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Antroquinonol, a natural ubiquinone derivative, induces a cross talk between apoptosis, autophagy and senescence in human pancreatic carcinoma cells☆

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Abstract

Pancreatic cancer is a malignant neoplasm of the pancreas. A mutation and constitutive activation of K-ras occurs in more than 90% of pancreatic adenocarcinomas. A successful approach for the treatment of pancreatic cancers is urgent. Antroquinonol, a ubiquinone derivative isolated from a camphor tree mushroom, *Antrodia camphorata*, induced a concentration-dependent inhibition of cell proliferation in pancreatic cancer PANC-1 and AsPC-1 cells. Flow cytometric analysis of DNA content by propidium iodide staining showed that antroquinonol induced G1 arrest of the cell cycle and a subsequent apoptosis. Antroquinonol inhibited Akt phosphorylation at Ser^{473}, the phosphorylation site critical for Akt kinase activity, and blocked the mammalian target of rapamycin (mTOR) phosphorylation at Ser^{2448}, a site dependent on mTOR activity. Several signals responsible for mTOR/p70S6K/4E-BP1 signaling cascades have also been examined to validate the pathway. Moreover, antroquinonol induced the down-regulation of several cell cycle regulators and mitochondrial antiapoptotic proteins. In contrast, the expressions of K-ras and its phosphorylation were significantly increased. The coimmunoprecipitation assay showed that the association of K-ras and Bcl-xL was dramatically augmented, which was indicative of apoptotic cell death. Antroquinonol also induced the cross talk between apoptosis, autophagic cell death and accelerated senescence, which was, at least partly, explained by the up-regulation of $p21^{Waf1/Cip1}$ and K-ras. In summary, the data suggest that antroquinonol induces anticancer activity in human pancreatic cancers through an inhibitory effect on PI3-kinase/Akt/mTOR pathways that in turn down-regulates cell cycle regulators. The translational inhibition causes G1 arrest of the cell cycle and an ultimate mitochondria-dependent apoptosis. Moreover, autophagic cell death and accelerated senescence also explain antroquinonol-mediated anticancer effect.

Keywords: Akt; mTOR; K-ras; Pancreatic cancer; Bcl-xL

1. Introduction

Pancreatic cancer is a malignant neoplasm of the pancreas. From the report of the National Cancer Institute, each year in the United States, more than 43,000 people are diagnosed with cancer of the pancreas. By the end of 2010, it is estimated that there will be about 43,140 cases and 36,800 deaths from the disease. The prognosis is poor. Without active treatment, patients with metastatic pancreatic cancer have a median survival of 3 to 5 months and 6 to 10 months for locally advanced disease [1]. Surgical operation is a major treatment of pancreatic cancer, and chemotherapy is used to gain a survival benefit and to improve the quality of life. In recent years, the molecular mechanism of pancreatic cancer has been better understood than ever before, leading to new approaches of the treatment.

The phosphatidylinositol 3-kinase (PI3-kinase)/Akt pathway is not only a major signaling for cell survival, growth, motility and metabolism, but also a resistant mechanism against a wide variety of cancer chemotherapeutic drugs [2,3]. Currently, therapeutics targeting PI3-kinase/Akt pathways are being developed against numerous types of cancers. Several lines of preclinical and early clinical evidence support this strategy [3,4]. However, the general role of PI3-kinase signaling in diverse biologic function raises concerns about its therapeutic use. Further strategies for selectivity issues are needed to diminish its impact in normal cellular function. Recently, PI3-kinase/Akt/the mammalian target of rapamycin (mTOR) signaling pathway has been highlighted since the pathway is activated in various types of cancers [5,6]. The mammalian target of rapamycin is a serine/threonine protein kinase that regulates cell growth by integrating nutrient- and growth-factor-derived signals [7,8]. Currently, it is known that mTOR exists in two function complexes, mTORC1 and mTORC2. Rapamycin and its analogs are selective for mTORC1 as anticancer agents reported in numerous preclinical and clinical studies [9]. Because of the constitutively activated PI3-kinase/mTOR pathway in cancers, selective dual PI3-kinase/mTOR inhibitors have been developed [6,10].

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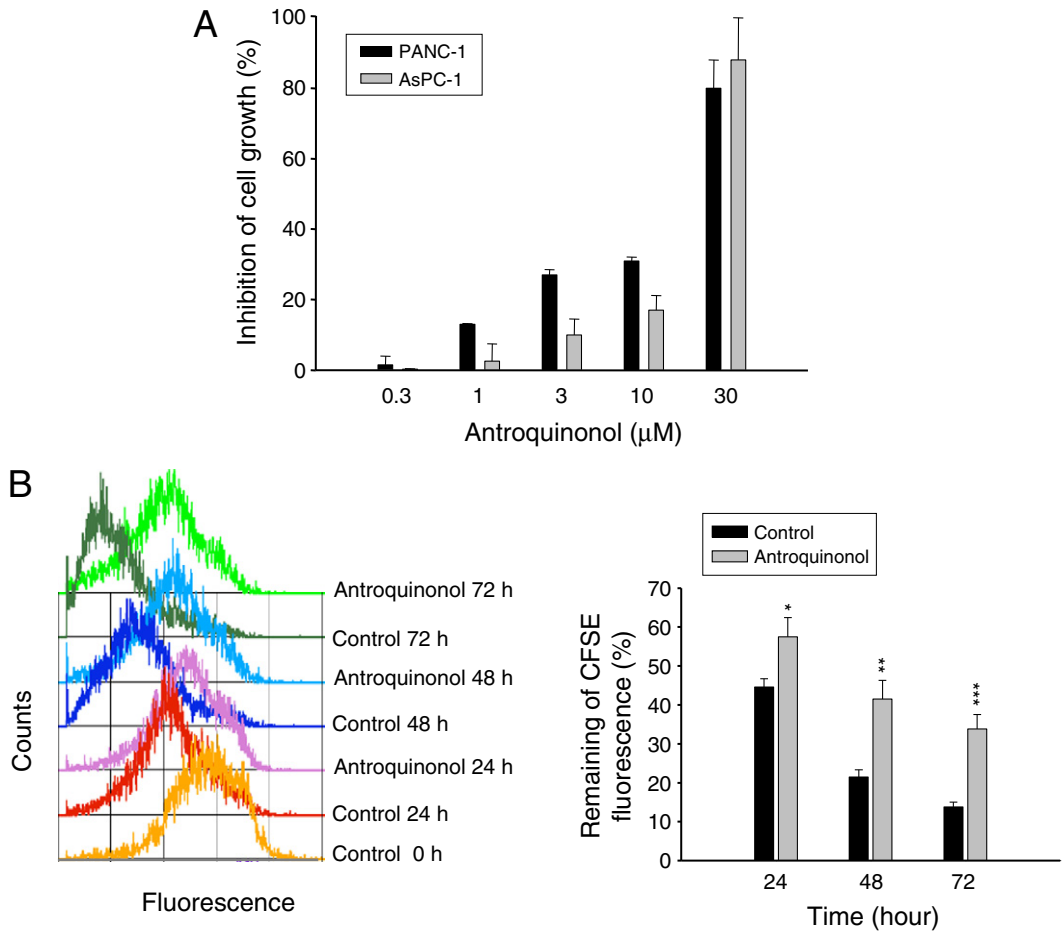

Fig. 1. Effect of antroquinonol on cell proliferation in AsPC-1 cells. (A) The graded concentrations of antroquinonol were added to cells for 48 h. The cells were fixed and stained with SRB, and the data were analyzed. Data are expressed as mean±S.E.M. of five determinations (each in triplicate). (B) Cells were labeled with CFSE and treated with vehicle (0.1% DMSO, control) or antroquinonol (30 μM) for the indicated times. The fluorescence intensity was determined by flow cytometric analysis. Data are expressed as mean±S.E.M. of three independent experiments. *P<.05, **P<.01 and ***P<.001 compared with the respective control.

Augmented activation of PI3-kinase/Akt/mTOR pathway has been reported in more than 50% of pancreatic cancers and has been associated with a poor prognosis [11,12]. To this end, dual PI3-kinase/mTOR inhibitors have been developed. Cao and colleagues reported that dual PI3-kinase/mTOR inhibitors produced significant antitumor activity in orthotopic xenografts inoculated with primary human pancreatic cancers [13]. Besides, the combination of both kinases inhibitors also demonstrated effective activity against pancreatic cancers [14]. These studies therefore suggest that targeting the PI3-kinase/mTOR pathway is a feasible strategy for pancreatic cancers that harbor activation of PI3-kinase/mTOR signaling pathway.

Antrodia camphorata, a camphor tree mushroom, is a precious traditional Chinese herbal medicine and shows pharmacological activities against several diseases. *Antrodia. camphorate* is rich in flavonoids, terpenoids, polyphenolics and polysaccharides and has been produced in agricultural manufacturing scales in Taiwan. Antroquinonol is a ubiquinone derivative isolated from *A camphorata*. Our previous study showed that antroquinonol displayed an anticancer activity against hepatocellular carcinoma (HCC) through activation of 5′adenosine-monophosphate-activated protein kinase (AMPK) and inhibition of mTOR pathway [15]. After further study, we found that antroquinonol is effective against pancreatic cancers through a distinct signaling pathway from that in HCC. It is of importance since pancreatic cancers are prone to be resistant to standard chemotherapies. Accordingly, several pharmacological and biochemical assessments have been used to delineate antroquinonol-mediated signaling cascade in pancreatic cancers.

2. Materials and methods

2.1. Materials

RPMI-1640 medium, fetal bovine serum (FBS), penicillin, streptomycin and all other tissue culture regents were obtained from GIBCO/BRL Life Technologies (Grand Island, NY, USA). Antibodies to cyclin D1, cyclin E, cyclin A, cyclin B1, Bcl-2, Bcl-xL, Bax, Bak, Mcl-1, $p21^{Waf1/Cip1}$, p53, N-ras, H-ras, K-ras and antimouse and antirabbit IgGs were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibodies to phospho-4E-$BP1^{Thr37/Thr46}$, phospho-4E-$BP1^{Thr70}$, 4E-BP1, phospho-$p70S6K^{Thr389}$, phospho-$p70S6K^{Thr421/Ser424}$, p70S6K, AMPKα, phospho-$AMPK\alpha^{Thr172}$, phospho-Akt^{Ser473}, phospho-Akt^{Thr308}, Akt, phospho-$mTOR^{Ser2448}$, m-TOR, phospho-$eIF4E^{Ser209}$, eIF4E, LC3 and α-tubulin were from Cell Signaling Technologies (Boston, MA, USA). Sulforhodamine B (SRB), propidium iodide (PI), phenylmethylsulfonyl fluoride (PMSF), leupeptin, dithiothreitol, EDTA, paclitaxel, trichloroacetic acid (TCA), Triton X-100, Rnase, sodium orthovanadate and aprotinin were obtained from Sigma-Aldrich (St. Louis, MO, USA). Carboxyfluorescein succinimidyl ester (CFSE) was purchased from Molecular Probes Inc. (Eugene, OR, USA). Antroquinonol was purified from *A. camphorate.* The purification and structure identification of antroquinonol were demonstrated elsewhere [16].

2.2. Cell lines and cell culture

Cancer cell lines including PANC-1 and AsPC-1 were from American Type Culture Collection (Rockville, MD, USA). Cells were cultured in RPMI-1640 medium with 10% FBS (vol/vol) and penicillin (100 U/ml)/streptomycin (100 μg/ml). Cultures were maintained in a humidified incubator at 37°C in 5% CO_2/95% air.