Biomedicine & Pharmacotherapy 79 (2016) 308–314

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The novel anthraquinone derivative IMP1338 induces death of human cancer cells by p53-independent S and G2/M cell cycle arrest

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ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 25 February 2016
Accepted 25 February 2016

Keywords:
Anthraquinone derivative
Selective cytotoxicity
Cell cycle arrest
Chemosensitizer
Radiosensitizer

ABSTRACT

To identify novel small molecules that induce selective cancer cell death, we screened a chemical library containing 1040 compounds in HT29 colon cancer and CCD18-Co normal colon cells, using a phenotypic cell-based viability assay system with the Cell Counting Kit-8 (CCK-8). We discovered a novel anthraquinone derivative, *N*-(4-[{(9,10-dioxo-9,10-dihydro-1-anthracenyl)sulfonyl}amino]phenyl)-*N*-methylacetamide (IMP1338), which was cytotoxic against the human colon cancer cells tested. The MTT cell viability assay showed that treatment with IMP1338 selectively inhibited HCT116, HCT116 $p53^{-/-}$, HT29, and A549 cancer cell proliferation compared to that of Beas2B normal epithelial cells. To elucidate the cellular mechanism underlying the cytotoxicity of IMP1338, we examined the effect of IMP1338 on the cell cycle distribution and death of cancer cells. IMP1338 treatment significantly arrested the cell cycle at S and G2/M phases by DNA damage and led to apoptotic cell death, which was determined using FACS analysis with Annexin V/PI double staining. Furthermore, IMP1338 increased caspase-3 cleavage in wild-type p53, p53 knockout HCT116, and HT29 cells as determined using immunoblotting. In addition, IMP1338 markedly induced the phosphorylation of histone H2AX and Chk1 in both cell lines while the combination of 5-fluorouracil (5-FU) and radiation inhibited the viability of HCT116, HCT116 $p53^{-/-}$, and HT29 cells compared to 5-FU or radiation alone. Our findings indicated that IMP1338 induced p53-independent cell death through S and G2/M phase arrest as well as DNA damage. These results provide a basis for future investigations assessing the promising anticancer properties of IMP1338.

1. Introduction

Cancer is a leading cause of death globally, and there were 8.2 million cancer-related deaths in 2012 worldwide according to the estimates of the International Agency for Research on Cancer (IARC) [1]. Although economically developed countries expect to experience a decline in cancer deaths, the estimated future global cancer death rate will probably increase due to the growth of the aging population and increased cancer risk in developing countries [1,2]. Chemotherapy is a common, effective strategy for cancer treatment. Therefore, the continued discovery and development of anticancer drugs have been intensively pursued using various approaches such as non-targeted cytotoxic and targeted therapies as well as immunotherapy.

Strategies for the discovery of anticancer drug treatments have evolved from cell-based cytotoxicity assays to target-specific screens. Recent rapid advancements in scientific technologies such as next generation sequencing (NGS) and the various omics tools provide molecular targets for targeted cancer therapies. Although a target-specific-based screening offers obvious advantages to facilitating the identification of novel anticancer agents, phenotypic screening still forms the basis of drug development [3]. Indeed, hits for lead optimization identified through target-based screens are examined to determine their phenotypes and to carefully discard compounds with nonspecific phenotypes. Furthermore, hits obtained through phenotypic screens subsequently require the determination of their active molecules or molecular

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http://dx.doi.org/10.1016/j.biopha.2016.02.034
0753-3322/

targeting mechanisms. Therefore, although both screening approaches appear to differ, drug discovery is likely to require both strategies, often in reverse order. Evasion of growth suppressors, one of the hallmarks of cancer cells as described by Hanahan and Weinberg [4], may be a potentially druggable target given that uncontrolled proliferation or dysregulation of the cell cycle is a dominant phenotype of cancer cells. Indeed, the development of anti-proliferative drugs is still a critical strategy in cancer drug discovery [5] because agents that target the proliferative cancer cell phenotype are cytotoxic or at least perturb the cell cycle.

One of the major limitations of potential anticancer compounds identified during screening is the compounds' average or low cytotoxicity. To overcome this limitation, we performed cell viability assay screening of chemical libraries consisting of 1040 small compounds using the CCD18-Co normal and HT29 cancer cell lines and identified a novel small molecule *N*-(4-[{(9,10-dioxo-9,10-dihydro-1-anthracenyl) sulfonyl}amino] phenyl)-*N*-methylacetamide (designated IMP1338) as a potential antitumor agent. IMP1338 exhibited selective killing effect with p53-independent cell cycle arrest in cancer cells.

2. Materials and methods

2.1. Cell culture and reagents

Human colon cancer HCT116 and HT29, colon epithelial CCD18-Co, NSCLC A549, and bronchial epithelial BEAS2B cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). HCT116 $p53^{-/-}$ cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD, USA). The cells were maintained at 37 °C in RPMI 1640 or MEM (CCD18-Co cells) supplemented with 10% fetal bovine serum (FBS, Gibco BRL, Grand Island, NY, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin. 5-Fluorouracil (5-FU) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

2.2. Chemical library

A chemical library containing 1040 compounds was purchased from Probiond Co., Ltd., Seoul, Korea. The chemical screening was performed as described in the cell viability assay in Section 2.3.

2.3. Cell viability assay

Cell proliferation was assessed using a Cell Counting Kit-8 (CCK-8, Dojindo, Japan) and the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (Sigma–Aldrich, St. Louis, MO, USA) according to the manufacturers' instructions. HT29 and CCD18-Co cells were plated in 96-well plates at a density of 5×10^3 cells/well in triplicate, and were subsequently treated with the library compounds or dimethyl sulfoxide (DMSO, vehicle control) for 2 h before irradiation. To determine cell viability, either CCK-8 solution (10 μL) or MTT (0.5 mg/mL) was added to each well for 3 h, followed by absorbance measurements at 450 or 540 nm,

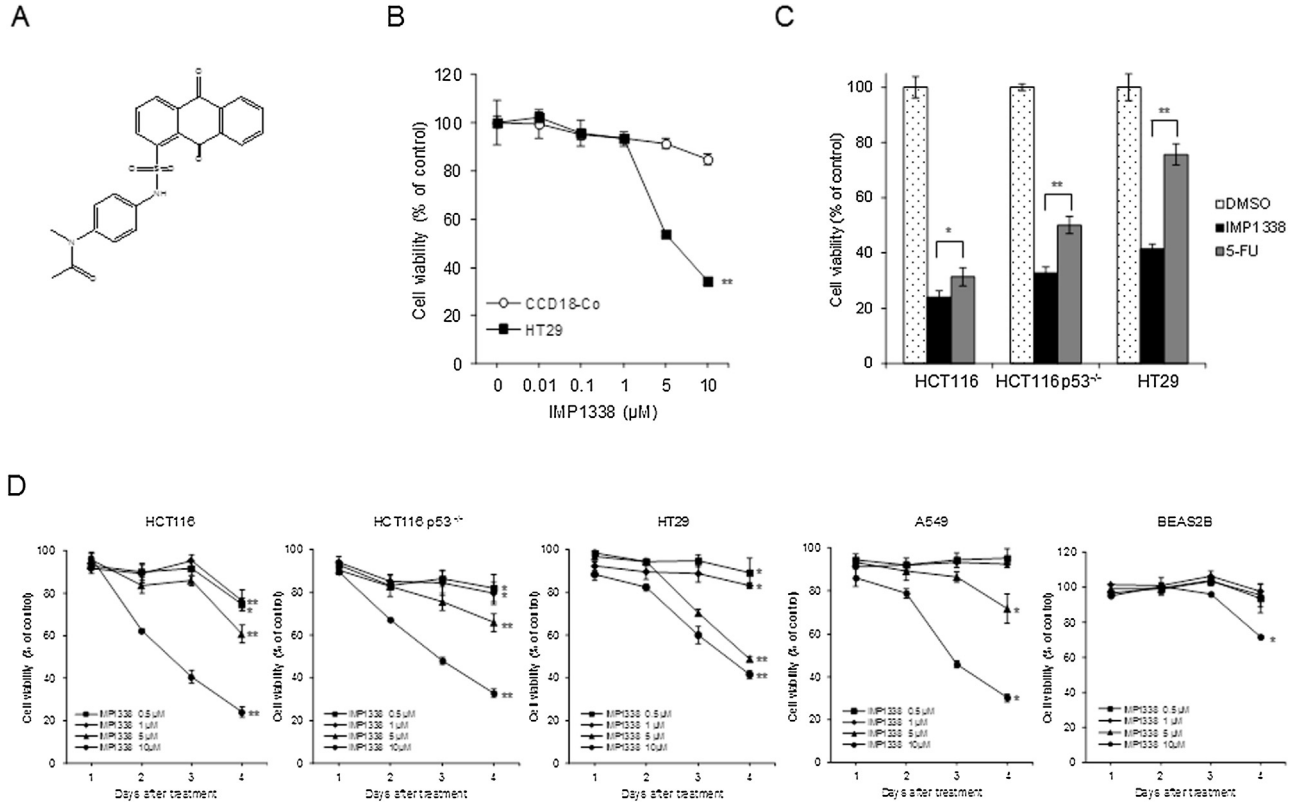

Fig. 1. The effects of IMP1338 on the proliferation of human cancer and non-tumorigenic cells. (A) Chemical structure of IMP1338. (B) HT29 and CCD18-Co cells were treated with either DMSO or 10 μM IMP1338, and cytotoxicity was determined 3 days after treatment using a CCK assay kit. (C) HCT116, HCT116 $p53^{-/-}$, and HT29 cells were treated with either 10 μM IMP1338 or 5-FU for 4 days and cytotoxicity was measured using the MTT assay. (D) Human cancer cells (HCT116, HCT116 $p53^{-/-}$, HT29, and A549 cells) and human non-tumorigenic cells (BEAS2B) were treated with IMP1338 at the indicated concentrations for 1–4 days. Cytotoxicity was measured using the MTT assay. Cell viability was normalized to that in the presence of DMSO (vehicle control), which was set as 100% viability for each cell line in all experiments. The data represent the means ± SD of three independent experiments (n = 3; $^*p < 0.05$, $^{**}p < 0.001$ *vs.* vehicle control).