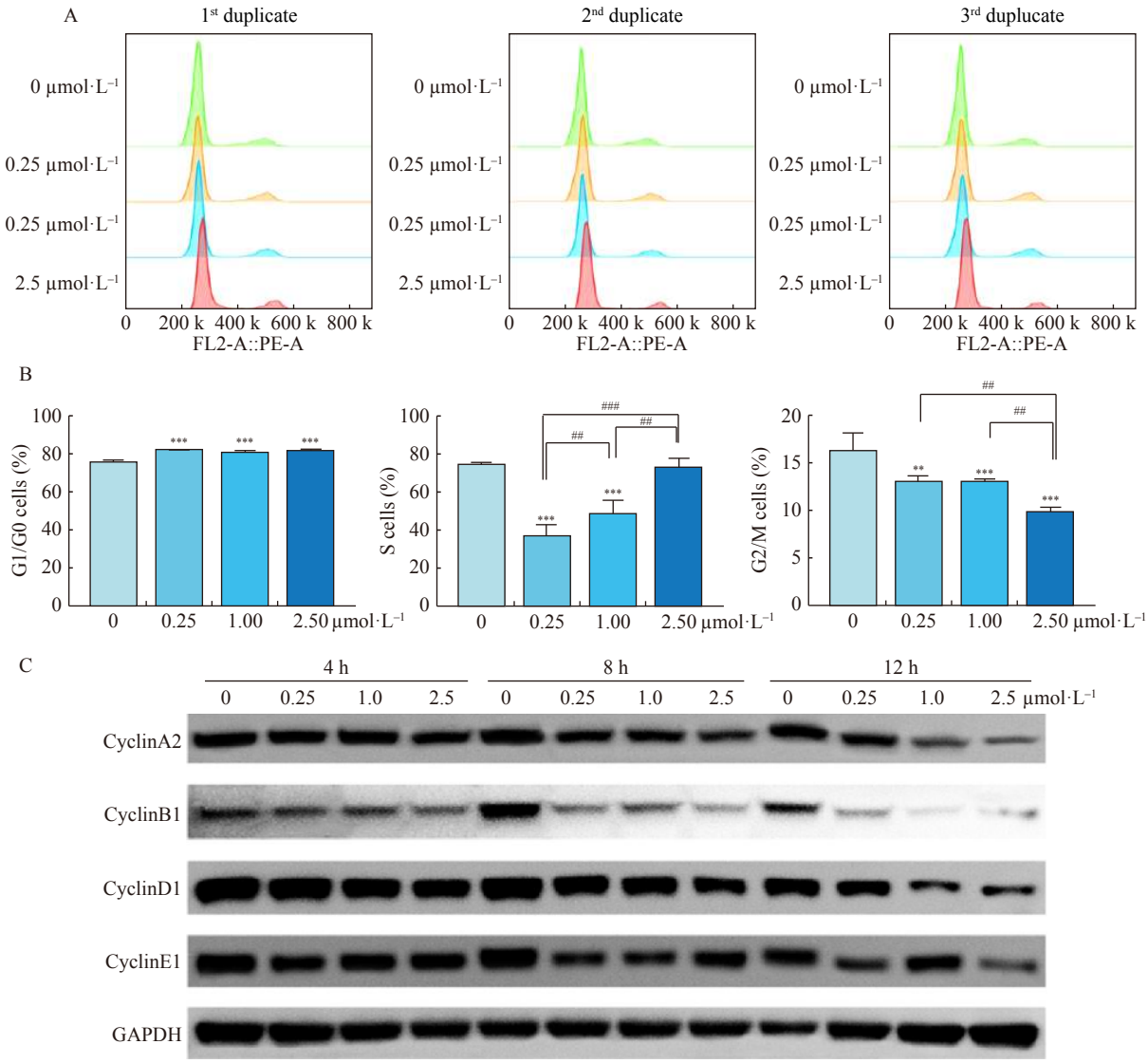


Fig. 3 Cell cycle analysis after treatments of cucurbitacin E. Cell were treated as in apoptosis and used for cycle analysis. (A) Charts of cell signal peaks; (B) Statistical charts. All the data are from 3 independent repeats per treatment and presented as mean ± SD. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs* carrier control groups. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ between treatments groups; (C) Western blotting analysis on cells treated with CuE at the concentration of 0, 0.25, 1.0 and 2.5 μmol·L^{-1} respectively for 4, 8 and 12 h for the cell cycle regulators indicated. DMSO (0.1%) was used as carrier controls

binding between EGFR and tyrosine kinase inhibitors [41], which is confirmed by the present work. The results of a 20 ns MD simulation for the CuE-EGFR complex have been shown in Fig. 5B. The protein backbone atoms essentially remain at dynamical equilibrium throughout the simulation, with average RMSD value of 0.31 ± 0.05 nm. In contrast with EGFR, CuE undergoes a more drastic conformational change, with average RMSD value of 0.56 ± 0.13 nm. Since CuE possesses a tetracyclic triterpenoid skeleton, which is too large to be accommodated within the binding pocket and, unfortunately, protrudes out of the cavity (Fig. 5A) and, hence, disrupts the binding stability of CuE toward EGFR. In conclusion, the binding mode of the CuE-EGFR complex observed from molecular docking is consistent with their binding stability assessed by MD simulation.

Conclusion

Treatment of CuE induced STAT3 dependent apoptosis cell cycle arrested at G1/G0 phase. Apoptosis and cell cycle arrest in treatments were attributed to the EGFR/MAPK signaling suppression by altering the activation of EGFR and other kinases. Molecular simulation also brought an insight on the interaction mechanism between CuE and EGFR. Therefore, CuE acted as a tyrosine kinase inhibitor interfering with the EGFR/MAPK signaling pathway and exerted its anti-proliferative effect against A549 cells.

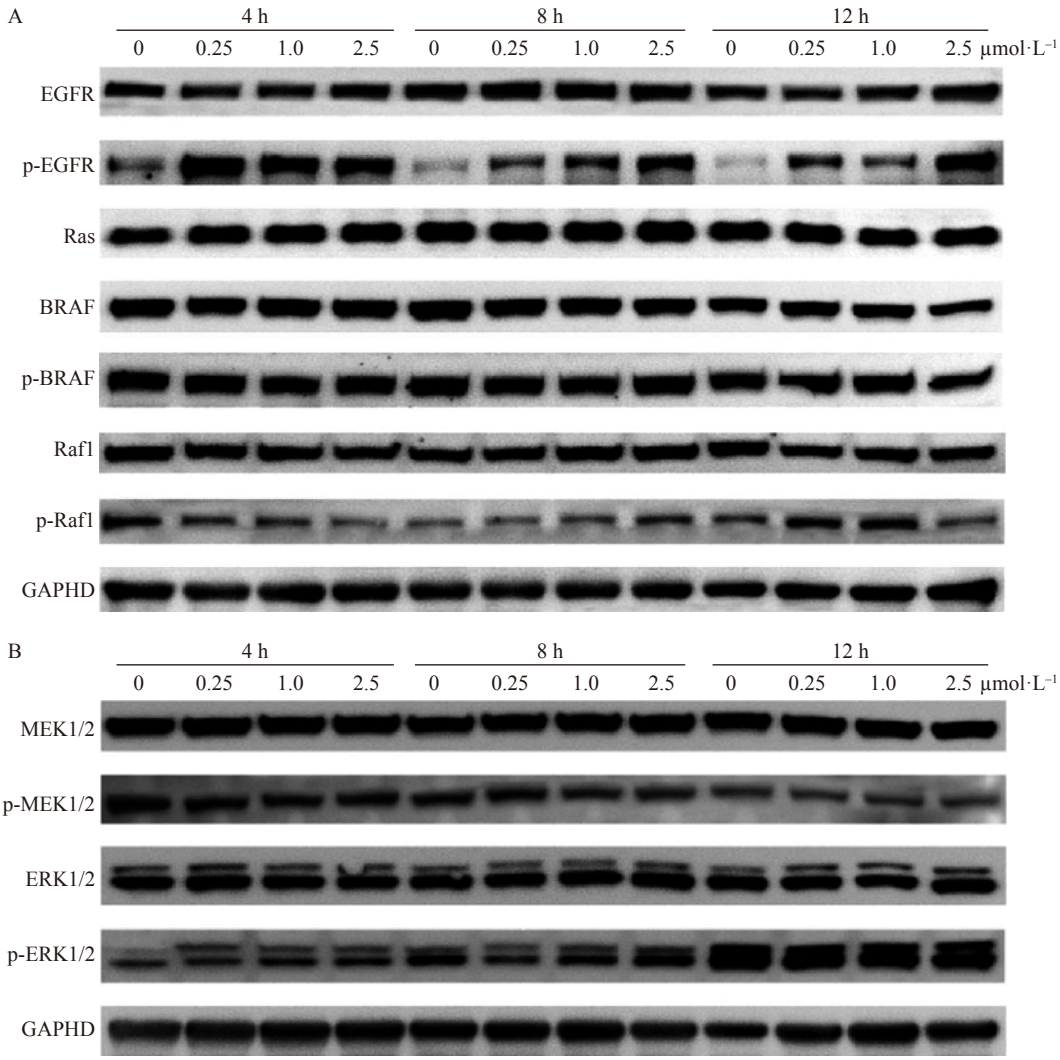


Fig. 4 The effect of cucurbitacin E treatment to participants in EGFR/MAPK pathway. Cells were treated with CuE at the concentration of 0, 0.25, 1.0 and 2.5 μmol·L^{-1} respectively for 4, 8 and 12 h. (A) Western analysis for EGFR, Ras, BRAF and Raf1; (B) Western analysis for MEK1/2 and ERK1/2. DMSO (0.1%) was used as carrier controls

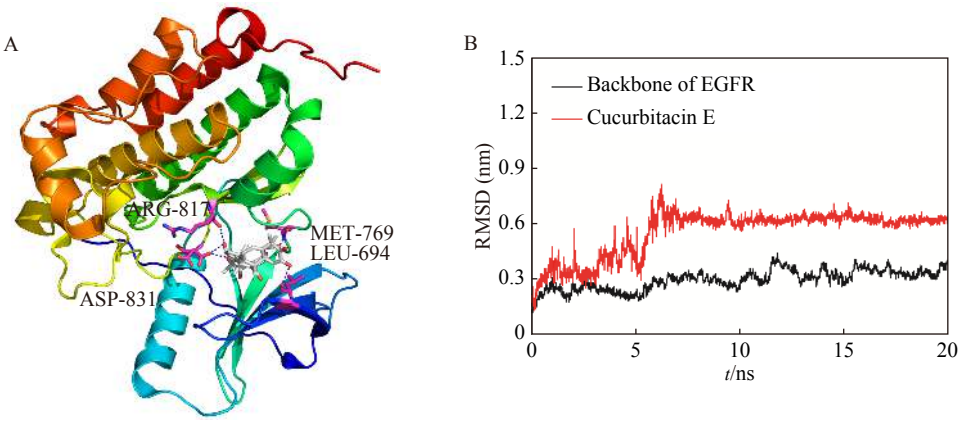


Fig. 5 The results of molecular simulation. (A) The binding conformation of cucurbitacin E in the binding pocket of EGFR. Hydrogen bonds are highlighted as blue dashed lines; (B) The root mean squared deviation (RMSD) values of EGFR and cucurbitacin E in the dynamic binding process

References

[1] Zhang T, zhong S, li T, *et al*. Saponins as modulators of nuclear receptors [J]. *Crit Rev Food Sci*, 2020, **60**(1): 94-107.

[2] Salehi B, Capanoglu E, Adrar N, *et al*. *Cucurbits* plants: a key emphasis to its pharmacological potential [J]. *Molecules*, 2019, **24**(10): 1854.

[3] Jevtić B, Djedović N, Stanisavljević S, *et al*. Cucurbitacin E potently modulates the activity of encephalitogenic cells [J]. *J Agric Food Chem*, 2016, **64**(24): 4900-4907.

[4] Lee DH, Iwanski GB, Thoennissen NH. Cucurbitacin: ancient compound shedding new light on cancer treatment [J]. *The Scientific World J*, 2010, **10**: 413-418.

[5] Wu D, Wang Z, Lin M, *et al*. *In vitro* and *in vivo* antitumor activity of cucurbitacin C, a novel natural product from cucumber [J]. *Front Pharmacol*, 2019, **10**: 1287.

[6] Alghasham AA. Cucurbitacins-a promising target for cancer therapy [J]. *Int J Health Sci (Qassim)*, 2013, **7**(1): 77-89.

[7] Cattel L, Balliano G, Caputo O, *et al*. Biosynthesis of cucurbitacins in *Bryonia dioica* seedlings [J]. *Planta Med*, 1981, **41**(4): 328-336.

[8] Du Q, Xiong X, Ito Y. *Separation of cucurbitacin B and cucurbitacin E from fruit base of Cucumis melo L. by high-speed countercurrent chromatography* [M]. *ACS Publications*, 1995.

[9] Zhou Y, Ma Y, Zeng J, *et al*. Convergence and divergence of bitterness biosynthesis and regulation in cucurbitaceae [J]. *Nat Plants*, 2016, **2**(12): 16183.

[10] Hung CM, Chang CC, Lin CW, *et al*. GADD45*γ* induces G2/M arrest in human pharynx and nasopharyngeal carcinoma cells by cucurbitacin E [J]. *Sci Rep-UK*, 2014, **4**: 6454.

[11] Luo W, Zhao W, Lu J, *et al*. Cucurbitacin B suppresses metastasis mediated by reactive oxygen species (ROS) *via* focal adhesion kinase (FAK) in breast cancer MDA-MB-231 cells [J]. *Chin J Nat Med*, 2018, **16**(1): 10-19.

[12] Qiao J, Xu LH, He J, *et al*. Cucurbitacin E exhibits anti-inflammatory effect in RAW 264.7 cells *via* suppression of NF-*κ*B nuclear translocation [J]. *Inflamm Res*, 2013, **62**(5): 461-469.

[13] Hsu PC, Tian B, Yang YL, *et al*. Cucurbitacin E inhibits the Yes-associated protein signaling pathway and suppresses brain metastasis of human non-small cell lung cancer in a murine model [J]. *Oncol Rep*, 2019, **42**(2): 697-707.

[14] Dong Y, Lu B, Zhang X, *et al*. Cucurbitacin E, a tetracyclic triterpenes compound from Chinese medicine, inhibits tumor angiogenesis through VEGFR2-mediated Jak2–STAT3 signaling pathway [J]. *Carcinogenesis*, 2010, **31**(12): 2097-2104.

[15] Wu Y, Zhang Y, Yao Y, *et al*. Cucurbitacin E ameliorates hepatic fibrosis *in vivo* and *in vitro* through activation of AMPK and blocking mTOR-dependent signaling pathway [J]. *Toxicol Lett*, 2016, **258**: 147-158.

[16] Liu Q, Yu S, Zhao W, *et al*. EGFR-TKIs resistance *via* EGFR-independent signaling pathways [J]. *Mol Cancer*, 2018, **17**(1): 53.

[17] Takimoto CH. Epidermal growth factor receptor and other molecular targets in the treatment of cancer [J]. *Clin Colorectal Canc*, 2003, **2**(4): 252.

[18] Nakai K, Hung MC, Yamaguchi H. A perspective on anti-EGFR therapies targeting triple-negative breast cance [J]. *Am J Cancer Res*, 2016, **6**(8): 1609-1623.

[19] Padfield E, Ellis HP, Kurian KM. Current therapeutic advances targeting EGFR and EGFRvIII in glioblastoma [J]. *Front Oncol*, 2015, **5**: 5.

[20] Alsayari A, Halaweish FT, Gurusamy N. The role of cucurbitacins in combating cancers: a mechanistic review [J]. *Pharmacogn Rev*, 2018, **12**(24): 157-165.

[21] Liu P, Xiang Y, Liu X, *et al*. Cucurbitacin B induces the lysosomal degradation of EGFR and suppresses the CIP2A/PP2A/Akt signaling axis in gefitinib-resistant non-small cell lung cancer [J]. *Molecules*, 2019, **24**(3): 647.

[22] Saglam AY, Alp E, Elmazoglu Z, *et al*. Treatment with cucurbitacin B alone and in combination with gefitinib induces cell cycle inhibition and apoptosis *via* EGFR and JAK/STAT pathway in human colorectal cancer cell lines [J]. *Hum Exp Toxicol*, 2016, **35**(5): 526-543.

[23] Zhou J, Zhao T, Ma L, *et al*. Cucurbitacin B and SCH772984 exhibit synergistic anti-pancreatic cancer activities by suppressing EGFR, PI3K/Akt/mTOR, STAT3 and ERK signaling [J]. *Oncotarget*, 2017, **8**: 103167-103181.

[24] Lopez-Haber C, Kazanietz MG. Cucurbitacin I inhibits Rac1 activation in breast cancer cells by a reactive oxygen species-mediated mechanism and independently of janus tyrosine kinase 2 and P-Rex1 [J]. *Mol Pharmacol*, 2013, **83**(5): 1141-1154.

[25] Sadzuka Y, Hatakeyama H, Sonobe T. Enhancement of doxorubicin concentration in the M5076 ovarian sarcoma cells by cucurbitacin E co-treatment [J]. *Int J Pharm*, 2010, **383**(1-2): 186-191.

[26] Feng H, Zang L, Zhao Z, *et al*. Cucurbitacin-E inhibits multiple cancer cells proliferation through attenuation of Wnt/β-catenin signaling [J]. *Cancer Biother Radiopharm*, 2014, **29**(5): 210-214.

[27] He X, Gao Q, Qiang Y, *et al*. Cucurbitacin E induces apoptosis of human prostate cancer cells *via* cofilin-1 and mTORC1 [J]. *Oncol Lett*, 2017, **13**(6): 4905-4910.

[28] Huang W, Yang J, Lin M, *et al*. Cucurbitacin E induces G2/M phase arrest through STAT3/p53/p21 signaling and provokes apoptosis *via* Fas/CD95 and mitochondria-dependent dathways in human bladder cancer T24 cells [J]. *Evid Based Complement Alternat Med*, 2012, **2012**: 952762.

[29] Kong Y, Chen J, Zhou Z, *et al*. Cucurbitacin E induces cell cycle G2/M phase arrest and apoptosis in triple negative breast cancer [J]. *PLoS ONE*, 2014, **9**(7): e103760..

[30] E.M.Saeed M, C.Boulos J, Elhaboub G, *et al*. Cytotoxicity of cucurbitacin E from citrullus colocynthis against multidrug-resistant cancer cells [J]. *Phytomedicine*, 2019, **62**: 152945.

[31] Ma G, Luo W, Lu J, *et al*. Cucurbitacin E induces caspase-dependent apoptosis and protective autophagy mediated by ROS in lung cancer cells [J]. *Chem Biol Interact*, 2016, **253**: 1-9.

[32] Silva IT, Geller FC, Persich L, *et al*. Cytotoxic effects of natural and semisynthetic cucurbitacins on lung cancer cell line A549 [J]. *Invest New Drugs*, 2016, **34**(2): 139-148.

[33] Stamos J, Sliwkowski MX, Eigenbrot C. Structure of the epidermal growth factor receptor kinase domain alone and in complex with a 4-anilinoquinazoline inhibitor [J]. *J Biol Chem*, 2002, **277**: 46265-46272.

[34] Tong X, Jiang P, Li Y, *et al*. Combined treatment with triptolide and tyrosine kinase inhibitors synergistically enhances apoptosis in non-small cell lung cancer H1975 cells but not H1299 cells through EGFR/Akt pathway [J]. *Chem Pharm Bull*, 2019, **67**(8): 864-871.

[35] FBromberg J, Wrzeszczynska MH, Devgan G, *et al*. Stat3 as an oncogene [J]. *Cell*, 1999, **98**(3): 295-303.

[36] Li F. Role of survivin and its splice variants in tumorigenesis [J]. *Brit J Cancer*, 2005, **92**(2): 212-216.

[37] Mu X, Zhang Y, Qu X, *et al*. Ubiquitin ligase Cbl-b is involved in lcotinib (BPI-2009H)-induced apoptosis and G1 phase arrest of EGFR mutation-positive non-small-cell lung cancer [J]. *Biomed Res Int*, 2013, **2013**: 726375.

[38] Baranski Z, Booij TH, Kuijjer ML, *et al*. MEK inhibition induces apoptosis in osteosarcoma cells with constitutive ERK1/2 phosphorylation [J]. *Genes Cancer*, 2015, **6**(11−12): 503-512.

[39] Kausar H, Munagala R, Bansal S, *et al*. Cucurbitacin B potently suppresses non-small-cell lung cancer growth: identification of intracellular thiols as critical targets [J]. *Cancer Lett*, 2013, **332**(1): 35-45.

[40] Silva IT, Carvalho A, Lang KL, *et al*. *In vitro* and *in vivo* antitumor activity of a novel semisynthetic derivative of cucurbitacin B [J]. *PLoS ONE*, 2015, **10**(2): e0117794.

[41] Singh P, Bast F. In silico molecular docking study of natural compounds on wild and mutated epidermal growth factor receptor [J]. *Med Chem Res*, 2014, **23**(12): 5074-5085.

Cite this article as: JING Si-Yuan, WU Zi-Dan, ZHANG Tie-Hua, ZHANG Jie, WEI Zheng-Yi. *In vitro* antitumor effect of cucurbitacin E on human lung cancer cell line and its molecular mechanism [J]. *Chin J Nat Med*, 2020, **18**(7): 483-490.