Medicinal herbs and bioactive compounds overcome the drug resistance to epidermal growth factor receptor inhibitors in non-small cell lung cancer (Review)

HIU YAN JENNIFER LEE[1], MINGJING MENG[2], YULONG LIU[1], TAO SU[2] and HIU YEE KWAN[1]

[1]School of Chinese Medicine, Hong Kong Baptist University, Hong Kong, SAR; [2]International Institute for Translational Chinese Medicine, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong 510006, P.R. China

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Abstract. Lung cancer is the leading cause of cancer-related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for ~85% of all lung cancer cases. Patients harboring epidermal growth factor receptor (EGFR) mutations usually develop resistance to treatment with frontline EGFR-tyrosine kinase inhibitors (EGFR-TKIs). The present review summarizes the current findings and delineates the molecular mechanism of action for the therapeutic effects of herbal extracts and phytochemicals in overcoming EGFR-TKI resistance in NSCLC. Novel molecular targets underlying EGFR-TKI resistance in NSCLC are also discussed. This review provides valuable information for the development of herbal bioactive compounds as alternative treatments for EGFR-TKI-resistant NSCLC.

Correspondence to: Dr Tao Su, International Institute for Translational Chinese Medicine, School of Pharmaceutical Sciences, Guangzhou University of Chinese Medicine, 232 Waihuan East Road, Guangzhou, Guangdong 510006, P.R. China
E-mail: sutao@gzucm.edu.cn

Dr Hiu Yee Kwan, School of Chinese Medicine, Hong Kong Baptist University, 7 Baptist University Road, Kowloon Tong, Hong Kong, SAR, P.R. China
E-mail: hykwan@hkbu.edu.hk

Abbreviations: CIP2A, cancerous inhibitor of protein phosphatase 2A; EGFR, epidermal growth factor receptor; ERK, extracellular signal-regulated kinase; MMP, matrix metalloproteinase; MTA-1, metastasis-associated protein-1; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor-κB; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol 3-kinase; PFS, progression-free survival; PP2A, protein phosphatase-2A; ROS, reactive oxygen species; SHP2, SH2 domain-containing phosphatase-2; STAT3, signal transduction and activator of transcription 3; TKI, tyrosine kinase inhibitor; USP8, ubiquitin-specific peptidase 8; VEGF, vascular endothelial growth factor; COX-2, cyclooxygenase-2; CDK, cyclin-dependent kinase

Key words: non-small cell lung cancer, epidermal growth factor receptor, herbal medicine, Chinese medicine, drug resistance

Contents

1. Introduction

Lung cancer is the most commonly occurring cancer worldwide, and ~6.3% of the population will be diagnosed with lung or bronchial cancer at some point during their lifetime (1).

Lung cancer can be categorized into small cell lung cancer (SCLC) and non-SCLC (NSCLC). NSCLC is a type of epithelial cancer that accounts for ~85% of all lung cancer cases (2). As reported by the American Cancer Society, based on the individuals diagnosed with NSCLC between 2010 and 2016, the 5-year survival rate for NSCLC is 63% for patients with localized tumors, but only 35% for those with early-stage tumors and 7% for those with distant tumors (2). Among the different types of NSCLC, the most common types are squamous cell carcinoma, large cell carcinoma and adenocarcinoma (2). Squamous cell carcinoma refers to cancer arising in the flat cells lining the inside of the airways of the lungs (2). Large cell carcinoma develops in the outer regions of the lungs and the cancer cells are large in size when viewed under the microscope (2). Lung adenocarcinoma is a subtype that begins in the cells lining the alveoli (2).

2. Clinical challenges of treating NSCLC

NSCLC is the most common subtype of lung cancer. In total, 10-35% of NSCLC cases harbor activating epidermal growth factor receptor (EGFR)-mutations (3). EGFR mutations in NSCLC have given rise to the opportunity to use EGFR-tyrosine kinase inhibitors (TKIs) for treatment, and these inhibitors have become one of the frontline treatments for NSCLC. First-generation EGFR-TKIs include gefitinib and erlotinib, which reversibly bind to the ATP-binding site of the intracellular TK-domain of EGFR and impede EGFR autophosphorylation and the subsequent activation of the downstream signaling pathways. Second-generation EGFR-TKIs include afatinib and dacomitinib, which not only inhibit the TK-domain of EGFR, but also inhibit other members of the EGFR family such as the ErbB receptors (4).

Several clinical studies have, however, reported adverse events involving EGFR-TKI treatments. For example, gefitinib induces diarrhea (5,6), causes various levels of hepatotoxicity (7), and results in liver dysfunction (7), skin disorders (8), stomatitis and mucositis (8). Furthermore, EGFR-TKI-induced paresthesia causes insensitivity and numbness of the limbs (7,8). In more severe cases, the patients will lose sensation (7,8). The third-generation EGRF-TKI, osimertinib, also causes grade 3 adverse events, as reported in a previous clinical trial (ClinicalTrials.gov number, NCT02296125) (9).

In addition to the reported side effects, the initial response to EGFR-TKIs is transient and most patients develop resistance to EGFR-TKI treatment, with a median progression-free survival (PFS) time of only 9-13 months (10,11). With regard to the intrinsic resistance to EGFR-TKIs, genomic profiling of tumor specimens by high-throughput next generation sequencing analyses has highlighted gene mutations, including single nucleotide variants, point mutations, gene insertions and deletions, copy number variations and dysregulated expression of oncogenes (4,12-14). Furthermore, activation of alternative signaling pathways and phenotypic transformation of NSCLC to SCLC has also led to the development of drug resistance (11).

More importantly, ~40% of lung cancer patients are diagnosed at the advanced stage (2), making treatment difficult. In a study analyzing data from patients enrolled in the Surveillance, Epidemiology and End Results (SEER) cancer registry, patients with stage III NSCLC treated with chemo-radiotherapy had a 3-year survival probability of only 24.1% (15). Although clinical results show that the first-line treatment with EGFR-TKIs significantly enhances the PFS time compared with standard chemotherapy in patients with advanced NSCLC harboring EGFR mutations, the reported adverse events and the development of drug resistance remain a clinical challenge for treatment.

3. Treatment with medicinal herbs prolongs the PFS time for patients with advanced NSCLC treated with EGFR-TKIs

With advancements in technology, recent studies have shown that treatment with medicinal herbs is an effective adjuvant to anti-EGFR therapy for the treatment of advanced NSCLC (16,17). Compared with EGFR-TKI monotherapy, the combination of herbal medicine and EGFR-TKIs achieves a significantly higher objective response rate [risk ratios (RR), 1.34; 95% CI, 1.15-1.57; P=0.0002], disease control rate (RR, 1.8; 95% CI, 1.09-1.27; P<0.0001), 1-year survival rate (RR, 1.21; 95% CI, 1.01-1.44; P=0.04) and 2-year survival rate (RR, 1.91; 95% CI, 1.26-2.89; P=0.002), and improves the Karnofsky performance status (RR, 1.38; 95% CI, 1.26-1.51; P<0.00001) (18). Another clinical trial reported that the median PFS time was significantly longer in the herbal medicine and EGFR-TKI combination treatment group (13.50 months; 95% CI, 11.20-16.46) compared with that in the EGFR-TKI mono-treatment group (10.94 months; 95% CI, 8.97-12.45) (hazard ratio, 0.68; 95% CI, 0.51-0.90; P=0.0064) (17). Furthermore, population-based cohort studies clearly demonstrate that adjunctive herbal medicine improves the survival of patients with advanced-NSCLC who have received EGFR-TKI treatment (19-23).

Brucea javanica is a well-known medicinal herb in Asia. Clinical research shows that *B. javanica* not only reduces the incidence of nausea, vomiting and leukopenia in patients with advanced NSCLC (24), but that it also potentiates the efficacy of EGRF-TKI treatment in NSCLC harboring L858R/T790M EGFR mutations (25). It is also a safe adjuvant option to chemotherapy that can maximize the chemotherapeutic effects for NSCLC treatment (26). *Marsdenia tenacissima* (Roxb.) Wight et Arn., is also commonly used in herbal medicine. Experimental research shows that *M. tenacissima* improves the sensitivity of TKI-resistant NSCLC cells to gefitinib treatment. The combination of *M. tenacissima* and gefitinib significantly potentiates the inhibitory effect of gefitinib on cancer growth by inhibiting the signaling pathways that are activated by EGFR, such as the phosphatidylinositol 3-kinase (PI3K)/Akt, mammalian target of rapamycin (mTOR) and Ras/Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase/ERK signaling cascades (27,28). Another study suggested that *M. tenacissima* treatment overcomes EGFR-TKI resistance by inhibiting the receptor tyrosine kinases c-MET and Axl. MET and Axl are activated by hepatocyte growth factor and growth arrest-specific 6, respectively. Upon activation, c-MET and Axl regulate the key processes in tumorigenesis implicated in EGFR activation (29). Combined *M. tenacissima* and EGFR-TKI treatment significantly inhibits the c-Met and Axl-activated signaling pathways and overcomes erlotinib and gefitinib resistance in NSCLC (30). With regard to the C-21 steroidal glycosides isolated from *M. tenacissima*, some have anticancer effects (31,32). However, the bioactive compounds in *M. tenacissima* that reverse EGFR-TKI resistance in NSCLC have not been identified. *Herba epimedium* (Horny Goat Weed) is a Chinese medicinal herb. A previous study showed that the combined treatment of *Epimedium koreanum* Nakai extract and gefitinib had an antiproliferative effect in T970M EGFR-resistant NSCLC cells (33). Further *in vitro* and *in vivo* studies showed that the extract decreased the phosphorylation of EGFR family members such as EGFR, HER-2, HER-3 and EGFR, and inhibited the downstream signaling pathway PI3K/Akt/mTOR, thus inhibiting the EGFR-resistant cell migration, invasion, angiogenesis and other transformation activities (34,35).

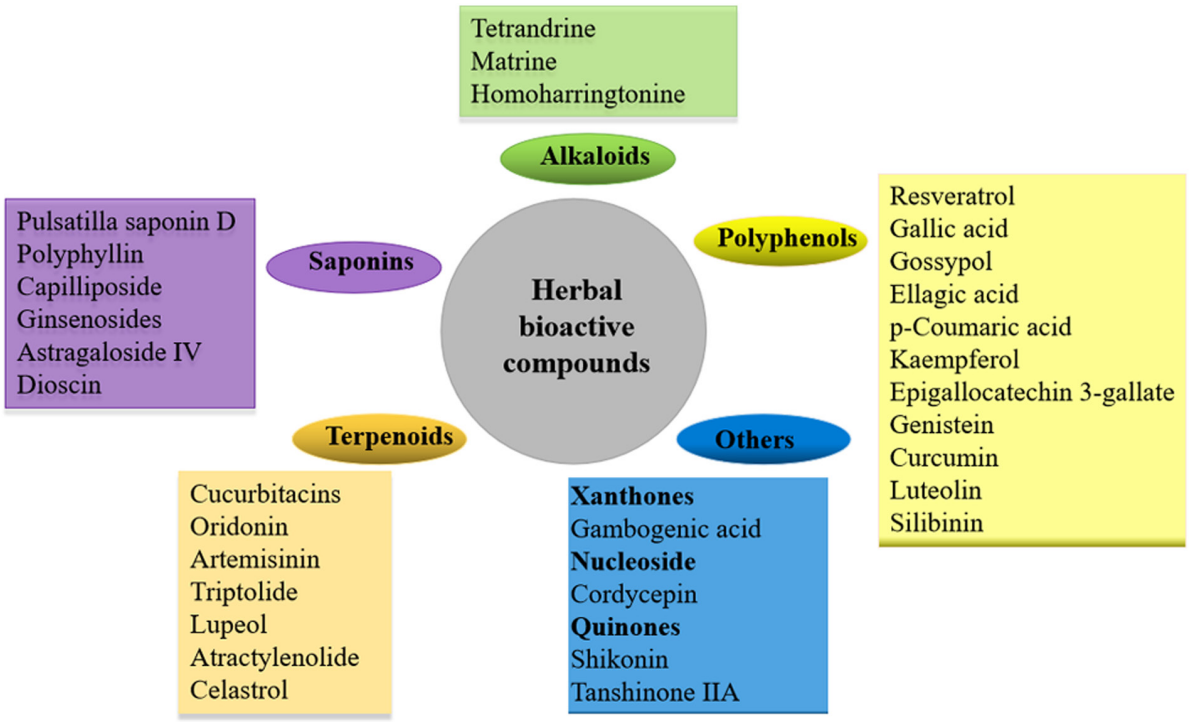

Figure 1. Herbal bioactive compounds that potentiate the therapeutic effects of EGFR-TKIs. Different natural products are marked in different colors. The green box presents the alkaloids, the purple box presents the saponins, the orange box presents the terpenoids, the yellow box presents the polyphenols and the blue box presents the other compounds, including the resins, nucleosides and quinones.

4. Herbal bioactive compounds can overcome drug resistance to EGFR-TKIs and enhance anti-EGFR therapeutic effects in NSCLC

A number of plant-derived compounds serve as the basis for the development of evidence-based pharmaceutical drugs. Studies have demonstrated that some of the bioactive compounds in medicinal herbs overcome drug resistance to EGFR-TKIs and potentiate the therapeutic effects of EGFR-TKIs. These compounds include polyphenols, saponins, terpenoids, alkaloids, quinones, resins and nucleosides (Fig. 1).

Polyphenols

Resveratrol. Resveratrol belongs to a class of polyphenolic compounds called stilbenes. This natural polyphenol can be detected in >70 plant species (36). Resveratrol decreases CYP1A1 and ABCG2 expression, and impairs gefitinib elimination from cells, increasing intracellular gefitinib concentration, which helps to overcome gefitinib resistance (37). The accumulated gefitinib triggers apoptosis, autophagy and senescence in gefitinib-resistant NSCLC cells (37). Therefore, co-treatment with gefitinib and resveratrol potentiates the anti-NSCLC effects of gefitinib.

Gallic acid. Gallic acid is a member of the hydroxybenzoic acids commonly found in a number of herbs and fruits. Gallic acid accelerates EGFR turnover (38), inhibits the Src-signal transducer and activator of transcription-3 (STAT3) signaling pathway, and induces apoptosis and cell cycle arrest in EGFR-TKI-resistant NSCLC, but not in EGFR-TKI-sensitive NSCLC, suggesting that gallic acid overcomes the acquired resistance to EGRF-TKI resistance in NSCLC (39).

Curcumin. Curcumin, also called diferuloylmethane, is isolated from the rhizome of *Curcuma longa*. Curcumin has anti-NSCLC effects; it inhibits NSCLC cell proliferation and induces apoptosis by increasing caspase-3 activity and the expression of miR-192-5p, which suppresses the phosphoinositide 3-kinase (PI3K)/Akt signaling pathway in NSCLC (40). miR-192-5p mimics enhance the effects of curcumin on cell viability and apoptosis, and suppress the PI3K/Akt signaling pathway in NSCLC cells, while anti-miR-192-5p mimics reverse the effect of curcumin on NSCLC cells (40). Curcumin also suppresses the proliferation and invasion of NSCLC by inhibiting the metastasis-associated protein-1 (MTA-1)-activated Wnt/β-catenin pathway (41). MTA-1 promotes NSCLC cell invasion and metastasis (42). The decrease in MTA-1 by curcumin treatment inhibits NSCLC growth. Furthermore, curcumin induces autophagy in NSCLC and the autophagy inhibitor 3-methyladenine (3-MA) partly blocks the inhibitory growth effect of curcumin on cancer cells (43).

Curcumin also potentiates the therapeutic effects of gefitinib. Curcumin induces apoptosis in TKI-resistant NSCLC cells by decreasing EGFR phosphorylation and increasing EGFR degradation, thus inhibiting cancer growth (44). More importantly, the combination treatment of curcumin and EGFR-TKI markedly inhibits NSCLC growth by decreasing the expression of EGFR, c-MET and cyclin D1. The combination treatment confers a better survival rate and decreases intestinal mucosal damage in intestinal epithelial cells by regulating mitogen-activated protein kinase activity (45).

Furthermore, the combination of curcumin and gefitinib causes marked autophagy induction, autophagic cell death and autophagy-mediated apoptosis when compared with curcumin or gefitinib treatment alone. Pharmacological autophagy inhibitors bafilomycin A1 or 3-MA, or knockdown of beclin-1 or autophagy-related 7, ameliorate treatment-induced autophagic cell death (45). Erlotinib and afatinib are other EGFR-TKIs used for frontline treatment. Co-administration of erlotinib and curcumin significantly decreases NSCLC cell viability by inducing apoptosis and increasing the expression of IκB (46), which restricts nuclear factor-κB (NF-κB) to the cytoplasm and inhibit its DNA binding activity (47). The combination treatment also significantly increases apoptosis by decreasing the expression of EGFR and survivin, and inhibiting NF-κB activity in erlotinib-resistant NSCLC cells (48). The application of curcumin and afatinib as a combination treatment for gefitinib- and erlotinib-resistant NSCLC has already been patented (CN105476996A).

Curcumin also overcomes chemotherapeutic resistance in NSCLC. A recent study implicated HIF-1α in the development of chemotherapeutic resistance in cancer; therefore, targeting HIF-1α either by RNA-interference or small interfering RNA may overcome the resistance to cisplatin (49). It has been reported that combined curcumin and cisplatin treatment markedly inhibits cisplatin-resistant NSCLC cell proliferation and triggers apoptotic death, by promoting HIF-1α degradation and activating caspase-3, respectively (49). Curcumin also reverses cisplatin resistance by increasing cisplatin-induced apoptosis by inducing generation of intracellular reactive oxygen species (ROS) and proteosomal degradation of Bcl-2 in NSCLC (50).

Saponins

Ginsenosides. Ginsenosides are the main saponins in ginseng roots, fruits, stems and leaves. To date, >60 ginsenosides have been isolated and identified. Although the basic structure of ginsenosides are similar, the different arrangements in the four rings of steroid nuclei are different in different sub-types, such as the A-Panaxadiol group (e.g., Rb1, Rb2, Rb3, Rc, Rd, Rg3 and Rh2), the B-Panaxatriol group (e.g. Re, Rg1, Rg2 and Rh1) and the C-Oleanolic acid group (e.g. Ro) (51). One study showed that ginsenoside upregulated vaccinia-related kinase 1 and activated the tumor suppressor p53-binding protein-1, thus inhibiting NSCLC cell proliferation (52).

Ginsenosides also promote the antitumor activity of gefitinib in NSCLC. It has been demonstrated that ginsenoside Rg3 increases the cytotoxic effects of gefitinib in NSCLC cells in a dose- and time-dependent manner (53). The combination treatment of gefitinib and ginsenoside Rg3 significantly increases apoptosis in NSCLC cells by increasing the expression of the pro-apoptotic protein Bax and the activity of caspase-3, and by decreasing the expression of the anti-apoptotic protein Bcl-2 (53). The combination treatment also inhibits NSCLC cell migration by decreasing the protein expression of Snail and Slug (53), which are master regulators that regulate numerous genes that contribute to epithelial-mesenchymal transition (EMT), including E-cadherin (54). Osimertinib is the third-generation EGFR-TKI. A recent study revealed that osimertinib-resistant NSCLC cells displayed stronger stemness than the parental cells, which conferred to them greater metastatic potential (55). Notably, ginsenoside Rg3 attenuates the stemness of NSCLC cells and the resistance to osimertinib, as evidenced by the decreased expression of stemness markers and decreased spheroid formation ability, which is dependent on the Hippo signaling pathway (56). Furthermore, ginsenosides overcome resistance to chemotherapy drugs such as cisplatin (DDP), which is used for the treatment of NSCLC. Clinical research shows that ginsenoside Rg3 enhances the therapeutic effects of chemotherapy in patients with NSCLC. Rg3 treatment prolongs the overall survival time and improves the quality of life for the patients (57). Rg3 restores leucocyte count, decreases vascular endothelial growth factor (VEGF) expression and increases the CD4/CD8 T-cell ratio in these patients. Clinical outcomes show that Rg3 combined with chemotherapy enhances the efficacy and overall survival time of patients with NSCLC. Experimental research also suggests that ginsenoside overcomes the resistance to cisplatin in NSCLC. In cisplatin-resistant NSCLC (A549/DDP) cells, ginsenoside inhibits the NRF2 pathway and significantly sensitizes A549/DDP cells to cisplatin. NRF2 knockdown attenuates the synergistic effects of ginsenoside in cisplatin-resistant cells (58). A previous study also reported that Rg3 alleviated the resistance of A549/DDP cells to cisplatin treatment by decreasing PD-L1 expression via the inhibition of NF-κB p65 and Akt; therefore, restoring the cytotoxicity of T cells to the cancer cells (59).

Astragaloside IV. Astragaloside IV is a bioactive saponin found in the roots of a Chinese medicinal herb *Astragalus membranaceus* (Fisch.) Bge. Astragaloside IV sensitizes NSCLC to gefitinib by regulating sirtuin 6 (SIRT6), and inhibiting SIRT6 abolishes the sensitization role of astragaloside IV in NSCLC cells including HCI-H1299, HCC827 and A549 cells (60). SIRT6 is an NAD-dependent deacetylase and promotes the metastasis of NSCLC. Depletion of SIRT6 inhibits transforming growth factor-β1-induced EMT in NSCLC cells, which underscores how astragaloside IV potentiates the anti-NSCLC effects of gefitinib.

Polyphyllin (PPI). PPI, PPII and PPVII are the main active saponins isolated from *Paris polyphylla*. PPI overcomes gefitinib resistance in NSCLC cells by decreasing cell viability and inducing apoptosis via downregulation of MALAT long non-coding RNA and inactivating the STAT3 signaling pathway (61). Both MALAT and STAT3 promote NSCLC growth. Overexpression of MALAT has been associated with a poor prognosis in patients with NSCLC (62), including recurrence and metastasis (63,64). Mechanistic studies show that MALAT activates Akt/mTOR signaling in NSCLC and promotes cancer cell migration and invasion. High expression of STAT3 in patients with NSCLC is also associated with a poor prognosis (65,66), and mediates drug resistance to EGFR-TKIs (67). Therefore, downregulating these two signaling molecules potentiates the anti-NSCLC effects of gefitinib. PPII restores sensitization of resistant NSCLC cells to gefitinib by inhibiting the PI3K/Akt/mTOR signaling pathway, thus potentiating the anti-proliferative effects of gefitinib and gefitinib-induced apoptosis (68). PPVII enhances the anti-proliferative effects of gefitinib and gefitinib-induced G_1 phase arrest by modulating the p21 signaling pathway

in gefitinib-resistant NSCLC cells (69). Another study by Lou *et al* also reported that PPI overcame erlotinib resistance in NSCLC by reversing EMT and modulating the IL-6/STAT3 signaling pathway. Co-treatment of erlotinib and PPI completely abrogated the growth of NSCLC (70).

Both PPI and PPVII have a chemo-sensitizing effect; they overcome cisplatin resistance in NSCLC by enhancing apoptosis, reversing EMT, suppressing the cancerous inhibitor of protein phosphatase 2A (CIP2A)/AKT/mTOR signaling axis and activating autophagy in cancer cells (71,72).

Dioscin. Dioscin is a natural steroidal saponin abundantly found in *Dioscorea nipponica* Makino. Dioscin overcomes EGFR-TKI resistance in NSCLC by downregulating the expression of SH2 domain-containing phosphatase-2 (SHP2) at the transcriptional level through the induction of ROS production, and therefore decreases the binding of p53 to the SHP2 promoter (73). It is known that SHP2 is required for the growth of KRAS-mutant NSCLC, and the inhibition of SHP2 in KRAS-mutant NSCLC provokes a senescence response (74).

Capilliposide. Capilliposide is a saponin in the Chinese medicinal herb *Lysimachia capillipes*; it inhibits AKT activity, increases the pro-apoptotic effect of gefitinib and decreases EGFR phosphorylation in gefitinib-resistant NSCLC cells (75).

Terpenoids

Cucurbitacins. Cucurbitacins, such as cucurbitacin B and D, are tetracyclic triterpenes isolated from the Cucurbitaceae plant family. Cucurbitacin B potently suppresses NSCLC growth by inducing G_2/M cell-cycle arrest and mitochondrial apoptosis. The anti-NSCLC effects of cucurbitacin B are attenuated by the thiol antioxidant N-acetylcysteine, while glutathione synthesis inhibitor butithione-sulfoxime exacerbates the anti-NSCLC effects (76). Further research suggests that NSCLC cells treated with cucurbitacin B have a significant decrease in the ratio of thiols and glutathione to oxidized glutathione, suggesting that disruption of the cellular redox balance mediates the anti-NSCLC effects of cucurbitacin B (77).

Cucurbitacin B and D also overcome gefitinib resistance in NSCLC cells. CIP2A inhibits protein phosphatase-2A (PP2A) (78), which suppresses tumor growth through dephosphorylation of oncogenic kinases and transcription factors (79). Cucurbitacin B suppresses the CIP2A/PP2A/Akt signaling axis in gefitinib-resistant cells and inhibits cancer growth and metastasis (80). Furthermore, the cucurbitacin B-mediated inhibition of the CIP2A/PP2A/Akt axis also induces the lysosomal degradation of EGFR in NSCLC cells (80). In a previous study, cucurbitacin D overcame gefitinib resistance by blocking EGF binding to EGFR, as demonstrated by a solid-phase EGF-EGFR interaction assay. In the analysis, cucurbitacin D directly inhibited the interaction between EGF and EGFR (81). Hence, cucurbitacin D decreases EGFR phosphorylation in gefitinib-resistant NSCLC cells (81).

Oridonin. Oridonin is a diterpenoid compound extracted from Isodon plant *Rabdosia rubescens*, *Isodon japonicus* Hara and *I. trichocarpus* (82). Oridonin inhibits the growth of gefitinib-resistant NSCLC cells. Oridonin decreases the expression of matrix metalloproteinase-12 (MMP-12) and CIP2A, suggesting that oridonin inhibits the growth and metastasis of gefitinib-resistant NSCLC by suppressing the EGFR/ERK/MMP-12 and CIP2A/Akt signaling pathways (83).

Artemisinin. Artemisinin is a sesquiterpene lactone in the Chinese medicinal herb *Artemisia annua* L. In EGFR-TKI-resistant NSCLC cells, artemisinin induces apoptosis and cell cycle arrest at the G_1 phase. Artemisinin decreases Wnt/β-catenin protein levels by stimulating the release of naked cuticle homolog 2 (NKD2) and Axin2 (84). Both NKD2 and Axin2 are negative regulators of the Wnt/β-catenin signaling pathway. Downregulation of NKD2 causes Wnt activation and increases the invasive potential of NSCLC (85). Axin promotes the phosphorylation and the consequent degradation of β-catenin, and thus suppresses the Wnt/β-catenin signaling pathway (86). One study reported that the combination of artemisinin and onconase, a RNase of ribonuclease A, synergistically inhibited NSCLC growth (87). However, whether the combination of artemisinin and onconase can overcome gefitinib resistance in NSCLC is not known.

Dihydroartemisinin is the semi-synthetic derivative of artemisinin. It has been reported that treatment with dihydroartemisinin and gefitinib synergistically inhibits NSCLC cell growth and induces apoptosis via the Akt/mTOR/STAT3 pathway. Furthermore, this combination of treatment induces NSCLC cell cycle arrest in the G_2/M phase, which is associated with decreased expression of G_2/M regulatory proteins, including cyclin B1 and cyclin-dependent kinase 1. Combined treatment with dihydroartemisinin and gefitinib also significantly decreases the metastatic potential of NSCLC compared with treatment with gefitinib alone (88).

Alkaloids

Matrine. Matrine is an alkaloid isolated from *Sophora flavescens*. Treatment with a combination of matrine and afatinib, an EGFR-TKI, results in increased growth inhibitory effects of NSCLC cells by inhibiting the JAK/STAT3 signaling pathway (89), suggesting that matrine can overcome drug resistance to afatinib in NSCLC.

Furthermore, matrine also overcomes resistance to chemotherapy. In cisplatin-resistant NSCLC cells, matrine inhibits β-catenin activation and survivin expression, and induces apoptotic death, coupled with the loss of mitochondrial membrane potential and activation of caspase-9 and -3 (90).

Oxymatrine. Oxymatrine is one of the alkaloid components in *Sophora flavescens*; it inhibits NSCLC growth by suppressing the EGFR signaling pathway by inhibiting the activity of wild-type EGFR and EGFR with exon 19 deletion and L858R/T790M mutation (91). Further studies are needed to verify whether oxymatrine overcomes EGFR-TKI resistance in NSCLC.

Quinonoids

Shikonin. Shikonin is a naphthoquinone compound extracted from the root of the Chinese medicinal herb

Lithospermum erythrorhizon. Shikonin exhibits cytotoxicity, increases ROS production and induces apoptosis in NSCLC cells (92). Inhibiting ROS production blocks shikonin-induced apoptosis (92). Furthermore, shikonin also induces necroptosis, which is necrosis in a programmed fashion, mediated by signal transduction from receptor-interacting serine/threonine kinase (RIP)-1 to RIP-3. It has been reported that shikonin increases the expression of RIP-1 and induces necroptosis in NSCLC (93). Notably, the shikonin-induced necroptosis is enhanced by the inhibition of autophagy in NSCLC cell (93). Shikonin also sensitizes NSCLC to EGFR-TKI treatment. Shikonin in combination with erlotinib or gefitinib significantly enhances ROS-mediated apoptosis in NSCLC when compared with EGFR-TKI treatment alone (92,94). Furthermore, the combination of shikonin and gefitinib has synergistic anticancer effects via the inhibition of pyruvate kinase M2 (PKM2) and its downstream signaling molecules, including STAT3 and cyclin D1, in NSCLC cells (95). However, there is lack of consistent evidence suggesting the correlation of PKM2 and tumor grade in NSCLC (96). Another study has suggested that shikonin sensitizes gefitinib-resistant NSCLC cells by enhancing TRAIL-induced cytotoxicity via modulation of the STAT3, c-Jun N-terminal kinase and AKT signaling pathways (97). Preclinical research has shown that overexpression of STAT3 is correlated with chemoresistance and radioresistance in NSCLC cells (65). Further studies may clarify the role of STAT3 in gefitinib resistance in NSCLC. In addition, shikonin suppresses EGFR phosphorylation and increases EGFR proteasomal degradation in NSCLC (92). Decreasing EGFR expression inhibits its downstream signaling. Cbl is a RING E3/protein-ubiquitin ligase recruited to EGFR that mediates EGFR degradation. However, the role of Cbl in shikonin-mediated EGFR degradation has not been studied.

Tanshinone IIA. Tanshinone IIA is a diterpenoid naphthoquinone derived from the roots of *Salvia miltiorrhiza*. A recent study shows that it synergistically enhances the cytotoxic activity of geftinib in gefitinib-resistant NSCLC cells (98). Both *in vitro* and *in vivo* studies suggest that tanshinone IIA potentiates the anti-proliferative and anti-metastatic effects of geftinib in TKI-resistant cancer cells, which is attributed to the increased level of cleaved caspase 3 and the inhibition of the VEGF receptor-2/Akt signaling pathway (98).

Xanthones

Gambogenic acid. Gambogenic acid is a polyprenylated xanthone isolated from the traditional Chinese medicinal herb gamboge. Gambogenic acid induces apoptosis in NSCLC cells by inhibiting the JAK/STAT3 signaling pathways (99); it also triggers autophagy by initiating fusion between autophagosomes and lysosomes, and inhibiting acidification in lysosomes, leading to cancer cell death (100). Furthermore, in a previous study, gambogenic acid abrogated the resistance to erlotinib in NSCLC, as demonstrated in a xenograft mouse model and patient-derived xenograft model by inhibiting c-Met activity and decreasing EGFR phosphorylation (99).

Gambogenic acid also overcomes resistance to cisplatin in NSCLC. In cisplatin-resistant NSCLC cells, gambogenic acid arrests the cell cycle at the G_1 phase by decreasing the expression of cyclin D1, cyclin dependent kinase (CDK)4 and CDK6, and upregulating p53 and p21. Moreover, gambogenic acid induces apoptosis by activating caspase-3 and -7 (101).

Nucleosides

Cordycepin. *Cordyceps* species such as *C. Sinensis* are traditional medicinal fungi. The main constituent of the extract derived from this fungus is cordycepin (102,103). Cordycepin, or 3'-deoxyadenosine, is a derivative of the nucleoside adenosine, differing from the nucleoside only by the absence of the hydroxy group in the 3' position of its ribose moiety. Cordycepin was first found in fermented broth of the medicinal mushroom *Cordyceps militaris* (102). Cordycepin induces cell cycle arrest at the G_0/G_1 phase and apoptosis in lung cancer cells by inhibiting the phosphorylation of EGFR, Akt and ERK1/2 (104), and by interacting with and activating AMP-activated protein kinase (105,106). Cordycepin is also capable of inhibiting NSCLC cell cycle progression. NSCLC cells containing EGFR mutations are more sensitive to cordycepin treatment than those without EGFR mutations. More importantly, the therapeutic potency of cordycepin is comparable to afatinib and gefitinib, suggesting cordycepin itself, or in combination with gefitinib, can be a novel therapeutic for treating drug-resistant NSCLC (107).

5. Perspective

Preclinical studies show that many bioactive compounds in the family of saponins, terpenoids, polyphenols and alkaloids overcome EGFR-TKI resistance in NSCLC via different mechanisms of action, such as the induction of apoptosis, autophagy and necroptosis, increased EGFR degradation, regulation of protein kinases and signaling activities, and the inhibition of transcription factors such as STAT3 and NF-κB.

Recently, studies have revealed other potential mechanisms that may underlie the development of EGFR-TKI resistance in NSCLC. Targeting these novel mechanisms and therapeutic targets may be a novel pragmatic approach to discover new drugs that can overcome EGFR-TKI resistance. For example, ubiquitin-specific peptidase 8 (USP8) may be a novel therapeutic target in gefitinib-resistant NSCLC cells. Knockdown of USP8 selectively kills gefitinib-resistant NSCLCs. It has been reported that synthetic USP8 inhibitor markedly decreases the viability of gefitinib-resistant and gefitinib-sensitive NSCLC cells by decreasing receptor tyrosine kinase expression while having no effect on normal cells (108). Ghrelin receptor could be another therapeutic target. Ghrelin receptor inhibitor reverses gefitinib resistance in NSCLC. A previous study showed that treating gefitinib-resistant NSCLC with antagonist for ghrelin-R (D-lys-3-GHRP-6) significantly decreased the phosphorylation of AKT and ERK1/2, inhibited cell proliferation and induced apoptosis (109). Inhibiting autophagy also enhances gefitinib cytotoxicity in gefitinib-resistant NSCLC cells. Computational protein-ligand docking and virtual drug screening with the AutoDock suite or the Local Move Monte Carlo-based approach may help to provide a direct and rational drug discovery with known therapeutic targets. These approaches allow us to model the interaction between

a small molecule and a protein at the atomic level, and characterize the behavior of the small molecule in the binding site of the target proteins (110,111). Subsequent functional studies can validate the function of the binding between the target protein and the small molecules or the bioactive compounds in NSCLC cells. Furthermore, post-translational modification of EGFR may also help to overcome resistance to EGFR-TKI. Upon EGF-binding, EGFR undergoes conformational changes and dimerization, resulting in kinase activation, autophosphorylation and activation of downstream signaling molecules. Sialylation, the covalent addition of sialic acid to the terminal end of glycoproteins, suppresses EGFR dimerization and EGFR phosphorylation. In EGFR-TKI-resistant NSCLC cells harboring EGFR L858R/T790M mutations, sialylation partially suppresses the phosphorylation at Y1068, Y1086 and Y1173 of EGFR and enhances EGFR sensitivity to EGRF-TKI (112). Therefore, screening bioactive compounds that can enhance sialylation in NSCLC may also overcome the resistance to EGFR-TKI treatment.

Targeting NF-κB and cyclooxygenase-2 (COX-2) may also be an alternative approach to overcome EGFR resistance. EGFR mutations have been reported to be associated with increased COX-2 expression in NSCLC (113). Notably, there is increased downregulation of COX-2 and EGFR phosphorylation when NSCLC cells are treated with celecoxib and gefitinib compared with that when treated with either agent alone (113).

The upregulation of COX-2 in NSCLC may also be attributed to enhanced NF-κB activity. NF-κB activity is elevated in lung cancer and is considered as a therapeutic target (114). A number of studies have reported that COX-2 is a downstream target of NF-κB in various cancer types, including lung cancer (115-117). Therefore, suppressing NF-κB activity will also decrease COX2 expression. Numerous natural compounds inhibit NF-κB activity, including the triptolide isolated from *Tripterygium wilfordii (*118), *Coix lacryma-jobi* (119) and usolic acid (120). Additionally, epigallocatechin-3-gallate, genistein, luteolin, silibinin, deguelin, gallic acid, parthenolide, flavopiridol, anthocyanin, quinoclamine, dehydroxymethylepoxyquinomicin, guggulsterone, betulinic acid, emodin, gingerol, flavopiridol, zerumbone, indole-3-carbinol, elagic acid, anethole, green tea catechins, S-allyl cysteine, lycopene, genistein, quercetagenin, sasanquol, leuteolin, apigenin, wogonin and diosgenin also have NF-κB suppressive functions (121-123). Among these compounds, some of the more well-known compounds such as berberine can suppress the nuclear translocation of p50/p65 NF-κB proteins and the binding of NF-κB to the COX-2 promoter, thus decreasing COX-2 expression (124). Curcumin and other curcuminoids from the ginger family can regulate the activity of NF-κB and therefore the expression of the NF-kB regulated gene products, including COX-2, in NSCLC (125). Resveratrol has been reported to suppress TNF-α-induced phosphorylation and nuclear translocation of the p65 subunit of NF-κB and NF-κB-δependent reporter gene transcription in lung cancer (126).

6. Conclusion

EGFR-TKI resistance underlines the failure of frontline NSCLC treatments. Natural herbal resources provide a vast pool for screening compound candidates that can specifically overcome EGFR-TKI resistance in NSCLC. Subsequent preclinical and clinical studies will help to develop these natural compounds as adjunct therapies for treating EGFR-TKI-resistant NSCLC and will benefit the affected patients.

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Availability of data and materials

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Authors' contributions

HYJL, YL, TS and HYK were responsible for conceptualization. Data curation and formal analysis were completed by HYJL and MM. HYJL, TS and HYK wrote the original manuscript. TS and HYK reviewed the manuscript. All authors have read and approved the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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