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Methyl protodioscin induces G_2/M arrest and apoptosis in K562 cells with the hyperpolarization of mitochondria

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Abstract

Methyl protodioscin is a furostanol bisglycoside with antitumor properties. The present study investigated its effects on human chronic myelogenous leukemia K562 cells. Cell cycle analysis showed that methyl protodioscin caused distinct G_2/M arrest, with the appearance of polyploidy population. The levels of cyclin B1 decreased, whereas Cdc2 kept at a steady level. Subsequent apoptosis after G_2/M blockage was demonstrated through DNA fragmentation and the annexin V staining assay. Methyl protodioscin induced a biphasic alteration (i.e. an early hyperpolarization, followed by depolarization) in mitochondrial membrane potential of K562 cells. The transient decline of intracellular Ca^{2+} concentration was observed at early stage. The generation of reactive oxygen species was also detected. The anti-apoptotic Bcl-x_L transiently increased and then decreased. And the pro-apoptotic Bax was markedly up-regulated. Taken together, these data demonstrated that methyl protodioscin inhibits K562 cell proliferation via G_2/M arrest and apoptosis, with mitochondrial hyperpolarization and the disruption of Ca^{2+} homeostasis playing important roles.

Keywords: Methyl protodioscin; Apoptosis; G2/M arrest; Ca^{2+} homeostasis; Mitochondrial hyperpolarization

1. Introduction

The up-to-date rational drug development in cancer therapy appears to concentrate on the discovery of effective pharmaceutical agents that can intervene diverse signaling pathways [1,2]. The regulatory system that control normal cell proliferation and cell death stay balanced through orchestrated cascades of multiple interacting signaling routes. When this rhythmic scheme is perturbed by biochemical agents, it often results in the breakdown of cell cycle machinery, subsequently entering into apoptosis. Checkpoint molecules establish the timing and strength of arrest, repair or apoptosis responses to the damage [3]. Specifically, it has been reported that the induction of a cell cycle arrest at G_2/M could be attributed to a reduction in CDK1-associated kinase activity that determines entry into mitosis [4]. Molecules regulating cell division, such as cyclin-dependent kinases (CDKs) and inhibitors for

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CDKs, are also implicated in the regulation of apoptosis.

Apoptosis is a highly regulated process of cell suicide, in which mitochondria integrate diverse stimuli into a core intrinsic death pathway [5]. Apoptosis induced by extracellular cues and internal insults such as DNA damage is mainly dependent on mitochondria, with the perturbation of inner membrane as an early event. It may be caused by the opening of the permeability transition pore (PTP), resulting in the decline of mitochondrial membrane potential (MMP, $\Delta\Psi_m$) and the release of pro-apoptotic proteins, such as cytochrome *c* and AIF [6]. Cytochrome *c* then activates caspase cascades to disassemble the structure of the cells [7]. Bcl-2 family members appear to regulate the commitment to survive or die by controlling the integrity of mitochondrial membrane [8]. In the early stage of apoptosis, pro-apoptotic Bax translocates from cytosol to mitochondria, leading to the efflux of cytochrome *c*. Mitochondria also modulate and synchronize Ca^{2+} signaling. Their central function in Ca^{2+}-regulated cell death program was well established [9]. Disruption of intracellular Ca^{2+} homeostasis is a critical event in the initiation of apoptosis [10], leading to mitochondrial Ca^{2+} uptake and Ca^{2+} overloading, which have detrimental effects in terms of enhanced production of reactive oxygen species (ROS). The defects of Ca^{2+}-cycling may also result in the decline of MMP through the opening of the PTP, finally culminating in mitochondrial dysfunction and apoptosis [11].

Diosgenyl saponins comprise a diverse class of plant glycosides that possess a broad range of biological activities. Their sapogenins are secondary metabolites whose biosynthetic precursors are sterols, especially cholesterol. They constitute one of the major components in Chinese herbal medicine, with the various carbohydrate residues being covalently conjugated to the sapogenin backbone. Some of these glycosides have been used to treat malaria, helminthes infections, and snake bites. Others are good antifungal and antibacterial agents. Methyl protodioscin is a furostanol bisglycoside (Fig. 1). It was tested for in vitro cytotoxicity against 60 human cancer cell lines in the NCI's (National Cancer Institute, USA) anticancer drug screen and showed potent activity [12,13]. Recent study found that it has antiosteoporotic activity without side effect on the uterus [14] and it has been totally synthesized from its aglycon diosgenin [15].

Concerning this attractive kind of natural product, it is necessary to study its pharmacological mode of action at cellular and molecular levels. The present study is to investigate the antiproliferative effects of methyl protodioscin on human chronic myelogenous leukemia (CML) K562 cells. The results showed that methyl protodioscin inhibited proliferation via blocking cell cycle progression at G_2/M phase and subsequently progressing into apoptosis. The underlying events relevant to mitochondria were studied in detail, including the alteration of MMP, ROS generation and failure control of Ca^{2+} homeostasis. The involvement of Bcl-2 family members was also demonstrated.

2. Materials and methods

2.1. Cell culture

Human CML cell line K562 was kindly provided by Prof. Li-Sheng Wang (Academy of Military Medical Sciences, Beijing). Human promyelocytic leukemia NB_4 cells were presented by Prof. Zhu Chen (Rui-jin Hospital, Shanghai Second Medical University). Human colon adenocarcinoma cell line HT-29 was obtained from Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences. These cells were cultured in RPMI 1640 medium (Invitrogen Corporation, CA), with 10% fetal bovine serum (FBS) (Hyclone Laboratories Inc., UT), 100 IU/mL penicillin, and 100 μg/mL streptomycin in humidified air at 37 °C with 5% CO_2.

2.2. Materials

Methyl protodioscin, 3-*O*-[α-L-rhamnopyranosyl-(1→2)-{α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranosyl]-26-*O*-[β-D-glucopyranosyl]-22-methoxy-25(*R*)-furost-5-ene-3β,26-diol, was isolated from *Polygnotum zanlanscianse* Pamp [16]. It was dissolved in physiological saline (50 μM) and diluted with the fresh medium to achieve the desired concentration. 3-(4,5-dimethyl-thiazol-2-yl)-2,5-Diphenyl-tetrazolium bromide (MTT), 12-*O*-tetradecany-phorbol-acetate