Heliyon 9 (2023) e15805

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

The topoisomerase inhibitor CPT-11 prevents the growth and metastasis of lung cancer cells in nude mice by inhibiting EGFR/MAPK signaling pathway

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ARTICLE INFO

Keywords:
CPT-11
Topoisomerase inhibitor
EGFR
MAPK pathway
Lung cancer
Subcutaneous xenograft tumor model
Metastatic tumor model

ABSTRACT

Objective: The topoisomerase inhibitor CPT-11 has been applied in treatment of multiple cancer types. Here, we probed into the possible mechanism of CPT-11 in affecting growth and metastasis of lung cancer (LC) cells, with involvement of the EGFR/MAPK pathway.
Methods: The target protein of CPT-11 was screened through bioinformatics analysis, and the LC-related microarray datasets GSE29249, GSE32863 and GSE44077 were obtained for differential analysis for identifying the target protein. A subcutaneous xenograft tumor model and a metastatic tumor model were constructed in nude mice for in vivo mechanism verification of the regulatory role of CPT-11 in LC through modulation of EGRF/MAPK pathway.
Results: Bioinformatics analysis showed that EGFR was the target protein of CPT-11. In vivo animal experiments confirmed that CPT-11 enhanced LC cell growth and metastasis in nude mice. CPT-11 could inhibit activation of EGFR/MAPK pathway. EGFR promoted LC cell growth and metastasis in nude mice through activation of the MAPK pathway.
Conclusion: The topoisomerase inhibitor CPT-11 may prevent LC growth and metastasis by inhibiting activation of EGFR/MAPK pathway.

1. Introduction

Lung cancer (LC) is regarded as a complicated malignancy consisting of a diversity of histological and molecular types [1]. In spite of improved understanding of LC in many aspects such as the risk and treatment options, this malignancy remains to be a leading contributor to cancer-related death [2]. Lung cancer is featured by rapid proliferation as well as metastatic potential [3]. Metastasis in LC is accountable for the high mortality of the cancer and involves enhanced motility and capacity to form new tumors [4]. In this context, it is of importance to curb the rapid cell growth and metastasis in LC.

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https://doi.org/10.1016/j.heliyon.2023.e15805
Received 7 December 2022; Received in revised form 11 April 2023; Accepted 21 April 2023
Available online 27 April 2023

The topoisomerase enzymes are believed to exert crucial functions on DNA metabolism, their inhibitors are of potential for developing novel anticancer drugs [5]. Since clinically introduced in 1998, the topoisomerase I inhibitor irinotecan (CPT-11) has been widely applied for treating solid tumors including LC [6]. At present, CPT-11 has been suggested as one of the mainstay first-line treatments for metastatic small-cell LC (SCLC) [7]. Of note, our bioinformatics analysis discovered EGFR as the target protein of CPT-11. Intriguingly, the active metabolite of CPT-11 (SN-38) in combination with gefitinib possesses great potentials in modulation of LC cell lines expressing wild-type EGFR [8]. EGFR, a transmembrane glycoprotein, is aberrantly activated by diverse mechanisms and links to the progression of different human cancers [9]. As previously documented, EGFR has served as a crucial therapeutic target for managing non-small cell LC (NSCLC) [10]. EGFR has also been highlighted as an oncogenic gene of NSCLC and its inhibition can produce dramatic tumor responses [11]. Previous research has indicated that EGFR activation might bring about activated MAPK pathway in LC cells [12]. Activation of the MAPK pathway by lncRNA TUC338 contributes to the promotion of the invasion of LC [13]. Interestingly, the activated EGFR/MAPK pathway by STAM binding protein (STAMBP) is capable of accelerating the metastasis of lung adenocarcinoma [14]. Considering the aforementioned reports, we might hypothesize that CPT-11 could inhibit LC cell growth and metastasis by disrupting the EGFR/MAPK pathway. Such hypothesis was validated utilizing the established subcutaneous xenograft tumor model and metastatic tumor model.

2. Materials and methods

2.1. Ethical approval

The Medical Animal Care & Welfare Committee of Shantou University Medical College (No. SUMC2022-230) approved the study. All procedures in the animal experiment were carried out obeying the guidelines for the care and use of laboratory animals.

2.2. Data download

Using the GEO database, LC-related microarray datasets were obtained, including GSE29249, GSE32863 and GSE44077. Six 6 adjacent tissue samples and 6 LC tissue samples were included in GSE29249, 58 LC and 58 adjacent tissue samples were included in GSE32863, and 55 LC and 66 normal lung tissue samples were included in GSE44077.

2.3. Differential gene screening

Differential analysis was performed on the microarray datasets GSE29249, GSE32863 and GSE44077. Differentially Expressed Genes (DEGs) were screened using R "limma" package, with the threshold set as $p < 0.05$.

2.4. Drug-target protein prediction

Through the STITCH online website, the target proteins of CPT-11 were predicted, and the top 15 target proteins were displayed in the corresponding figure.

2.5. Experimental animals

Eighty BALB/c nude mice (6 weeks, No.401, Vitalriver; Beijing, China) were fed in separated cages in a SPF laboratory, with 60–65% humidity at 22–25 °C for 1 week before experiment.

2.6. Construction of lentiviral vectors

The lentiviral overexpression vector pCDH-CMV-MCS-EF1-copGFP (System Biosciences, CD511B-1) was purchased. A lentivirus-based EGFR overexpression vector was constructed, and lentiviral particles carrying oe-EGFR or NC were packaged into HEK-293 T cells (iCell Bioscience Inc., Shanghai, China, iCell-h237). After 48 h, cell supernatants were harvested as lentivirus at a titer of 1×10^8 TU/mL.

2.7. Establishment of a xenograft model in nude mice

For establishment of a xenograft model, A549 cells (iCell Bioscience Inc., iCell-h011) in logarithmic growth period were made into suspension (5×10^7/mL). Then, 0.2 mL cell suspension was taken with a 1 mL syringe and inoculated in the left axilla of mice subcutaneously. When the tumor grew up to 25 mm^3 (about one week), nude mice were randomly injected with DMSO, CPT-11, DMSO + oe-NC, CPT-11 + oe-NC, CPT-11 + oe-EGFR, oe-NC + DMSO, oe-EGFR + DMSO or oe-EGFR + sesamolin (each n = 3).

Oe-EGFR (0.1 mL) and its NC (0.1 mL) were intratumorally injected into nude mice with a titer of 1×10^8 TU/mL. CPT-11 (30 mg/kg) (HY-16562, MedChemExpress, Monmouth Junction, NJ) and MAPK pathway inhibitor sesamolin (5 mg/kg) (HY–N0809, MedChemExpress) were intraperitoneally injected into mice twice a week, lasting 4 weeks.

After inoculation, on days 7, 14, 21, 28 and 35, tumor growth was observed. Then, mice were sacrificed on day 36 by means of cervical dislocation. The tumor tissue was removed, and tumor was weighed.

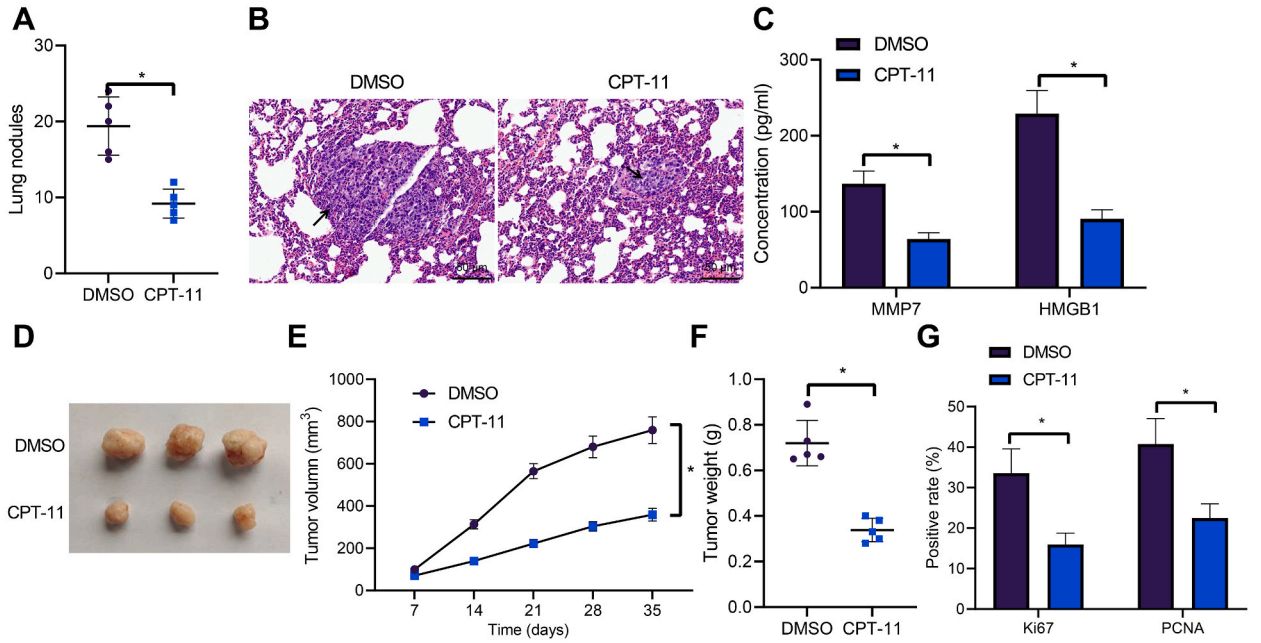

Fig. 1. CPT-11 can effectively inhibit LC cell growth and metastasis in mice. A, Number of pulmonary nodules in lung tissue in the metastatic tumor mouse model upon CPT-11. B, The lung metastasis in nude mice upon CPT-11 as observed using HE staining. The black arrows indicate metastatic sites. C, Expression of MMP7 and HMGB1 in lung tumor tissues of mice upon CPT-11 as detected by ELISA. D, Diagram of the tumor body of subcutaneous xenograft tumor models upon CPT-11. E, Statistical chart of subcutaneous xenograft tumor volume upon CPT-11. F, Weight statistics of subcutaneous xenograft tumor upon CPT-11. G, The positive expression of PCNA and Ki67 upon CPT-11 as detected by immunohistochemistry. There were 5 mice in each group. *$p < 0.05$. Measurement data, which were expressed as mean ± SD, were compared with unpaired *t*-test.

2.8. Establishment of metastatic tumor model in nude mice

Nude mice (each n = 3) were grouped into DMSO, CPT-11, DMSO + oe-NC, CPT-11 + oe-NC, CPT-11 + oe-EGFR, oe-NC + DMSO, oe-EGFR + DMSO or oe-EGFR + sesamolin groups. The stably transfected LC cell line A549 was made into 5×10^7/mL cell suspension, 0.2 mL of which was injected through tail vein into mice. Intraperitoneal injection of 30 mg/kg CPT-11 and 5 mg/kg sesamolin was performed twice a week, lasting 5 weeks. After 36 days, mice were sacrificed as above with the lung tissue removed.

2.9. IHC

Tumor tissues were subjected to antigen retrieval for 20 min and probed at 4 °C overnight with primary antibodies including rabbit *anti*-PCNA (ab92552, 1:200, Abcam, Cambridge, MA) and rabbit *anti*-Ki67 (ab15580, 1:200, Abcam). After that, biotinylated secondary antibody (goat anti-rabbit, ab150077, 1:500, Abcam) was added for further incubation for 30 min. Then, DAB (Solarbio, Beijing, China, DA1015) was added for development and hematoxylin (Solarbio, G1080) was used for counterstaining. The sections were dehydrated, cleared, and sealed before microscopic observation in five representative fields.

2.10. HE staining

The lung tumor tissues in nude mice were dewaxed to water, and HE staining was performed according to the HE staining kit (Bogoo Biological Technology Co., Ltd., Shanghai, China, PT001). First, hematoxylin was applied for 10-min staining at room temperature. Then, the tissue sections were differentiated with 1% alcohol hydrochloride for 30 s, and stained with eosin for 1 min. After dehydration, clear, and sealing, the morphological changes of lung tumor tissue in each group were observed with an Olympus microscope (BX50).

2.11. ELISA

The tumor tissue from each group of nude mice was made into tissue homogenate. By following the ELISA kit instructions, the expression of Matrix Metallopeptidase 7 (MMP7) and High-Mobility Group Box 1 (HMGB1), and the phosphorylation levels of JNK and p38 in tumor tissues were detected. ELISA kits used in the experiment were MMP7 (No: JL29243, Shanghai Jianglai Industrial Co., Ltd., Shanghai, China), HMGB1 (JL51819, Shanghai Jianglai Industrial Co., Ltd.), JNK and *p*-JNK (ab176662, Abcam), p38 and p-p38 (ab221013, Abcam) kits.

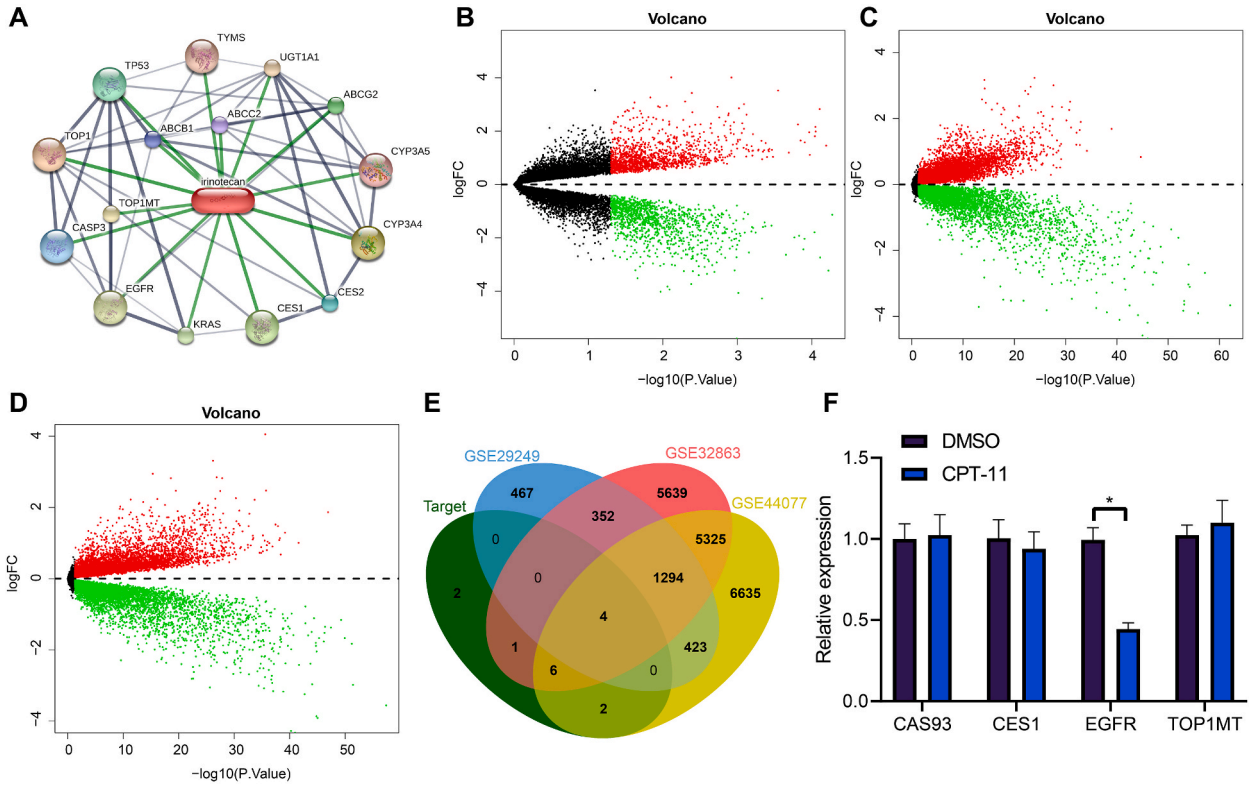

Fig. 2. Prediction of action target proteins of the topoisomerase inhibitor CPT-11 in LC. A, The STITCH online website predicted the target protein of CPT-11 (Irinotecan). The rectangle represents CPT-11. The circles represent the target proteins. Each green line refers to relationship between drug and the target protein. Each purple line refers to relationship between the target proteins, and the line thickness represents the tightness of the relationship. B-D, Volcano map of differential genes in the microarray datasets GSE29249 (B), GSE32863 (C) and GSE44077 (D). Black dots represent non-differentially expressed genes, green dots represent down-regulated DEGs and red dots represent up-regulated DEGs. The microarray GSE29249 included 6 cancer and 6 adjacent cancer tissue samples. The microarray GSE32863 included 58 cancer and 58 adjacent cancer tissue samples. The microarray GSE44077 included 55 cancer and 66 normal lung tissue samples. E, Venn plot of intersections of drug target (green) and DEGs in microarray datasets GSE29249 (blue), GSE32863 (red), and GSE44077 (yellow). F, The expression of CASP3, CES1, EGFR and TOP1MT as determined by RT-qPCR. There were 5 mice in each group. *$p < 0.05$. Measurement data, which were expressed as mean ± SD, were compared with unpaired *t*-test. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.12. RT-qPCR

Total RNA from tissues was extracted by TRIzol (15,596,026, Thermo Fisher Scientific, Rockford, IL). The mRNA was reversely transcribed to cDNA following the instruction of PrimeScript RT reagent Kit (Takara, Otsu, Shiga, Japan, RR047A). TaKaRa designed and synthesized the primers for Epidermal Growth Factor Receptor (EGFR), Caspase 3 (CASP3), Carboxylesterase 1 (CES1), and Topoisomerase I, Mitochondrial (TOP1MT) (Supplementary Table 1). RT-qPCR was performed using a 7500 Fast RT-qPCR system (4,351,106, Thermo Fisher Scientific). Relative transcription level of target genes was determined with $2^{-\triangle\triangle CT}$, normalized to GAPDH.

2.13. Statistical analysis

The statistical analysis of data, which were expressed as mean ± SD, was performed using SPSS 21.0. Measurement data were. Comparison on two-group data was performed by unpaired *t*-test. One-way ANOVA with Tukey's tests or repeated measures ANOVA was adopted for multi-group data comparison.

3. Results

3.1. CPT-11 could effectively inhibit LC cell growth and metastasis in vivo

For exploration purpose, we constructed the subcutaneous xenograft model and metastatic tumor model using A549 cells in nude mice, followed by treatment of CPT-11. As shown in Fig. 1A and B, injection of CPT-11 led to reduced lung metastases. The ELISA

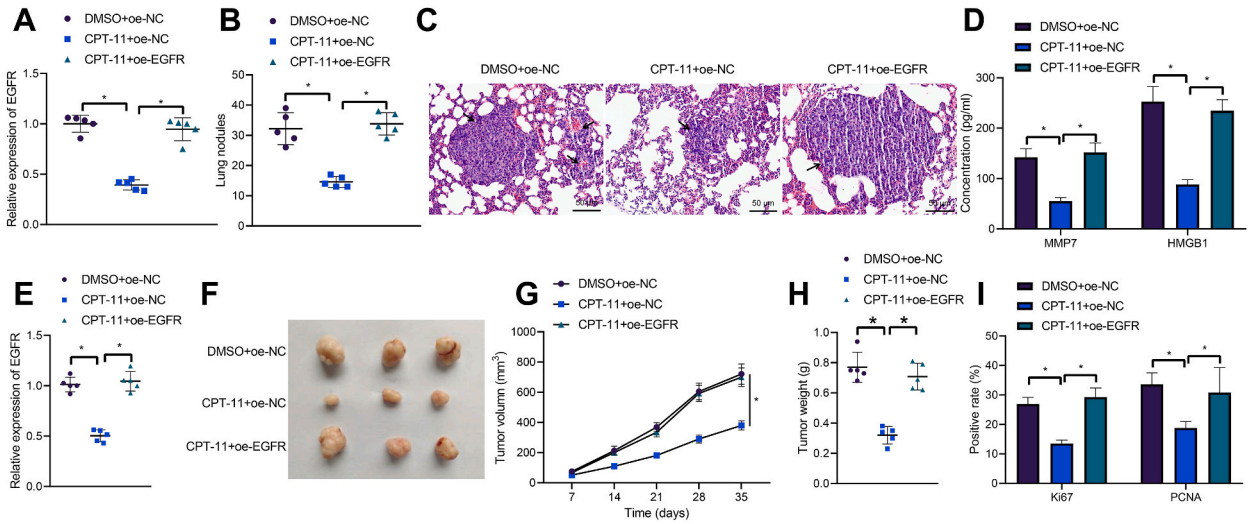

Fig. 3. The topoisomerase inhibitor CPT-11 inhibits LC cell growth and metastasis in mice mainly by down-regulating EGFR. A, Expression of EGFR in lung tissue in the metastatic tumor model in response to CPT-11 or oe-EGFR as detected by RT-qPCR. B, Number of pulmonary nodules in lung tissue in the metastatic tumor model in response to CPT-11 or oe-EGFR overexpression. C, The lung metastasis in nude mice in response to CPT-11 or oe-EGFR overexpression as observed using HE staining. The black arrows indicate metastatic sites. D, MMP7 and HMGB1 protein expression in lung tumor tissues in response to CPT-11 or oe-EGFR as detected by ELISA. E, The expression of EGFR in lung tissue in the metastatic tumor model in response to CPT-11 or oe-EGFR. F, Diagram of the tumor body of subcutaneous xenograft tumor in response to CPT-11 or oe-EGFR. G, Statistical chart of subcutaneous xenograft tumor volume in response to CPT-11 or oe-EGFR. H, Weight statistics of subcutaneous xenograft tumor in response to CPT-11 or oe-EGFR. I, Ki67 and PCNA positive expression in response to CPT-11 or oe-EGFR as detected by immunohistochemistry. There were 5 mice in each group. *$p < 0.05$. Measurement data, which were expressed as mean ± SD, were compared with one-way ANOVA, combined with Tukey's tests.

results showed (Fig. 1C) decreased protein expression of MMP7 and HMGB1 in the nude mice injected with CPT-11. The above results showed that CPT-11 could effectively inhibit metastasis of LC cells in nude mice. Furthermore, tumor volume and weight were down-regulated in the nude mice injected with CPT-11 (Fig. 1D–F). IHC results showed decreased positive rates of Ki67 and PCNA in tumor tissues of mice following CPT-11 injection (Fig. 1G).

The above results showed that the topoisomerase inhibitor CPT-11 could effectively suppress LC cell growth and metastasis in vivo.

3.2. *EGFR might be a key action target protein of CPT-11*

As shown in Fig. 2A, 15 target proteins of CPT-11 were predicted from the STITCH website. The differential analysis of the GSE29249, GSE32863 and GSE44077 datasets yielded 2540, 12,621 and 13,689 DEGs, respectively (Fig. 2B–D). The target proteins were intersected with the DEGs of CPT-11 (irinotecan), and thus CASP3, CES1, EGFR and TOP1MT were obtained (Fig. 2E).

RT-qPCR showed decreased EGFR expression yet no difference in CASP3, CES1 and TOP1MT expression in the nude mice treated with CPT-11 versus that in the nude mice treated with DMSO (Fig. 2F). Therefore, we hypothesized that EGFR might be a key action target of CPT-11, and CPT-11 could suppress EGFR expression.

3.3. *CPT-11 inhibited LC cell growth and metastasis in vivo mainly by down-regulating EGFR expression*

RT-qPCR results (Fig. 3A) showed that EGFR expression was significantly decreased in tumor tissues of metastatic tumor mouse models in response to CPT-11, and additional overexpression of EGFR led to opposing trend. Pathological observations (Fig. 3B and C) showed that lung metastasis was significantly decreased by CPT-11, and this trend was reversed by further oe-EGFR treatment. ELISA results (Fig. 3D) showed that the protein expression of MMP7 and HMGB1 was markedly decreased in response to CPT-11, and this effect was reversed by further oe-EGFR treatment.

In the subcutaneous xenograft model in mice, RT-qPCR results (Fig. 3E) showed decreased EGFR expression in the presence of CPT-11, the trend of which could be reversed by further overexpression of EGFR. Moreover, as shown in Fig. 3F–H, tumor volume and weight in lung tissue of mice were notably decreased in response to CPT-11, and this trend was reversed by further oe-EGFR treatment. IHC results (Fig. 3I) showed that CPT-11 decreased the positive rates of Ki67 and PCNA, and the additional overexpression of EGFR could reverse this effect.

The above results showed that CPT-11 mainly inhibited LC cell growth and metastasis in mice by downregulating EGFR expression.

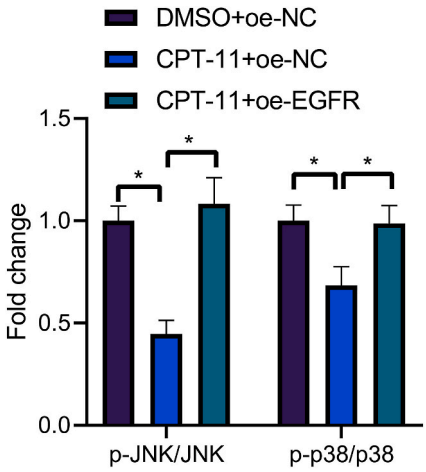

Fig. 4. CPT-11 inhibits EGFR/MAPK pathway activation. ELISA was adopted to detect phosphorylation levels of the MAPK pathway-related proteins in lung tissue in the subcutaneous xenograft model in response to CPT-11 or oe-EGFR. There were 3 mice in each group. *$p < 0.05$. Measurement data, which were expressed as mean ± SD, were compared with one-way ANOVA combined with Tukey's tests.

3.4. CPT-11 inhibited the activation of EGFR/MAPK pathway

EGFR relates to MAPK pathway activation [15], and MAPK pathway can promote LC progression and metastasis [16]. We then focused on the regulatory mechanism between CPT-11 and EGFR/MAPK pathway. ELISA results (Fig. 4) showed decreased phosphorylation levels of JNK and p38 in the presence of CPT-11, and additional EGFR overexpression could reverse the changing tendency.

Therefore, we speculated that CPT-11 might prevent LC growth and metastasis by inhibiting EGFR/MAPK pathway activation.

3.5. EGFR promoted LC cell growth and metastasis in vivo by activating MAPK pathway

RT-qPCR and ELISA (Fig. 5A and B) showed that EGFR expression and phosphorylation levels of JNK and p38 in lung tumor tissue of metastatic tumor mouse models were significantly increased in the presence of oe-EGFR + DMSO relative to oe-NC + DMSO. Compared with that upon oe-EGFR + DMSO, the expression of EGFR in lung tumor tissue in response to oe-EGFR + sesamolin had no significant difference, but the phosphorylation levels of JNK and p38 were decreased significantly. Pathological observations (Fig. 5C and D) demonstrated that lung metastasis was increased in response to EGFR overexpression, while opposite trends were seen after further treatment with sesamolin. Furthermore, ELISA (Fig. 5E) showed increased protein expression of MMP7 and HMGB1 in lung tumor tissue in the presence of oe-EGFR + DMSO, the trend of which was reversed by additional treatment with sesamolin.

RT-qPCR and ELISA performed in the subcutaneous xenograft model in nude mice (Fig. 5F and G) showed that EGFR expression and phosphorylation levels of JNK and p38 in lung tumor tissue of mice were significantly increased by EGFR overexpression, while further sesamolin treatment caused no difference in EGFR expression in lung tumor tissue of mice but reduced the phosphorylation levels of JNK and p38. Furthermore, as shown in Fig. 5H–J, the tumor volume and weight were significantly increased by EGFR overexpression, which could be reversed by additional treatment with sesamolin. IHC results (Fig. 5K) revealed that the positive rates of Ki67 and PCNA upon EGFR overexpression were increased significantly, while additional treatment with sesamolin could reverse the trend. The above results showed that EGFR promoted LC cell growth and metastasis in mice through activation of the MAPK pathway.

4. Discussion/conclusion

Lung cancer is considered to be a lethal tumor type because of the high rate of metastasis as well as recurrence post treatment [17]. Importantly, anticancer agents targeting Topoisomerase I and II have been widely applied for cancer treatment, showing effective effect [18]. In this study, we intend to explore the possible molecular mechanism of the topoisomerase inhibitor CPT-11 in regulating LC development and found that it could disrupt LC cell growth and metastasis by down-regulating the EGFR/MAPK pathway.

First, we confirmed through in vivo animal experiment that CPT-11 could effectively inhibit LC cell growth and metastasis in mice. Similar with our results, SN-38, the active metabolite of CPT-11, was found to effectively suppress growth of human advanced NSCLC cells [19]. It was documented that CPT-11 in combination with stem cells could exert therapeutic efficacy of apoptosis in primary and advanced metastatic LC tissues [20]. Besides, CPT-11 combined with nedaplatin could produce good efficacy for treatment of advanced SCLC [21]. Mechanistically, we discovered EGFR as the key target protein of CPT-11 through bioinformatics analysis, and further confirmed in the in vivo animal experiments that CPT-11 could delay advanced LC cell growth and metastasis by inhibiting EGFR. It should be noted that the regulatory relationship between CPT-11 and EGFR has been previously suggested in LC and other cancer types. For instance, the use of GE11, the antibody of EGFR, could partially enhance the uptake of CPT-11 in A549 cells in nude

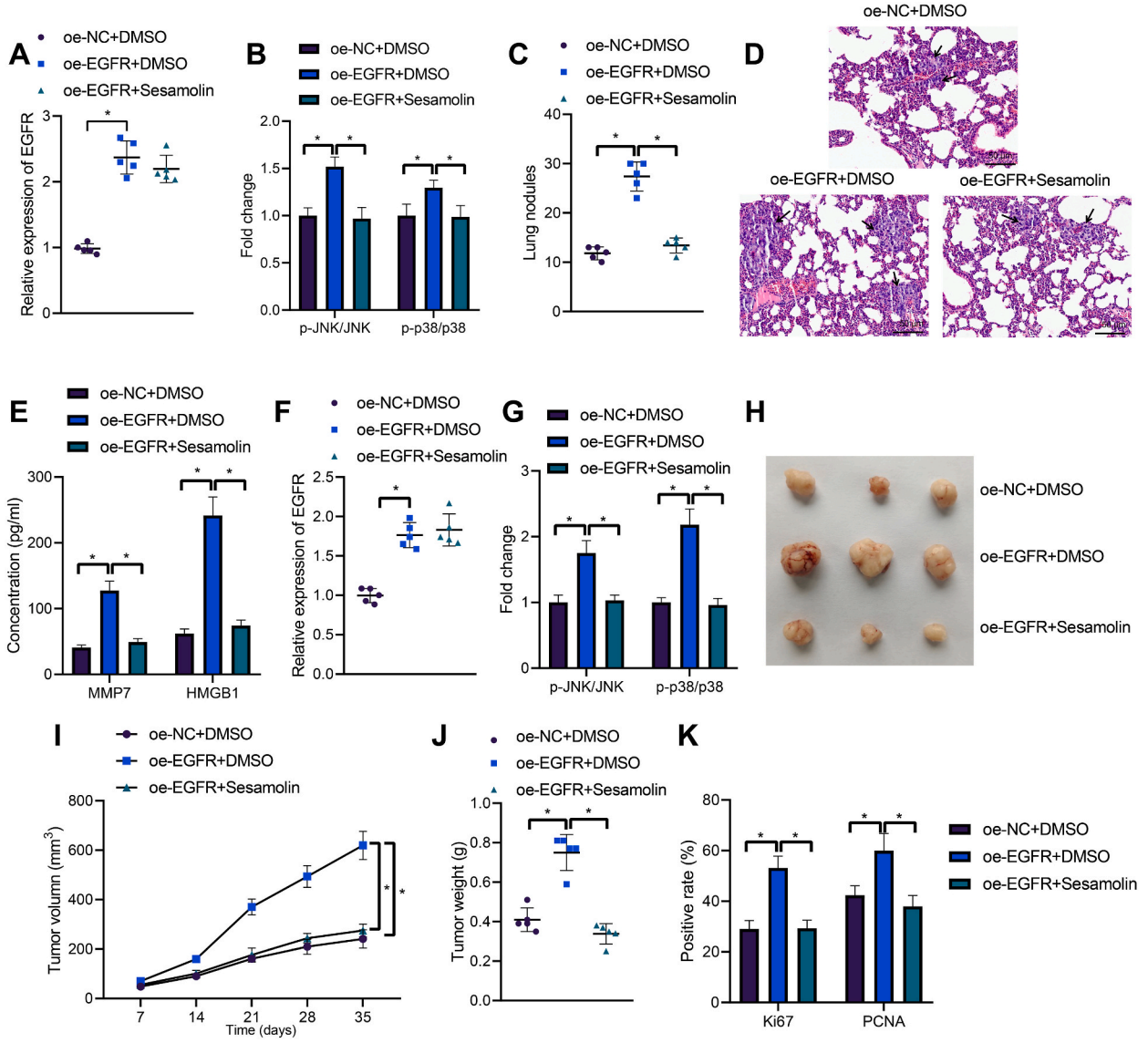

Fig. 5. EGFR promotes LC cell growth and metastasis in mice by activating MAPK pathway. A, EGFR expression in lung tissue in the metastatic tumor model upon EGFR overexpression or sesamolin as detected by RT-qPCR. B, The phosphorylation levels of MAPK pathway-related proteins in lung tissue in the metastatic tumor model upon EGFR overexpression or sesamolin as determined by ELISA. C, Number of pulmonary nodules in lung tissue in the metastatic tumor model upon EGFR overexpression or sesamolin. D, The lung metastasis in nude mice upon EGFR overexpression or sesamolin as observed using HE staining. The black arrows indicate metastatic sites. E, Expression of MMP7 and HMGB1 in lung tumor tissues upon EGFR overexpression or sesamolin as detected by ELISA. F, The expression of EGFR in lung tissue in the subcutaneous xenograft tumor model upon EGFR overexpression or sesamolin. G, The phosphorylation levels of the MAPK pathway-related proteins in lung tissue in the subcutaneous xenograft tumor model upon EGFR overexpression or sesamolin as determined by RT-qPCR. H, Diagram of the body of subcutaneous xenograft tumor upon EGFR overexpression or sesamolin. I, Statistical chart of subcutaneous xenograft tumor volume upon EGFR overexpression or sesamolin. J, Weight statistics of subcutaneous xenograft tumor upon EGFR overexpression or sesamolin. K, The positive expression of Ki67 and PCNA upon EGFR overexpression or sesamolin as detected by immunohistochemistry. There were 5 mice in each group. Measurement data, which were expressed as mean ± SD, were compared with one-way ANOVA, combined with Tukey's tests or repeated measures ANOVA. *$p < 0.05$.

mice, thereby augmenting the efficacy for treating LC [22]. Notably, co-treatment of gefitinib and SN-38 was demonstrated to regulate LC cells that expressed wild-type EGFR [8]. Besides, resistance to CPT-11 could result in EGFR activation in LoVo advanced colon cancer cells [23]. Furthermore, in the current study, we revealed that EGFR could promote LC cell growth and metastasis in mice by activating MAPK pathway. EGFR and MAPK are important in regulating LC. Targeted therapy with EGFR-TKIs is a standard regimen for treating advanced NSCLC with EGFR-mutation [24]. It was unfolded that the suppression of MAPK/ERK pathway due to treatment of lupeol could produce an anti-metastatic effect in LC [25]. Moreover, repressed MAPK by PP4C could aid in facilitating LC cell growth while inhibiting the apoptosis [26]. Notably, it was previously unveiled that the inhibition of MAPK signal transduction by

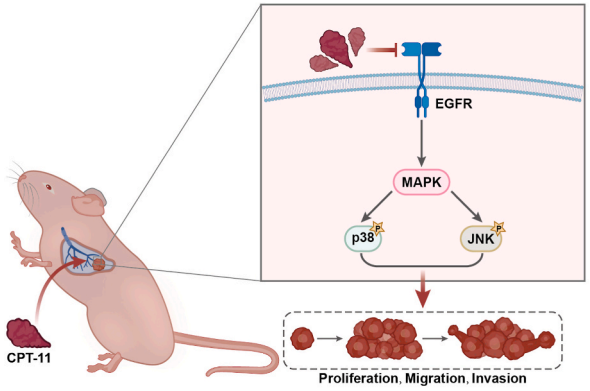

Fig. 6. Molecular mechanism diagram of the role of the topoisomerase inhibitor CPT-11 in affecting LC growth and metastasis by regulating the EGFR/MAPK pathway. CPT-11 inhibits LC cell growth and metastasis by inhibiting activation of EGFR/MAPK pathway.

microRNA-195 could result in repressed cell apoptosis in SCLC [27]. Furthermore, the interaction between EGFR and MAPK has been unveiled largely in LC. MAPK pathway is a downstream of EGFR, and *p*-MAPK/EGFR combined with *p*-mTOR/p-AKT can serve as a promising predictive marker for survival of NSCLC patients [28]. Binding the ligand to the EGFR could lead to activation of the intracellular signal transduction involving that of MAPK, contributing to NSCLC cell proliferation [29]. Intriguingly, the activated EGFR pathway could regulate MAPK to increase the expression of circRNAC190, thereby promoting the development of NSCLC [30]. These previous reports support our findings regarding the inhibition of CPT-11 in LC cells achieved through blockage of EGFR/MAPK pathway.

To sum up, the current study has demonstrated that the topoisomerase inhibitor CPT-11 inhibits LC cell growth and metastasis by suppressing the activation of EGFR/MAPK pathway (Fig. 6). This finding may offer a new direction for understanding the mechanism of CPT-11 in LC development; nevertheless, further validation is still warranted.

5. Statement

5.1. Statement of ethics

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. SUMC2022-230) granted by the Medical Animal Care & Welfare Committee of Shantou University Medical College, in compliance with institutional guidelines for the care and use of animals.

Author contribution statement

Ying-Qiu Yin; Shan-Qiang Zhang: Conceived and designed the experiments; Performed the experiments; Wrote the paper.
Jun-Ling Xie; Ling-Zhi Yin; Hui-Jing Situ and Feng Peng: Analyzed and interpreted the data; Wrote the paper.
Li-Ming Tan; Yun Xiao; Hui-Ying Zheng: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding

This work was supported by 2021 Science and Technology Plan Project of Shaoguan-Supporting Scientific Research Workers Project (Grant numbers 210803154532880) and Scientific Research Projects of Shaoguan Municipal Health Commission (Grant numbers Y21070).

Data availability statement

The authors are unable or have chosen not to specify which data has been used.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e15805.

References

[1] Jaime Rodriguez-Canales, Edwin Parra-Cuentas, Ignacio I. Wistuba, Diagnosis and molecular classification of lung cancer, Cancer Treat Res. 170 (2016) 25–46.
[2] Brett C. Bade, Charles S Dela Cruz, Lung cancer 2020: epidemiology, etiology, and prevention, Clin. Chest Med. 41 (1) (2020) 1–24.
[3] Xiao-Lin Su, Jiang-Wei Wang, Hui Che, Chang-Fu Wang, Hai Jiang, Xia Lei, Wan Zhao, et al., Clinical application and mechanism of traditional Chinese medicine in treatment of lung cancer, Chin. Med. J. (Engl). 133 (24) (2020) 2987–2997.
[4] Pithi Chanvorachote, Supakarn Chamni, Chuanpit Ninsontia, Preeyaporn Plaimee Phiboonchaiyanan, Potential anti-metastasis natural compounds for lung cancer, Anticancer Res. 36 (11) (2016) 5707–5717.
[5] Xiaoxia Liang, Qiang Wu, Shangxian Luan, Zhongqiong Yin, Changliang He, Lizi Yin, Yuanfeng Zou, A comprehensive review of topoisomerase inhibitors as anticancer agents in the past decade, Eur. J. Med. Chem. 171 (2019) 129–168.
[6] Femke M de Man, Andrew K.L. Goey, Ron H N van Schaik, Ron H.J. Mathijssen, Bins Sander, Individualization of irinotecan treatment: a review of pharmacokinetics, pharmacodynamics, and pharmacogenetics, Clin. Pharmacokinet. 57 (10) (2018) 1229–1254.
[7] Jan P van Meerbeeck, Dean A. Fennell, Dirk K M De Ruysscher, Small-cell lung cancer, Lancet 378 (9804) (2011) 1741–1755.
[8] Tatsu Shimoyama, Fumiaki Koizumi, Hisao Fukumoto, Katsuyuki Kiura, Mitsune Tanimoto, Nagahiro Saijo, Kazuto Nishio, Effects of different combinations of gefitinib and irinotecan in lung cancer cell lines expressing wild or deletional EGFR, Lung Cancer 53 (1) (2006) 13–21.
[9] Davinder Singh, Bhupinder Kumar Attri, Rupinder Kaur Gill, Jitender Bariwal, Review on EGFR inhibitors: critical updates, Mini Rev. Med. Chem. 16 (14) (2016) 1134–1166.
[10] Gilda da Cunha Santos, Frances A. Shepherd, Ming Sound Tsao, EGFR mutations and lung cancer, Annu. Rev. Pathol. 6 (2011) 49–69.
[11] Xiaomin Liu, Ping Wan, Caiyan Zhang, Zhongliang Ma, Epidermal growth factor receptor (EGFR): a rising star in the era of precision medicine of lung cancer, Oncotarget 8 (30) (2017) 50209–50220.
[12] Lin Yang, Hanyin Zhang, Dan Chen, Peikun Ding, Yunchang Yuan, Yandong Zhang, EGFR and Ras regulate DDX59 during lung cancer development, Gene 642 (2018) 95–102.
[13] Y.-X. Zhang, J. Yuan, Z.-M. Gao, Z.-G. Zhang, LncRNA TUC338 promotes invasion of lung cancer by activating MAPK pathway, Eur. Rev. Med. Pharmacol. Sci. 22 (2) (2018) 443–449.
[14] Hui Xu, Xiaomei Yang, Xiaofeng Xuan, Di Wu, Jieru Zhang, Xinchun Xu, Yuanjie Zhao, et al., STAMBP promotes lung adenocarcinoma metastasis by regulating the EGFR/MAPK signaling pathway, Neoplasia 23 (6) (2021) 607–623.
[15] Xiao-Lin Su, Jiang-Wei Wang, Hui Che, Chang-Fu Wang, Hai Jiang, Xia Lei, Wan Zhao, et al., Clinical application and mechanism of traditional Chinese medicine in treatment of lung cancer, Chin. Med. J. (Engl). 133 (24) (2020) 2987–2997.
[16] Xiaochen Li, Xiaopei Cao, Hanqiu Zhao, Mingzhou Guo, Xiaoyu Fang, Ke Li, Qin Lu, et al., Hypoxia activates Notch 4 via ERK/JNK/P38 MAPK signaling pathways to promote lung adenocarcinoma progression and metastasis, Front. Cell Dev. Biol. 9 (2021), 780121.
[17] Yan Lu, Yushuang Zheng, Yuhong Wang, Dongmei Gu, Jun Zhang, Fang Liu, Kai Chen, et al., FlowCell-enriched circulating tumor cells as a predictor of lung cancer metastasis, Hum. Cell 34 (3) (2021) 945–951.
[18] Fei You, Changshou Gao, Topoisomerase inhibitors and targeted delivery in cancer therapy, Curr. Top. Med. Chem. 19 (9) (2019) 713–729.
[19] Dharmendra Kumar Maurya, Rie Ayuzawa, Chiyo Doi, Deryl Troyer, Masaaki Tamura, Topoisomerase I inhibitor SN-38 effectively attenuates growth of human non-small cell lung cancer cell lines in vitro and in vivo, J. Environ. Pathol. Toxicol. Oncol. 30 (1) (2011) 1–10.
[20] Bo-Rim Yi, Seung U. Kim, Kyung-Chul Choi, Co-treatment with therapeutic neural stem cells expressing carboxyl esterase and CPT-11 inhibit growth of primary and metastatic lung cancers in mice, Oncotarget 5 (24) (2014) 12835–12848.
[21] Jun Fu, Sanyou Fang, Wen Yin, Yanbing Wang, Xin Yin, Daofeng Wang, The efficacy and safety of irinotecan combined with nedaplatin in the treatment of small cell lung cancer, J BUON 25 (4) (Jul-Aug 2020) 1707–1713.
[22] Liang Zhang, Haixin Cui, HAase-sensitive dual-targeting irinotecan liposomes enhance the therapeutic efficacy of lung cancer in animals, Nanotheranostics 2 (3) (2018) 280–294.
[23] Ming-Cheng Chen, Nien-Hung Lee, Tsung-Jung Ho, Hsi-Hsien Hsu, Chia-Hua Kuo, Wei-Wen Kuo, Yueh-Min Lin, et al., Resistance to irinotecan (CPT-11) activates epidermal growth factor receptor/nuclear factor kappa B and increases cellular metastasis and autophagy in LoVo colon cancer cells, Cancer Lett. 349 (1) (2014) 51–60.
[24] Jingyi He, Zhengrong Huang, Linzhi Han, Yan Gong, Conghua Xie, Mechanisms and management of 3rd-generation EGFR-TKI resistance in advanced non-small cell lung cancer (Review), Int. J. Oncol. 59 (5) (2021) 90.
[25] Mital Bhatt, Mitesh Patel, Adnan Mohd, Mandadi N. Reddy, Anti-metastatic effects of lupeol via the inhibition of MAPK/ERK pathway in lung cancer, Anti Cancer Agents Med. Chem. 21 (2) (2021) 201–206.
[26] Bin Wang, Xun-Xia Zhu, Lin-Yue Pan, He-Feng Chen, Xiao-Yong Shen, PP4C facilitates lung cancer proliferation and inhibits apoptosis via activating MAPK/ERK pathway, Pathol. Res. Pract. 216 (5) (2020), 152910.
[27] Jichun Tong, Jiawei Lu, Yajun Yin, Yeming Wang, Ke Zhang, microRNA-195 promotes small cell lung cancer cell apoptosis via inhibiting Rap2C protein-dependent MAPK signal transduction, Technol. Cancer Res. Treat. 19 (2020), 1533033820977546.
[28] Haruhisa Kitano, Joon-Yong Chung, Kris Ylaya, Catherine Conway, Mikiko Takikita, Junya Fukuoka, Yoshinori Doki, et al., Profiling of phospho-AKT, phospho-mTOR, phospho-MAPK and EGFR in non-small cell lung cancer, J. Histochem. Cytochem. 62 (5) (2014) 335–346.
[29] Agnieszka Rybarczyk-Kasiuchnicz, Rodryg Ramlau, Katarzyna Stencel, Treatment of brain metastases of non-small cell lung carcinoma, Int. J. Mol. Sci. 22 (2) (2021) 593.
[30] Afeez Adekunle Ishola, Chian-Shiu Chien, Yi-Ping Yang, Yueh Chien, Aliaksandr A. Yarmishyn, Ping-Hsing Tsai, Jerry Chieh-Yu Chen, et al., Oncogenic circRNA C190 promotes non-small cell lung cancer via modulation of the EGFR/ERK pathway, Cancer Res. 82 (1) (2022) 75–89.