Research Article

Antitumor effect of β-elemene in non-small-cell lung cancer cells is mediated via induction of cell cycle arrest and apoptotic cell death

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Abstract. β-Elemene is a novel anticancer drug, which was extracted from the ginger plant. However, the mechanism of action of β-elemene in non-small-cell lung cancer (NSCLC) remains unknown. Here we show that β-elemene had differential inhibitory effects on cell growth between NSCLC cell lines and lung fibroblast and bronchial epithelial cell lines. In addition, β-elemene was found to arrest NSCLC cells at G2-M phase, the arrest being accompanied by decreases in the levels of cyclin B1 and phospho-Cdc2 (Thr-161) and increases in the levels of p27kip1 and phospho-Cdc2 (Tyr-15). Moreover, β-elemene reduced the expression of Cdc25C, which dephosphorylates/activates Cdc2, but enhanced the expression of the checkpoint kinase, Chk2, which phosphorylates/inactivates Cdc25C. These findings suggest that the effect of β-elemene on G2-M arrest in NSCLC cells is mediated partly by a Chk2-dependent mechanism. We also demonstrate that β-elemene triggered apoptosis in NSCLC cells. Our results clearly show that β-elemene induced caspase-3, -7 and -9 activities, decreased Bcl-2 expression, caused cytochrome c release and increased the levels of cleaved caspase-9 and poly(ADP-ribose) polymerase in NSCLC cells. These data indicate that the effect of β-elemene on lung cancer cell death may be through a mitochondrial release of the cytochrome c-mediated apoptotic pathway.

Key words. Lung cancer, NSCLC, elemene, cell cycle arrest, G2-M arrest, apoptosis.

Lung cancer remains the leading cause of mortality among men and women in the United States, with 195,000 deaths every year. This exceeds the sum of the next three leading causes of death due to breast, colon and prostate cancers. There are over one million deaths due to malignant tumors of the lung and 1.2 million new cases of lung cancer occur worldwide annually, making it an epidemic disease [1–3]. Lung cancer is classified into four major types, including squamous cell, adenocarcinoma, large-cell or undifferentiated cell, and small-cell, from a histological point of view. Non-small-cell lung cancer (NSCLC) comprises the first three types and accounts for approximately 75–85% of all cases [4, 5]. Although the best treatment for lung cancer is surgical resection, few patients with lung cancer present with surgically resectable disease and more than 75% of patients with NSCLC prove to be potential candidates for chemotherapy at some point during the course of their disease because of the development of metastases [6]. The most effective systemic chemotherapy for NSCLC is based on cisplatin combinations, and for more than two decades, platinum-based combinations have remained the standard first-line chemotherapy for advanced NSCLC [7–9]. However, chemotherapy for NSCLC seems to have reached a
plateau, and the current prognosis for most patients with this disease remains poor, with an overall survival at 5 years of only 10–15% [7; 8; 10]. Therefore, there is a need to develop compounds that can effectively treat this disease.

One candidate drug for this role is elemene. Elemene (1-methyl-1-vinyl-2,4-diisopropenyl-cyclohexane), an extract from the ginger plant *Rhizoma zedoaria*, is a novel anticancer drug. The extract of elemene is a mixture of β-, δ- and γ-elemene, with β-elemene as the main component (fig. 1), which accounts for 60–72% of the three isoforms. β-Elemene has been effective in China in the treatment of leukemia and carcinomas of the brain, breast, liver and other tissues [11–13], and is now in application for clinical studies in the United States. The major advantages of β-elemene as an anticancer drug are that (i) it has a broad-spectrum antitumor effect in many types of cancer, including drug-resistant tumors, (ii) it does not directly multidrug resistance and can reverse the resistance to other drugs and (iii) it has low toxicity and is therefore well tolerated and accepted by cancer patients [12; 13]. The mechanism of action of β-elemene in cancers remains unknown. Recent studies showed that β-elemene-inhibited cell proliferation was correlated to G2-M phase arrest in leukemia HL-60 and K562 cells [12, 13]. In addition, β-elemene was found to trigger apoptosis in glioma SHG-44 cells and leukemia K562 cells [11–13], and the apoptosis induced by β-elemene was associated with reduction of Bcl-2 protein expression. However, the mechanisms underlying G2-M arrest and apoptotic cell death triggered by β-elemene are not elucidated.

The goal of the chemotherapy of human malignancies is inhibition of cell proliferation and/or induction of cell apoptosis, and although the primary intracellular targets and the pharmacological mechanisms of action of the anticancer drugs vary, drug-induced cell killing is, at least partially, mediated by cell cycle arrest and apoptotic cell death [14, 15]. The cell cycle is regulated by cyclins, cyclin-dependent kinases (CDKs) and cyclin-dependent kinase inhibitors (CKIs) [16]. CDK activity is modulated through three distinct mechanisms: by cyclin binding, by positive and negative phosphorylation events and by interaction with CKIs [17]. CDK1 (Cdc2) and cyclin B are the two major players for control of the G2-M transition in the cell cycle. Chk2 kinase also regulates G2-M transition by phosphorylating Cdc25C [18, 19], and the later activates Cdc2 by removing phosphate from Thr-14 and Tyr-15 on Cdc2 [20]. Cellular apoptosis is mediated through two major pathways in response to different types of stimuli, including genotoxic stresses and anticancer drugs [21, 22]. One pathway is activated by the ligation of death receptors, with the subsequently cleavage of caspase-8 and activated caspase-3 by active caspase-8 [23]. The other apoptosis-mediating route, which is activated by the majority of anticancer drugs, involves the release of cytochrome c from the mitochondria, which can trigger the activation of cytosolic caspase-9 in the complex with Apaf-1 in the presence of ATP [24; 25]. Subsequently, effector caspases, such as caspase-3, are activated also, these take care of the execution phase of the apoptotic process, causing the degradation of cellular proteins and the disassembly of the cell [26]. Thus caspases play a vital role in the apoptotic process [27].

To date, little has been known about the effect and mechanism of β-elemene in human NSCLC. Therefore, the aims of the present study were to examine the antitumor effect of β-elemene in human NSCLC cells and to determine the underlying mechanisms. We investigated whether the regulation of cell cycle control and apoptosis is involved in the mechanisms for the effect and activity of β-elemene in the human lung carcinoma cells. Here we report that β-elemene inhibits the growth of human NSCLC cells and causes G2-M phase cell cycle arrest and apoptosis in H460 cells, accompanied by marked alterations in the expression of key G2-M phase and apoptosis regulatory components.

**Materials and methods**

**Chemicals and immunoreagents**

(-)-β-Elemenum (98% purity) was obtained from Yuanda Pharmaceuticals (Dalian, China). Propidium iodide (PI), RNase and glycine were purchased from Sigma-Aldrich (St. Louis, Mo.). The primary antibodies against cyclin A, cyclin B1, p27kip1, Cdc2, CDK2, Chk2, Cdc25C, caspase 8, caspase-9, Bcl-2, cytochrome c, poly(ADP-ribose) polymerase (PARP) and β-actin, and the secondary antibodies HRP-conjugated goat anti-rabbit IgG, HRP-conjugated goat anti-mouse IgG, as well as nitrocellulose, blotto, and the chemiluminescence luminal reagent were all from Santa Cruz Biotechnology (Santa Cruz, Calif.). Antibodies anti-phospho-Cdc2 (Thr-161), anti-phospho-Cdc2 (Tyr-15), and anti-phospho-CDK2 (Thr-160) were purchased from Cell Signaling Technology (Beverly, Mass.). The CellTiter 96 Aqueous ONE Solution Cell Proliferation Assay Kit was from Promega Corporation (Madison, Wis.). Cell Death Detection ELISA PLUS and TUNEL Label kits were obtained from Roche Diagnos-