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Anticancer mechanisms of YC-1 in human lung cancer cell line, NCI-H226

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ABSTRACT

As part of a continuing search for potential anticancer drug candidates, 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1) was evaluated in the Japanese Cancer Institute's (JCI) *in vitro* disease-oriented anticancer screen. The results indicated that YC-1 showed impressive selective toxicity against the NCI-H226 cell line. Therefore, the molecular mechanism by which YC-1 affects NCI-H226 cell growth was studied. YC-1 inhibited NCI-H226 cell growth in a time- and a concentration-dependent manner. YC-1 suppressed the protein levels of cyclin D1, CDK2 and cdc25A, up-regulated p16, p21 and p53, increased the number of NCI-H226 cells in the G0/G1 phase of the cell cycle. Long exposure to YC-1 induced apoptosis by mitochondrial-dependent pathway. In addition, YC-1 inhibited MMP-2 and MMP-9 protein activities to abolish tumor cells metastasis. These findings suggest a mechanism of cytotoxic action of YC-1 and indicate that YC-1 may be a promising chemotherapy agent against lung cancer.

1. Introduction

Cancer can be regarded as a heterogeneous group of proliferative diseases, resulting from the accumulation of genetic lesions. Despite considerable advances in our understanding of the molecular mechanisms of carcinogenesis, cancer remains the first or second major cause of death due to medical causes in the United States as well as many of the developed world [1,2]. Thus there is an urgent need for new, efficacious and specific anticancer approaches. The identification of genetic alterations in cancer cells such as mutations in tumor oncogenes and tumor suppressor genes, the impaired ability of cancer cells to undergo apoptosis and the discovery of potent and specific, rationally designed drugs against molecular targets have developed into the era of targeted therapy [3].

In our previous works, we have synthesized a series of 1-arylmethyl-3-aryl-imidazole derivatives and found that

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Abbreviations: bFGF, basic fibroblast growth factor; DMSO, dimethyl sulfoxide; ERK, extracellular signal-regulated kinase; FBS, fetal bovine serum; FACS, fluorescence-activated cell sorter; JNK, c-Jun NH_2-terminal kinase; MAPKs, mitogen-activated protein kinases; MMPs, matrix metalloproteinases; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PI, propidium iodide; sGC, soluble guanylate cyclase; SRB, sulforhodamine B; VEGF, vascular endothelial growth factor; YC-1, 1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole.

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1-benzyl-3-(5-hydroxymethyl-2-furyl)indazole (YC-1) was the most promising anti-platelet agent [4,5]. Subsequent investigation of its action mechanism demonstrated that YC-1 is a unique NO-independent, and NO-enhancing, activator of soluble guanylate cyclase (sGC) [6,7]. Because sGC is associated with many physiological functions, a lot of papers with the biological functions and pharmacological action of YC-1 have been reported in the last decade. These discrete effects include anticancer activity [8]. YC-1's anticancer effects seem to result from multiple action, including cell cycle arrest and apoptosis induction [9,10], anti-angiogenesis [11], anti-inflammation [12], and inhibition of matrix metalloproteinases (MMPs) [13]. Animal studies also indicate that YC-1 suppressed tumor growth and prolonged the medium survival periods in xenograft animal models carrying various human cancers (such as non-small cell lung cancer (NSCLC) [11], hepatoma [9] and prostate cancer [14]).

In this study, YC-1's cytotoxicity was examined against the Japanese Cancer Institute (JCI) human cancer cell line panel combined with database analysis to evaluate the possible application of YC-1 to cases of cancer. YC-1 exhibited middling cytotoxicity against 39 human cancer cell lines, but was more sensitive to NCI-H226 cells. We further investigated the anticancer mechanisms of YC-1 in NCI-H226 cells. Our results indicated that cell cycle arrest followed by apoptosis and the suppression of metastasis are likely to contribute to the anticancer effects of YC-1 in NCI-H226 cells. Furthermore, the possible mediating signaling pathways involved were also evaluated.

2. Materials and methods

2.1. Materials

YC-1 was synthesized and provided by one of our colleagues (Dr. Fang-Yu Lee). The purity is more than 99.0% by the examination of HPLC and NMR. In this study, YC-1 was dissolved in dimethyl sulfoxide (DMSO) to achieve the desired concentration before each experiment. The final concentration of DMSO in the culture medium was kept below 0.1%.

2.2. Human cancer cell line panel experiment

The system was developed according to the method of the National Cancer Institute [15,16], modified by the Japanese Cancer Institute [17,18]. The cancer panel experiment for YC-1 was carried out in JCI, and the inhibition profile was compared with those of more than 200 standard compounds including various anticancer drugs. The precise methods of experiments and data analysis have been described elsewhere [18]. We briefly showed the cell lines used and the method for detecting growth inhibition. The following human cancer cell lines were used in cancer panel experiments: breast cancer HBC-4, BSY-1, HBC-5, MCF-7 and MDA-MB-231; brain cancer U251, SF-268, SF-295, SF-539, SNB-75 and SNB-78; colon cancer HCC2998, KM-12, HT-29, HCT-15 and HCT-116; lung cancer NCI-H23, NCI-H226, NCI-H522, NCI-H460, A549, DMS273 and DMS114; melanoma LOX-IMVI; ovarian cancer OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8 and SK-OV-3; renal cancer RXF-631L and ACHN; stomach cancer St-4, MKN1, MKN7, MKN28, MKN45 and MKN74; and prostate cancer DU-145 and PC-3. The cell lines were cultured in RPMI-1640 (GIBCO/BRL, NY, USA) supplemented with 5% fetal bovine serum (FBS; GIBCO/BRL), penicillin (100 units/ml) (GIBCO/BRL) and streptomycin (100 μg/ml) (GIBCO/BRL) at 37 °C in humidified air containing 5% CO_2. Dose–response curves at five different concentrations between 10^{-4} and 10^{-8} M were obtained from computer analysis. The 50% growth inhibition (GI_{50}), total growth inhibition (TGI), and 50% lethal concentration (LC_{50}) values for these cell lines were determined using the sulforhodamine B (SRB) colorimetric method. Computer processing of these values produced differential activity patterns against the cell lines (mean graphs). The mean graph was compared with those of standard compounds, including various anticancer drugs, by using COMPARE analysis.

2.3. NCI-H226 cells and cell culture

Human non-small cell lung cancer cell line NCI-H226 was purchased from the American Type Culture Collection (Manassas, VA, USA). In this study, the cells were cultured in RPMI-1640 medium (GIBCO/BRL) supplemented with 10% FBS (GIBCO/BRL), 100 unit/ml penicillin/100 μg/ml streptomycin and 1% L-glutamine (GIBCO/BRL). All cells were grown in a humidified atmosphere containing 5% CO_2 at 37 °C.

2.4. Measurement of cell growth

NCI-H226 cells were seeded at 5×10^3 cells/well into 96-well plates. After 24 h incubation to allow for cell attachment, YC-1 with serial dilutions (0.1, 0.5, 0.75, 1 and 2 μM) was added to the plates, and the plates were incubated for 24, 36 and 48 h. The cell proliferation was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After treatment, cells were washed once with PBS and incubated with 1 mg/ml MTT (Sigma, St. Louis, MO, USA) for 2 h. Then the formazan precipitate was dissolved in 150 μl dimethyl sulfoxide and the absorbance was measured on an ELISA reader at a best wavelength of 570 nm.

2.5. Cell cycle analysis

NCI-H226 cells were seeded at 2×10^5 cells/well into 12-well plates. After 36 h incubation to allow for cell attachment, cells were treated with 2 μM YC-1 for 18, 24 and 48 h. Then both detached and attached cells were harvested and fixed with 70% ice-cold ethanol at −20 °C overnight. After fixation, cells were washed with PBS and stained with 1% Triton-X 100 (Sigma), 0.1 mg/ml RNase A (Sigma) and 4 μg/ml propidium iodide (PI, Sigma) at 37 °C for 30 min in the dark. Samples of 10,000 cells were then analyzed for DNA content by flow cytometer, using a fluorescence-activated cell sorter (FACS; Becton Dickinson, San Jose, CA, USA), and cell cycle phase distributions were analyzed by ModFit software.

2.6. Cell migration assay

Migrations of NCI-H226 cells were measured by the number of cells migrating through transwell chamber (Corning, NY,