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PARP-1 inhibitor sensitizes arsenic trioxide in hepatocellular carcinoma cells via abrogation of G2/M checkpoint and suppression of DNA damage repair

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ABSTRACT

Arsenic trioxide (ATO) is successfully used to treat hematological malignancies. However, the clinical application of the agent in solid tumors is largely limited by its dose-dependent toxicity which results from the high intrinsic resistance of the cancer cells. In this study, we firstly identified a series of sensitization effects of 4AN, a PARP-1 inhibitor, on human hepatocellular carcinoma cell line HepG2 to ATO treatment. We showed that treatment of HepG2 cells with 4AN promoted ATO-induced cell death in a synergistic manner. The ATO-sensitization by 4AN was associated with its effect on abrogation of ATO-induced G2/M checkpoint which impairs DNA damage repair and promotes cell apoptosis. Further analysis demonstrated that the ATO-induced G2/M checkpoint was closely related to a decrease in cyclin B1, a key G2/M mediator; whereas 4AN up-regulated the expression of cyclin B1 in ATO-treated cells, which may be at least partly responsible for its effect on abrogation of ATO-induced G2/M checkpoint. This was further supported by the result showing that down-regulation of cyclin B1 using siRNA could restore the G2/M checkpoint in cells co-treated with ATO and 4AN, thereby improving DNA damage repair and decreasing apoptosis. Our study indicates that the abrogation of G2/M checkpoint and the suppression of DNA damage repair contribute to ATO-sensitization by PARP-1 inhibitor in HepG2 cells, which provides a novel insight into the chemo-sensitization mechanism of PARP-1 inhibitor.

1. Introduction

Arsenic, a metalloid element existing ubiquitously on earth, has been used in medicine for over 2000 years. In last century, one form of inorganic arsenic, arsenic trioxide (ATO), was successfully utilized as an effective drug on the treatment of hematological malignancies, especially on acute promyelocytic leukemia (APL), with a complete remission rate up to 90% [26,43]. Since then, the anti-tumor activity of ATO has also been demonstrated in a variety of solid tumor cell lines including hepatocellular carcinoma, ovarian cancer, gastric cancer, esophageal cancer, etc. [4,45]. Numerous studies further revealed that ATO exerts its anti-cancer effects via generation of oxygen species (ROS) that induce oxidative DNA damage. The accumulation of DNA damage subsequently affects normal DNA synthesis, triggers the release of caspase-cascade, and thereby leading to cell death by apoptosis [6,26,39]. However, the curative benefit of ATO against solid tumors is largely limited in most phase II clinical trials, for the dosage of ATO required to exert detectable antitumor effect is much higher than that required in hematologic malignancies [1,25]. The high doses of ATO are prone to induce severe toxic effects such as hepatotoxicity, cardiotoxicity, nephrotoxicity, and even sudden death [13,41]. Therefore, the high intrinsic resistance of tumor cell, is primarily responsible for ATO-toxicity in solid tumor treatment. New strategies aiming at improving the sensitivity of cancer cells to ATO are of urgent significance for the development of ATO-mediated anticancer therapy.

Combination Poly (ADP ribose) polymerase (PARP)-1 inhibitors with ATO may be one of the most promising strategies in that one major mechanism of tumor resistance is the high DNA repair capacity of cancer cells, and that PARP-1 inhibitors are developed to target DNA repair in attempt to confer chemo- and/or radiosensitivity upon cancer cells [2,7,11,14]. As a DNA damage sensor,

Abbreviations: ATO, arsenic trioxide; PARP, Poly (ADP ribose) polymerase; 4AN, 4-amino-1,8-naphthalimide; HCC, hepatocellular carcinoma; ROS, reactive oxygen species; Comet assay, single cell gel electrophoresis; OTM, Olive tail moment; CCK-8, cell counting kit-8; CI, combination index.

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PARP-1 plays a key role in the repair of DNA damage including oxidized and methylated bases and DNA strand breaks [21]. When activated by DNA damage, PARP-1 binds to DNA nicks and catalyzes covalent attachment of long branched chains of Poly (ADP ribose) and then forms a scaffold that recruits and assembles necessary DNA repair proteins to keep DNA repair moving along [36]. Over-expression of PARP-1 has been identified in a range of tumor cells, i.e. in hepatocellular, lung, colorectal and breast cancer [9,27,38]. Such PARP-1 over-expression is traditionally regarded to be responsible, by directly enhancing DNA repair in cancer cells, for tumor resistance to chemo- and radio-therapeutics. However, it has become increasing clear that the role of PARP-1 in response to DNA damage far exceeds a simple repair pathway. The underlying molecular mechanisms are multifaceted and involve a wide range of cellular adaptation activities, among which the cell cycle checkpoints have aroused considerable attention due to their key roles in DNA damage response and DNA repair facilitation [22,35]. By comparison of the basal gene expression profile in PARP-1-deficient cells with their wild-type counterparts, Ogino et al. [30] found a direct relationship between PARP-1 and the components of cell cycle regulation machinery, such as E2F-A, FOXO1, c-Fos and Sp1. Later, accumulating evidence demonstrated that, besides enhancing DNA repair, the aberration of PARP-1 in tumor cells disturbs the expression of cell cycle genes, which stimulates cell proliferation thereby contributing to the development of tumor resistance [32,47]. Accordingly, suppression of PARP-1 activity with the use of inhibitors reversed such disturbance in cell cycle genes, inducing S and/or G0/G1 phase checkpoints which impedes the synthesis and replication of DNA, and thus confers a drug-sensitivity to cancer cells [7,14,19,22].

It is well known that certain cell cycle checkpoint can be triggered at different stages of cell cycle to guarantee the accuracy of DNA synthesis, replication and repair. The G0/G1 and S checkpoints prevent inappropriate DNA replication, and the G2/M checkpoint, via providing sufficient time for the cell to repair damaged DNA, plays a key role in DNA damage repair and cell survival promotion [11]. A number of studies have revealed a link between the chemo-sensitization effect of PARP inhibitor and G0-S phase arrest in a variety of cancer cells [5,46,47]. Interestingly, together with the prolonged G0-S phase, a considerable reduction in G2/M phase can also be tracked in many of these reports [17,18]. This indicates that the reduction in G2/M checkpoint may be also a common and important event in PARP-1 inhibitor-mediated cell cycle alterations [15]. Considering the necessity of G2/M phase for DNA repair and cell survival, such reduction in G2/M checkpoint may be of considerable significance in PARP-inhibitor-mediated chemo-sensitization. However, it seems that such reduction in G2/M checkpoint has always been covered up by the prolonged G0-S phase checkpoint. Recently, abrogation of G2/M checkpoint has been identified as an novel mechanism underlying the radio-sensitization effect of some new-developed drugs like CBP93872 and small survivin inhibitor YM155 in many tumor cell lines, such as esophageal squamous carcinoma cell lines Eca109 and TE13 as well as colon cancer cell lines HCT116 and HT29 [31,15]. Therefore, in the present study, we employed a solid tumor cell line, the hepatocellular carcinoma cell line HepG2, to investigate the ATO-sensitization effect of PARP-1 inhibitor, with a special emphasis on its interference with G2/M checkpoint. 4-amino-1,8-naphthalimide (4AN), a classical PARP-1 inhibitor, was utilized to detect the ATO-sensitization effect of PARP-1 inhibitor. Based on our previous study and other's showing that ATO-induced DNA damage could trigger a G2/M checkpoint in many cancer cells including HepG2 cells [23] and that the reduction in G2/M checkpoint is expected to be a potential mechanism underlying PARP-inhibitor-mediated chemo-sensitization, we hypothesized that the G2/M checkpoint induced by ATO may be a protective mechanism which provides sufficient time for DNA repair thus conferring an ATO-resistance upon HepG2 cells; while PARP-1 inhibitor 4AN could abrogate this G2/M checkpoint thereby suppressing ATO damage repair and leading to ATO-sensitivity. Since PARP-1 has been well documented as a mediator of cell cycle via regulation of a number of genes involved in cell cycle control [30], we further hypothesized that the effect of PARP-1 inhibitor on ATO-induced G2/M checkpoint may be fulfilled by its regulation of the core factors of G2/M phase such as cyclin B1. To test these hypotheses, a series of experiments were designed and performed in this study.

2. Materials and methods

2.1. Cell culture and reagents

The human liver hepatocellular carcinoma cell line HepG2 was purchased from the Type Culture Collection of the Chinese Academy of Science, Wuhan, China. Cells were routinely cultured in high glucose Dulbecco's Modified Eagle Medium (DMEM) (Invitrogen, Grand Island, NY, USA) with 10% (v/v) fetal bovine serum (FBS), penicillin (100 unit/ml) and streptomycin (100 μg/ml) at 37 °C in a 5% CO_2 incubator. PARP-1 inhibitor 4-amino-1,8-naphthalimide (4AN) was from Alexis Co., CA, USA and was dissolved in dimethylsulfoxide (DMSO). Therefore, cells treated with DMSO were served as control in some indicated experiments in our study.

2.2. Detection of cell viability by the CCK-8 assay

The cell viability was detected by using a cell counting kit-8 (CCK-8) assay. In brief, cells were seeded in 96-well plates at a density of 1×10^4 cells per well overnight. Then cells were treated with 4AN (range from 0 to 400 μM), ATO (range from 0 to 200 μM), or ATO (range from 0 to 200 μM) with 4AN (10 μM) for 24 h. After treatment, 10 μl of CCK-8 solution (KeyGen Co., Nanjing, China) was added to each well. Absorbance was read at 450 nm after 4 h incubation by using a Bio-Rad micro-plate reader (Bio-Rad, Hercules, CA, USA). Cell viability was calculated using the formula: Cell viability (%) = [(A450 of test group-A450 of blank group)]/[(A450 of control group-A450 of blank group)] × 100, while 50% inhibitory concentration (IC_{50}) was obtained by probit analysis.

2.3. Analysis of drug combination effect by calculation of CI value

The combination index (CI) was utilized to evaluate whether the two drugs have a synergistic, antagonistic or additive effect, according to the previous report [34]. The data obtained from CCK-8 assay was used to calculate CI value. CI is calculated as follows: $CI = DA/IC_{50}A + DB/IC_{50}B$. Here, $IC_{50}A$ or $IC_{50}B$, represents the 50% inhibitory concentration of single agent. DA, or DB, represents the dose of single agent when the combined inhibition effect is equivalent to 50%. A CI value less than, equal to, or greater than 1 indicates the two drugs are synergistic, additive, or antagonistic, accordingly.

2.4. Analysis of drug combination effect by isobologram

The isobologram was utilized to further evaluate the combination effect of ATO and 4AN, according to the previous report [48]. The data obtained from CCK-8 assay was used to plot isobologram. Briefly, the concentrations of the two individual drugs are plotted along the two axes in a Cartesian coordinate system. *Y*-axis represents 4AN alone and *X*-axis indicates ATO alone. The two diagonal points (0, *Y*1) and (*X*1, 0) represents the IC_{50} of 4AN alone