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Enhancement of non-homologous end joining DNA repair capacity confers cancer cells resistance to the novel selenophene compound, D-501036

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ABSTRACT

D-501036 is a promising anti-cancer compound that exhibits potent anti-proliferative activity against various types of human cancers through the induction of double strand DNA breaks. To determine drug resistance mechanism related to this class of DNA-damaging agents, a KB-derived D-501036-resistant cell line (S4) was established. Results showed that S4 cells exhibit enhanced DNA rejoining ability as compare to KB cells, through up-regulation of the non-homologous end joining activity. In conclusion, enhancement of NHEJ activity plays important role in the development of D-501036-resistance and targeting NHEJ-related molecules maybe able to overcome drug resistance to DNA damaging agents.

1. Introduction

D-501036 is a selenophene derivative of natural products extracted from *Echinops grijsii* has been demonstrated to have promising effect in animal model and further pre-clinical investigation is ongoing. The mechanisms of action of D-501036 include the generation of reactive oxygen species (ROS) and the formation of Se–DNA adducts in cancer cells, which results in DNA damage, arrests cells in the S phase of cell cycle, and further induces apoptosis [1]. D-501036-induced DNA damage also activates ataxia telangiectasia-mutated (ATM) nuclear protein kinase signaling pathway, including phosphorylation of its downstream components, Chk1 and Chk2.

Living organisms encounter various situations caused DNA damage through entire lifetime. Besides endogenous errors introduced during DNA replication, chemical carcinogens, ionize radiation, and ROS also introduce DNA lesions that interfere with DNA replication and gene transcription in cells [2]. In order to maintain the genetic integrity and the related physiological functions, mammalian cells can activate multiple DNA repair systems in response to various types of DNA damage [3,4]. However, cells experienced severe DNA damages that beyond repair can activate apoptosis and subsequently be removed from the tissue. Base on this fact, various natural or synthetic DNA damage agents such as cisplatin and oxaliplatin were widely used in cancer therapies. In addition, various novel DNA damage agents are currently undergoing different pre-clinical and clinical investigations.

The development of drug resistance cancer cells is a major problem in patients with prolonged chemotherapeutic treatments [5]. It has widely demonstrated that

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increased DNA repair ability confers cancer cells ability to evade the cytotoxicity induced by DNA damage agents. For example, activation of nucleotide excision repair (NER) was showed able to correct the cisplatin-induced lesion by removing DNA fragments that were linked with platinum adducts [6]. In addition, the level of base excision repair (BER) activity and resistance to the ROS damage induced by DNA damage agents were showed positively correlated in mouse cell line [7]. Direct reversion of the damaged bases by O^6-methylguanine-methyltransferase helps the repair of DNA damage produced by alkylating agents [8]. However, severe DNA damage can induce double strand DNA breaks (DSBs) that cannot be repaired simply by nucleotide replacement. DSBs are primarily repaired by homologous recombination (HR) and non-homologous end joining (NHEJ) in mammalian cells [9]. Due to template requirement, HR operates more effectively during S and G2 phase of cell cycle, whereas NHEJ activity keeps constant during entire cell cycle. Recruitment of the Mre11/Rad50/NBS1 (MRN) complex in response to DSB activates these two pathways with distinct repair mechanisms [10,11]. HR machinery is a group of protein composed with Rad51, Rad52, Rad54, Rad55, Rad57, Rdh54, Mre11 and Nbs1. 5′ end strand resection, strand invasion, and D-loop formation combine broken strand and another intact DNA molecule to perform an error-free repair [12]. HR enhancement has been demonstrated to induce cisplatin resistance in fusion tyrosine kinase (FTK) positive leukemia, and Rad51, the central factor in HR were up-regulated by FTK [13]. In contrast to HR, NHEJ simply links the broken end of two DNA strands, which is more an efficient error-prone repair mechanism. There are two main complexes involved in the NHEJ. When MRN complex stabilizes DSB ends, Ku70 and Ku80 recruit the DNA protein kinase catalytic subunit (DNA-PKcs) to form as a first detection complex. DNA-PKcs then bridges two ends of the DSB while Ku70/Ku80 protecting the DSB ends. The second complex is the NHEJ ligation complex, including the executors of ligation, Ligase IV and XRCC4. Although the mechanisms of action of D-501036 in cancer cells have been elucidated, the machinery(s) of DNA repair involved in response to D-501036 induced DNA damage remains unknown. To investigate the repair mechanism that is responsible to the causation of resistance to DNA damage agent, a D-501036-resistant cell line, S4, has been established in this study. Furthermore, we described the role of NHEJ plays inducing drug resistance.

2. Materials and methods

2.1. Reagents

D-501036 [2,5-bis(5-hydroxymethyl-2-selenienyl)-3-hydroxymethyl-N-methylpyrrole] was synthesized at the Department of Medicinal Chemistry, Development Center for Biotechnology, Taipei, Taiwan, Republic of China. Oxaliplatin was kindly provided by Sanofi (New York, NY, USA). Paclitaxel, etoposide (VP16), camptothecin (CPT), vincristine, and monoclonal antibody against α-tubulin were purchased from Sigma–aldrich (St. Louis, MO). Monoclonal antibody against phosphor-ATM (serine-1981), polyclonal antibodies against phosphor-Chk2 (threonine-68) and phosphor-Chk1 (Serine-345) were purchased from Cell Signaling Technology (Beverly, MA). Polycolonal antibodies against ataxia telangiectasia mutated- and Rad3-related kinase (ATR) were purchased from Novus Biologicals (Littleton, CO). Polycolonal antibodies against phosphor–minichromosome maintenance 2 (MCM2) (Serine-108) were purchased from GeneTex (Irvine, CA). Monoclonal antibodies against Ku80, DNA-PKcs, XRCC4, and DNA Ligase IV were purchased from BD bioscience (Franklin lakes, NJ). Monoclonal antibodies against α-actinin, and horseradish peroxidase-conjugated secondary antibody were purchased from Santa Cruz biotechnology (Santa Cruz, CA). Acrylamide/bis-acrylamide solution, ammonium persulfate, and N,N,N,N-tetramethylethylene diamine were purchased from Bio-Rad (Hercules, CA). Dichlorofluorescein diacetate was purchased form Molecular Probes (Eugene, OR). Western blot chemiluminescence reagent was purchased from Perkin–Elmer Life Sciences (Boston, MA).

2.2. Establishment of the D-501036 resistant cell line

Human cervical carcinoma KB cells were obtained from American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI-1640 medium, supplemented with 5% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 U/mL), and glutamate at 37 °C. D-501036 resistant KB-1036-S4 (S4) cells were established from KB cells by exposure to stepwise increasing concentrations of D-501036. Cells that grew in the present of 500 nM of D-501036 were sub-cloned by dilution plating in 96-well plates. Individual clones were isolated. For maintenance, cells were cultured under similar conditions to those used for KB, except with an addition of D-501036 (500 nM). Drug resistant cells were cultured in drug free medium 3 days prior to the treatment.

2.3. Growth inhibition assay

Cells were then seeded in 24-well plates and cultured overnight. Cells were incubated with various concentrations of test drug for three generation times. The methylene blue dye assay was performed to evaluate the growth inhibition ability of drugs in cells [14]. Inhibition concentration of 50% (IC_{50}) values was obtained by plot of relative survival rate versus drug concentrations.

2.4. Pulsed-field gel electrophoresis

KB and S4 cells were treated with various concentrations of D-501036 for 24 h. Cells were suspended in PBS and mixed with 1% low melting agarose, and the concentration was adjusted to 1×10^6 cells in 80 μL of agarose mixture. The mixture was loaded into casting plug and cells in jellified sample plugs were lysed in 0.5 mL of lysis buffer (10 mM Tris HCl pH8.0, 10 mM NaCl, 25 mM EDTA, 0.1% sodium lauryl sacrosine, and 1 mg/mL proteinase K) at 50 °C for 24 h. Sample plugs were then washed three times with wash buffer (10 mM Tris HCl pH8.0, 50 mM