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Inhibition of cell-cycle progression in human colorectal carcinoma Lovo cells by andrographolide

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ABSTRACT

In recent years, attention has been focused on the anti-cancer properties of pure components, an important role in the prevention of disease. Andrographolide (Andro), the major constituent of *Andrographis paniculata* (Burm. F.) Nees plant, is implicated towards its pharmacological activity. To investigate the mechanism basis for the anti-tumor properties of Andro, Andro was used to examine its effect on cell-cycle progression in human colorectal carcinoma Lovo cells. The data from cell growth experiment showed that Andro exhibited the anti-proliferation effect on Lovo cells in a time- and dose-dependent manner. This event was accompanied the arrest of the cells at the G1–S phase by Andro at the tested concentrations of 0–30 μM. Cellular uptake of Andro and Andro was confirmed by capillary electrophoresis analysis and the intracellular accumulation of Andro (0.61 ± 0.07 μM/mg protein) was observed when treatment of Lovo cells with Andro for 12 h. In addition, an accumulation of the cells in G1 phase (15% increase for 10 μM of Andro) was observed as well as by the association with a marked decrease in the protein expression of Cyclin A, Cyclin D1, Cdk2 and Cdk4. Andro also inducted the content of Cdk inhibitor p21 and p16, and the phosphorylation of p53. Further immunoprecipitation studies found that, in response to the treatment, the formation of Cyclin D1/Cdk4 and Cyclin A/Cdk2 complexes had declined, preventing the phosphorylation of Rb and the subsequent dissociation of Rb/E2F complex. These results suggested Andro can inhibit Lovo cell growth by G1–S phase arrest, and was exerted by inducing the expression of p53, p21 and p16 that, in turn, repressed the activity of Cyclin D1/Cdk4 and/or Cyclin A/Cdk2, as well as Rb phosphorylation.

1. Introduction

Andrographis paniculata (Burm. F.) Nees (Acathaceae) is an important herbal medicine widely used in China, India and other Southeastern Asian countries. The main components of *A. paniculata* are the diterpene lactones of which andrographolide (Andro; Fig. 1A) is the major component and constitutes 70% of the plant extract fraction [1]. Andro has been reported to have multiple pharmacological properties, including anti-inflammatory [2,3], anti-allergic

Abbreviations: Andro, andrographolide; Lovo, human colorectal carcinoma; Cdk, cyclin-dependent kinase; CKI, Cdk inhibitor; Rb, retinoblastoma; DMSO, dimethyl sulfoxide; TBS, tris-buffered saline; FACS, fluorescence-activated cell sorting; RNase, ribonuclease; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ECL, enhanced chemiluminescence; GI_{50}, the molar concentration that produces 50% growth inhibition; ROS, reactive oxygen species.

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[4], anti-platelet aggregation [5], hepatoprotective [6,7] and anti-HIV activities [8,9]. And this compound has been widely used in clinic for the treatment of fever, cold, inflammation, diarrhea and other infectious diseases. Recent studies suggested that Andro is an interesting pharmacophore with anti-cancer and immunomodulatory activities and hence has the potential to be developed as a chemotherapeutic agent [10,11]. Andro can be readily isolated in high yield and has anti-tumor effects against breast cancer models [10], whose anti-cancer activity is thought to be exerted through blockage of cell-cycle progression by the induction of Cyclin-dependent kinase inhibitors (CKIs) and with a concomitant decrease in Cyclin-dependent kinase (Cdk) expression [10,12].

Multiple genetic changes taking place during the process of carcinogenesis cause the abnormalities of cells. Recent advances in cell biology have illustrated the detailed mechanisms of the cell-cycle regulatory systems and have shown that an up-regulated cellular proliferation is a common characteristic in numerous cancers [13,14]. Eukaryotic cells have developed precise and well-regulated mechanisms to control progression through the cell cycle [15]. Regulation of the vertebrate cell cycle requires the periodic formation, activation, and inactivation of unique protein kinase complexes that consist of Cyclin (regulatory) and Cdk (catalytic) subunits. The associations of Cyclin D1 and Cdk4, Cyclin E, and Cdk2, and Cyclin A and Cdk2 have also been shown to phosphorylate retinoblastoma (Rb) in the G0–G1 and the G1–S phase transitions of the cell cycle [16]. Upon phosphorylation, Rb releases and activates a number of proteins such as the E2F family of transcription factors at the G1–S transition phase [17,18], which in turn regulates the expression of several genes involved in DNA replication, such as dihydrofolate reductase, thymidine kinase, and DNA polymerase [19]. Regulation of G1 Cyclin/Cdk activity is also dependent on CKIs, which can bind and inactivate Cyclin/Cdk complexes [20,21]. Several inhibitory proteins have been identified, including p27, p16, and p21, which have been reported to mediate G1 cell-cycle arrest [22]. Another major factor believed to plays a central role in cell-cycle regulation is p53. The major downstream effectors of p53 include p21 and Cyclin D1, which participate in cell-cycle arrest. The diverse phosphorylation sites of p53 have been demonstrated to play important roles in the regulation of many cellular responses, of which the phosphorylation of p53 at serine 15 is an important target for p53 activation and stabilization. Recent reports have revealed that some compounds could reactivate the p53 function to inhibit cancer cell proliferation through cell-cycle arrest and/or apoptosis, which opens new possibilities to fight cancer [23].

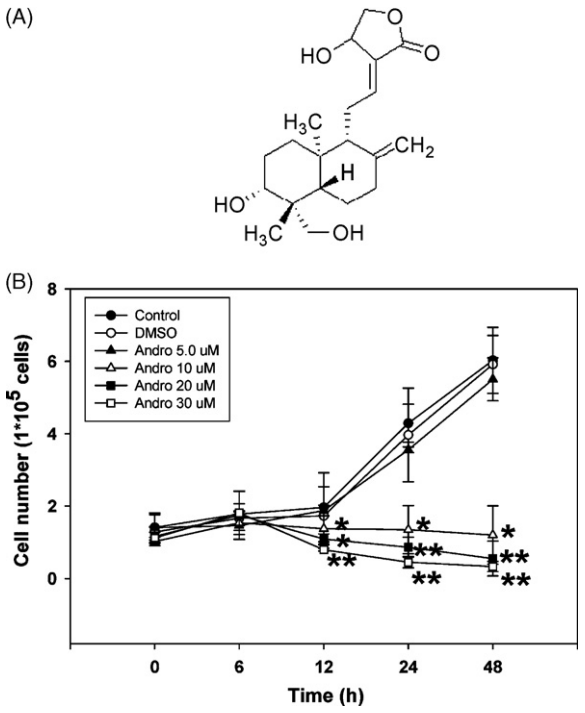

Fig. 1. (A) Chemical structure of Andro. (B) Effects of Andro on Lovo cell growth. Cultured cells were treated with various concentrations (0, 5, 10, 20 and 30 μM) of Andro for 6, 12, 24, and 48 h as described in the text. The number of cells was counted by trypan blue dye exclusion assay. The results were represented the mean ± S.D. of three independent experiments and the significant difference was established at $p < 0.05$. $^{*}p < 0.05$, $^{**}p < 0.005$ compared with the control group for the indicated time. DMSO served as the solvent control.

Cancer cells differ from normal mortal cells in that they are no longer regulated by the usual growth controlling mechanisms. Most of the anti-cancer agents currently in use are inducers of apoptosis, necrosis, cell-cycle arrest and cell differentiation; others might involve immunostimulating activity. Many traditional herbs have been reported to have these activities, but it appears likely that different pathways are involved in different types of cells [24,25] and at different concentrations. Andro, an herbal medicine exhibiting anti-inflammatory properties, was found to suppress breast cancer cell proliferation [10]. In the present study, we report evidences demonstrating that Andro inhibited human colorectal carcinoma Lovo cell-cycle progression by G1–S arrest.

2. Materials and method

2.1. Chemicals

Andrographolide (purity 98%), Tris–HCl, EDTA, SDS, phenylmethylsulfonyl fluoride, bovine serum albumin (BSA), leupeptin, nonidet p-40, deoxycholic acid, sodium orthovanadate, and aprotinin were purchased from the Sigma–Aldrich Chemical Co. (St. Louis, MO). Protein assay kits were obtained from Bio-Rad Labs. (Hercules, CA). F12 nutrient mixture, fetal-calf serum, trypsin-EDTA, and penicillin, streptomycin, and neomysin mixture (PSN) were purchased from Gibco/BRL (Gaithersburg, MD). Rb, E2F-1 monoclonal antibodies were purchased from BD-PharMingen (San Diego, CA). Cyclin A, Cyclin D1, Cyclin D3, Cdk2, Cdk4, p16, p21, p27 and p53 monoclonal and Cyclin E polyclonal antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The phospho-Ser15 p53 and phospho-Ser795 Rb rabbit polyclonal antibodies were purchased from Cell Signaling technology (Beverly, MA). Horseradish peroxidase-conjugated