Journal of Ethnopharmacology 206 (2017) 353–362

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

# *Aucklandia lappa* DC. extract enhances gefitinib efficacy in gefitinib-resistance secondary epidermal growth factor receptor mutations

Guan Huang[a,1], Yanli Tong[b,*,1], Qidi He[a], Jie Wang[a], Zuanguang Chen[a]

[a] School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China
[b] Department of Pharmacy, Guangdong Second Provincial General Hospital, Guangzhou 510317, China

ARTICLE INFO

*Keywords:*
*Aucklandia lappa* DC.
Non-small cell lung cancer
Epidermal growth factor receptor
T790M/L858R mutations
Caenorhabditis elegans

ABSTRACT

*Ethnopharmacological relevance:* *Aucklandia lappa* DC. is a widely used medicinal plant in China, India and Pakistan for a long time. Previously, a number of different pharmacological experiments *in vitro* and *in vivo* have convincingly demonstrated the abilities of it to exhibit anticancer activities. *Reynoutria japonica* Houtt. has also been widely used as traditional Chinese medicinal plant. Previous studies have demonstrated that it is bioactive to exhibit anticancer activities.

*Aim of the study:* This study aims to investigate whether the extracts of *Aucklandia lappa* DC. and *Reynoutria japonica* Houtt. are capable of treating drug-resistant non-small cell lung cancer (NSCLC), providing support for novel usage beyond traditional uses.

*Materials and methods:* Extracts combined with gefitinib have been tested taking the vulval development of transgenic *C. elegans* (*jgIs25*) as an effective and simple *in vivo* model system, evaluating their efficacy against acquired NSCLC. Synchronous larval 1 (L1) larvae were treated with extracts plus gefitinib and cultured to obtain mainly L4 larvae. The *multivulva* (Muv) phenotype was recorded at the adult stage.

*Results:* Our data showed that *Aucklandia lappa* DC. extract could significantly enhance the efficacy of gefitinib, suppressing the Muv phenotype of *jgIs25*. Meanwhile, it could also down-regulate the mRNA and protein expression of EGFR in *jgIs25*. Collectively, our results verified that the capability of *Aucklandia lappa* DC. to inhibit Muv phenotype may be based on the EGFR signaling pathway inhibition.

*Conclusion:* We demonstrated that the co-administration of *Aucklandia lappa* DC. with gefitinib may provide an effective strategy for the therapy of EGFR inhibitor resistant NSCLCs.

## 1. Introduction

*Saussurea lappa*, the synonyms of *Aucklandia lappa* DC., belongs to the Asteraceae family, and is usually utilized as an ethnologic medicine in India and Pakistan. Reestablishing vital energy, relieving pain, invigorating spleen and promoting digestion are four main traditional usages of the *Saussurea costus* (SC), root of *Saussurea lappa*. A number of different pharmacological experiments *in vitro* and *in vivo* have compellingly demonstrated the efficacy of SC, to exhibit anti-inflammatory (Cho et al., 2000; Damre et al., 2003; Gokhale et al., 2002; Jin et al., 2000; Lee et al., 1995; Lee et al., 1999), anti-ulcer and cholagogic (Venkataranganna et al., 1998; Yamahara et al., 1985), anticancer (Cho et al., 2004; Jung et al., 1998; Ko et al., 2004; Ko et al., 2005; Lee et al., 2001; Oh et al., 2004; Sun et al., 2003) and hepatoprotective activities (Chen et al., 1995), providing support to its traditional uses. Since the 1930s, *Aucklandia lappa* DC. has been cultivated in Southwest China. Its functional part, dried root, known as *Radix Aucklandiae* (RA), was called Muxiang in Chinese. It is listed in the Chinese Pharmacopoeia (part one, traditional Chinese medicines) for its extensive medicinal uses. RA has been traditionally used to treat various diseases in digestive system in China. In addition, it possesses antispasmodic (Shoji et al., 1986), anti-inflammatory (Damre et al., 2003), anticancer (Li et al., 2005a, 2005b), anti-ulcer (Taniguchi et al., 1995; Yamahara et al., 1985) and cholagogic (Mitra et al., 1996) efficacies. *Rhizoma et Radix Polygoni Cuspidati*, well known as Huzhang (HZ) in China, is the stem and root of *Reynoutria japonica* Houtt.. This herb has been used commonly as Chinese indigenous medicine for the therapy of hypertension, atherosclerosis, cough,

* Corresponding author.
*E-mail address:* tongh2008@126.com (Y. Tong).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.jep.2017.06.011
Received 9 January 2017; Received in revised form 6 June 2017; Accepted 10 June 2017
Available online 12 June 2017

gonorrhea, and suppurative dermatitis. HZ contains abundant active chemical ingredients such as stilbenes, flavones, anthraquinones and tannins. Previous studies have demonstrated that some bioactive chemical substances in HZ exhibit anti-cancer activities (Fu et al., 2014; Jayatilake et al., 1993; Lee et al., 2012; Leu et al., 2008; Park et al., 2004).

Herbal medicines are gradually becoming one of the sources of bioactive compounds for discover promising new drugs (Clardy and Walsh, 2004). A extensively studies suggested that many herbal medicines possessed many different kinds of therapeutic effects, including anticancer (Jia et al., 2013), antiaging (Li et al., 2010), anti-diabetes (Suresh et al., 2013), neuroprotective (Campos-Esparza and Torres-Ramos, 2010) and cardioprotective effects (Khan et al., 2014). Now and in the future, natural products are still major sources of innovative therapeutic agents for a variety of diseases, which could provide unlimited possibilities for new epidermal growth factor receptor (EGFR) inhibitors on account of the matchless availability of chemical diversity.

Despite recent progress in therapy, non–small cell lung cancer (NSCLC) is still the dominating cause of cancer-related death around the world. Most patients are diagnosed at the advanced stage, with an average survival of only 10–12 months even with active therapy (Scagliotti et al., 2012). Although chemotherapy assumes a large proportion of lung cancer patients, it remains little effective (Breathnach et al., 2001). The EGFR is an important part of a complex signal transduction network and is at the center of several key cellular processes. Since EGFR is usually found in NSCLC cells (Brabender et al., 2001), EGFR tyrosine kinase has become a significant therapeutic target for cancer treatment, especially for NSCLC. Targeted therapy with EGFR inhibitors remarkably benefits patients carrying activating mutations in EGFR. The first-generation EGFR inhibitors, gefitinib (ZD1839, Iressa), has been studied extensively to inhibit the tyrosine kinase activity of EGFR (Kris et al., 2003). It is effective to use tyrosine kinase inhibitors to target the EGFR gene in patients during their initial response (Lynch et al., 2004), which is limited, however, by the emergence of drug-resistance EGFR mutations and easily relapsed after a few months (Riely et al., 2006). The most common phenomenon is deletions in exon 19 or missense mutation in exon 21(L858R) (Jemal et al., 2006). Moreover, a secondary mutation (T790M) in exon 20 of the EGFR kinase domain easily leads patients acquire resistance to the first-generation EGFR inhibitors (Mulloy et al., 2007). Developing new and effective treatment for advanced disease remains a major challenge, indicating the necessity of developing more effective EGFR inhibitors.

*Caenorhabditis elegans* (*C. elegans*), a fascinating widely used multicellular animal model in the field of biological research, showed many advantages over other models, including small dimension, simplicity, convenience of propagation and maintenance, short lifespan, and cost-effectiveness. It can serve as a model organism to screen potential anti-cancer medicine. The *C. elegans* vulva, a basic structure of the nematode body, is an important instruction for cell-cell interactions in the development procedure, and its entire adult morphology is retained (Sharma-Kishore et al., 1999). The precise formation of this organ consists of a network of intercellular signaling, signal transduction and transcriptional regulation, connecting the hermaphrodite uterus and the outside of the nematode. So it can act as an outstanding studied sample of animal organogenesis. In addition, vulval development can also be used to investigate the EGFR signaling pathway for its well-established model system (Moghal and Sternberg, 2003). In 2012, Y.-K. Bae et al. developed an *in vivo* screening system that the TK domain inserted with the T790M-L858R mutations (LET-23::hEGFR-TK[T790M-L858R]). And in a wild-type *C. elegans* (N2) background, the activated mutant chimeras induced a *multivulva* (Muv) phenotype. The anti-cancer drugs gefitinib could suppress the Muv phenotype in LET-23::hEGFR-TK[L858R]-expressing transgenic animals, but could not work in LET-23::hEGFR-TK[T790M-L858R] transgenic animals (Bae et al., 2012).

The present study aimed to support the fundamental theory behind several of their traditional uses, investigate the *in vivo* anti-tumor activities of RA, HZ and SC combined with gefitinib, and determine the possible mechanisms involved.

## 2. Materials and methods

### 2.1. Chemical and reagents

Deoxyribonucleic acid (DNA) sequences were synthesized from Generay Biotechnology (Guangzhou, China). Cholesterol, Diethy pyrocarbonate (DEPC) and Total RNA Extractor (Trizol) were purchased from Sangon Biotech. Co., Ltd. (Shanghai, China). Dimethyl sulfoxide (DMSO) and gefitinib were aquired from Aladdin Chemistry Co., Ltd. (Shanghai, China). Sodium chloride (NaCl), potassium dihydrogen phosphate ($KH_2PO_4$), potassium phosphate dibasic ($K_2HPO_4$), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), potassium chloride (KCl), sodium hypochlorite (NaClO), citric acid monohydrate, manganese Chloride Tetrahydrate ($MnCl_2{\cdot}4H_2O$), copper sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), ethylene diaminetetra-acetate (EDTA), tri-potassium citrate monohydrate, ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), Zinc sulfate heptahydrate ($ZnSO_4{\cdot}7H_2O$) were purchase from Damao Chemical Reagent Co., Ltd. (Tianjing, China). Agar powder and peptone were from Sinopharm chemical reagent Co. Ltd. (Shanghai, China). Ethanol was from Manufacturer Guangdong Guangzhou Sci-Tech Co., Ltd. (Guangzhou, China). LB powder was obtained from Guangdong Huankai microbial SCI. & TECH. Co., Ltd. (Guangzhou, China). Acetonitrile, methanol and formic acid were obtained from BCR International trading Co., Ltd. (Shanghai, China). PrimeScript™ RT Reagent Kit (Perfect Real Time) and SYBR® Premix Ex Taq™ II(Tli RNase H Plus) were offered by Takara Biomedical Technology Co., Ltd. (Beijing, China). All other chemicals were of analytical grade unless otherwise required and purchased from Aladdin (Shanghai, China).

### 2.2. C. elegans strains and maintenance

Strains used in this study were N2 and *jgIs25* (LET-23::hEGFR-TK[T790M-L858R]), maintained at 20 °C on nematode growth medium (NGM) seeded with *E.coli* OP50 as described by Brenner (Brenner, 1974). N2 was achieved by the Caenorhabditis Genetics Center (CGC). *JgIs25* was a generous gift from Professor Jaegal Shim, Comparative Biomedicine Research Branch, National Cancer Center, Korea. All tests were conducted in 48-well plates with a final volume of 200 μL per well containing worms, cholesterol, drug and dead E. coli. About 50 larval 1 (L1) larvae were maintained in each well. Each test was performed in triplicate and repeated at least twice.

### 2.3. Plant materials and extract preparation

SC is the dried root of *Aucklandia lappa DC.* produced in India and Pakistan. And RA is the dried root of *Aucklandia lappa DC* produced in China. HZ comes from the stem and root of plant *Reynoutria japonica Houtt.*. Names of Aucklandia lappa DC. and *Reynoutria japonica* Houtt. have been checked as accepted names with online website www.theplantlist.org. RA and SC were bought from MedCom for Trading & Import. Co., Ltd. (Sana'a, Republic of Yemen). HZ was bought from Guangzhou Tiancheng Pharmaceutical Co., Ltd. (Guangzhou, China). They were authenticated by Professor Depo Yang (Sun Yat-sen University, Guangzhou, China). 10 g of Raw materials were decocted with 100 mL ethanol solution (ethanol: water=70 : 30) for 1 h, repeated twice. The extracts were filtered under vacuum and concentrated to dryness using a reduced pressure rotary evaporator, stored at −20 °C until use. The materials were dissolved in water to prepare a series of concentrations of the extract.