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Suppression of Cell Growth, Migration and Drug Resistance by Ethanolic Extract of *Antrodia cinnamomea* in Human Lung Cancer A549 Cells and C57BL/6J Allograft Tumor Model

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Abstract: The purpose of this study was to investigate the inhibitory activities of ethanolic extracts from *Antrodia cinnamomea* (EEAC) on lung cancer. Cell proliferation and cell cycle distribution were analyzed using (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) assay and flow cytometry, respectively. Wound-healing assay, Western blotting, and a murine tumor model were separately used to examine cell migration, protein expression, and tumor repression. Our results showed that EEAC induced cell cycle arrest at the G0/G1 phase resulting decreased cell viability in A549 cells. Moreover, EEAC up-regulated the growth-suppressing proteins, adenosine 5′-monophosphate-activated protein kinase (AMPK), p21 and p27, but down-regulated the growth-promoting proteins, protein kinase B (Akt), mammalian tarfet of rapamycin (mTOR), extracellular signal-regulating kinase 1/2 (ERK1/2), retinoblastoma protein (Rb), cyclin E, and cyclin D1. EEAC also inhibited A549 cell migration and reduced expression of gelatinases. In addition, our data showed that tumor growth was suppressed after treatment with EEAC in a murine allograft tumor model. Some bioactive compounds from EEAC, such as cordycepin and zhankuic acid A, were demonstrated to reduce the protein expressions of matrix metalloproteinase (MMP)-9 and cyclin D1 in A549 cells. Furthermore, EEAC enhanced chemosensitivity of A549 to paclitaxel by reducing the protein levels of caveolin-1. Our data suggests that EEAC has the potential to be an adjuvant medicine for the treatment of lung cancer.

Keywords: *Antrodia cinnamomea*; anti-migration; anti-proliferation; paclitaxel resistance; lung cancer

1. Introduction

In general, the five-year survival rate of the patients with lung cancer is only 15% [1]. Non-small cell lung cancer (NSCLC), a major histological type of lung cancer, shows lower susceptibility to traditional chemotherapy and radiation treatments. Therefore, a more effective agent should be investigated.

For treatment of tumor metastasis, cell cycle arrest and migration inhibition are the targets of drug development. Several molecules are involved in the regulation of cell cycle and cell growth, including cyclin-dependent kinase (CDKs) and CDK inhibitors (CDKi) [2,3]. Additionally, AMP-activated protein kinase (AMPK), a cellular energy sensor, is involved in the control of tumor growth via activating p53-/p21 cascade and inhibiting the mTOR-mediated pathway [4]. Besides, Akt protein, known as an upstream regulator of mTOR, has been shown to stimulate cell growth and migration as well as to provide anti-apoptotic activity for cancer survival [5].

Moreover, matrix metalloproteinases (MMPs), are highly expressed in various types of human cancers and play a critical pathological role in tumor growth, metastasis and angiogenesis [6]. The extracellular signal-regulated kinase (ERK) associated with regulation of MMP-2 and MMP-9 proteins has been reported [7]. Furthermore, chemotherapy resistance is also associated with clinical unresponsiveness in cancer patients who receive chemotherapy drugs. Ho et al. showed that up-regulation of caveolin-1 was correlated with drug-resistant and poor progression-free survival rates in NSCLC patients [8]. Silencing of *cav-1* has been evidenced to enhance doxorubicin-induced apoptosis and reduced lung metastasis in human renal cell carcinomas [9].

Antrodia cinnamomea (*A. cinnamonea*) is a medicinal fungus that only grows inside the rotten trunk of *Cinnamomum kanehirae*, a native tree species of Taiwan [10]. *A. cinnamomea* has been explored to evaluate its effect in different cancers or use of adjuvant medicine for chemotherapy [11,12]. Our previous studies identified two main constituents, zhankuic acid A and cordycepin, in ethanolic extracts of *A. cinnamomea* (EEAC) by HPLC/Mass-fingerprint analysis [13]. The present study attempted to evaluate the mechanisms of anti-cancer activities and synergistic effects of the EEAC in A549 human lung adenocarcinoma epithelial cells and a C57BL/6J allograft tumor model.

2. Results

2.1. EEAC Induced Cell-Cycle Arrest and Reduced Cell Viability of A549 Cells

Our results showed that various doses (12.5, 25, 50, 100, and 200 μg/mL) of EEAC reduced serum-stimulated cell growth of A549 cells in a dose-dependent manner (Figure 1a), and IC_{50} value of EEAC on A549 cells after a 24 h treatment was approximately 170 μg/mL. Moreover, the results obtained from flow cytometry demonstrated that growth inhibition of EEAC may be partially mediated by cell-cycle arrest at G0/G1 phase (Figure 1b). Specifically, the proportion of cells in the G0/G1 phase increased from 56% (control group) to 66% (25 μg/mL), 68% (50 μg/mL), and 71% (100 μg/mL).

2.2. Regulation of EEAC on Cell Growth-Associated Proteins in A549 Cells

Several critical molecules involved in the regulation of cell growth were examined to understand the growth-inhibitory mechanisms of EEAC on A549 cells. Experimental data indicated that EEAC significantly increased the phosphorylation level of a growth-suppression protein, AMPKα, as well as dose-dependently inhibited activations of several growth-promoting proteins, such as Akt, mTOR, ERK1/2 and Rb. However, EEAC did not influence the total protein levels of these proteins (Figure 2a and Table 1). Furthermore, the cell cycle regulatory proteins, such as p27, p21, cyclin E, and cyclin D1, were also examined in A549 cells treated with EEAC for 24 h. The protein levels of cyclin E and cyclin D1 were reduced, while the p21 and p27 protein levels were increased in A549 cells with EEAC treatment (Figure 2b).