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WRN translocation from nucleolus to nucleoplasm is regulated by SIRT1 and required for DNA repair and the development of chemoresistance

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ABSTRACT

When defective or absent, Werner syndrome protein (WRN) causes a genetic premature aging disorder called Werner syndrome. Several studies have reported that defects in WRN function are responsible for not only progeria syndrome but also genomic instability via the deregulation of DNA repair, replication, recombination, and telomere stability. Given the importance of WRN in the repair process, we herein investigated the potential role of WRN in drug response by evaluating the DNA repair following exposure to cisplatin in human cancer cell lines. We found that the down-regulation of SIRT1 and inhibition of SIRT1 deacetylase activity blocked the translocation of WRN from the nucleolus to the nucleoplasm in response to genotoxic stresses. In addition, cells expressing low levels of WRN responded favorably to cisplatin, whereas cells expressing high levels responded poorly to cisplatin. The forced expression of WRN protein in chemosensitive cells resulted in an approximately two-fold increase in cell viability in response to cisplatin compared with vector controls and promoted DNA repair, while WRN-deficient cells accumulate unrepaired double-strand breaks following cisplatin exposure. These results suggest that WRN is regulated by SIRT1 and increased expression of WRN might be one of the determinants for the development of chemotherapeutic drug resistance.

1. Introduction

Werner syndrome protein (WRN) is one of the five members of the human RecQ helicase family [1–4]. Defective proteins produced by *WRN* gene mutation cause a rare autosomal recessive genetic disorder, Werner syndrome (WS), characterized by the onset of premature aging and multiple age-related diseases [5]. WRN helicase, which also possesses exonuclease activity [6–8], plays important roles in DNA metabolism, including DNA replication, recombination, repair, and transcription, and in maintaining genomic stability via telomere maintenance [9,10].

A loss of WRN function deregulates DNA metabolism, resulting in detrimental consequences for various cellular processes. Fibroblasts derived from WS patients show a prolonged S-phase, a reduced life span, increased chromosomal aberrations [11] and an elevated sensitivity to DNA-damaging agents [12,13]. Similarly, WRN-deficiencies in fibroblasts, transformed human fibroblast, U-2OS, and lymphoblastoid cell lines (LCLs) result in more sensitivity to cisplatin (CDDP), camptothecin (CPT), 4-nitroquinoline-*N*-oxide (4NQC), hydroxyurea (HU), and some DNA cross-linking agents than wild type cells [14–18]. In addition, the transcriptional silencing of WRN via the hypermethylation of 5′CpG islands leads to hypersensitivity to chromosomal damage and apoptosis upon exposure to mitomycin C and CPT [19].

WRN collaborates with various proteins involved in the DNA repair process, supporting the idea that WRN plays a role in DNA repair. These proteins include the Ku complex, the Mre11-Rad50-NBS1 complex, PARP-1, RAD52, and p53 [10,20]. In response to DNA damage, key proteins required for the repair process are recruited to the site of the DNA lesion, which often requires the subcellular redistribution of proteins across localization sites [21]. Recent studies reported that the shuttling between cellular localizations and the catalytic activity of WRN is regulated by CBP/p300-mediated acetylation [22] and SIRT1-mediated deacetylation [23],

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although the exact consequences of acetylation/deacetylation remain unclear. SIRT1 (silent mating type information regulation 2 homolog1) belongs to the class III histone deacetylase family. SIRT1 has been implicated in diverse cellular processes, and it may play a key role in cell survival under oxidative and genotoxic stresses [24]. The upregulation of the SIRT1 deacetylase is involved in the development of drug resistance in neuroblast and prostate cancers [25,26], but the underlying mechanisms remain to be solved. These previous findings suggest that the aberrant regulation and expression of WRN might contribute to the onset of drug resistance to chemotherapeutic agents by promoting the repair process. Although much is known about the role of WRN in DNA repair, little is known about the contribution of WRN to the development of drug resistance. In this study, we investigated the potential involvement of WRN in drug resistance and the possible mechanism underlying WRN-mediated drug resistance. We found that the forced expression of WRN promoted DNA repair and resulted in an increase in resistance to cisplatin. Moreover, we confirmed that WRN interacts with the human sirtuin1 (SIRT1) protein and that SIRT1 is required for DNA damage-induced WRN translocation from the nucleoli to the nucleoplasm. These results suggest that controlling the level of WRN expression and WRN translocation are important in drug sensitivity, and we propose that WRN is a potential therapeutic target in human cancer.

2. Materials and methods

2.1. Cell lines, cell culture, and treatments

Human hepatocellular carcinoma cells (SNU-368, SNU-423, SNU-449, and SNU-475) and gastric cancer cell lines (SNU-601, SNU-668, and SNU-638) were obtained from the Korean Cell Line Bank (Seoul, Korea) and grown in RPMI 1640 medium with 10% fetal bovine serum (FBS). HepG2 and HeLa cells were purchased from ATCC® (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS. Cisplatin (*cis*-diammine-dichloroplatinum (II); CDDP) and sirtinol were purchased from Sigma–Aldrich (St. Louis, MO, USA). The cells were plated on culture dishes at a density of 10,000 cells per cm^2 and allowed to attach for 16–24 h in a normal culture medium before cisplatin or sirtinol treatment. After exposure to cisplatin or sirtinol, the cells were washed with serum free medium and grown in fresh medium.

2.2. Cytotoxicity assay

The cytotoxicity was determined by using the Cell Counting Kit-8 according to the manufacturer's instructions (Dojindo Laboratories, Kumamoto, Japan). The cells were plated at 5000 cells per well in a 96-well plate and allowed to attach for 16–24 h in normal culture medium. After exposure to different concentrations of cisplatin for 6 h, the cells were washed with serum free medium and cultured in fresh culture medium for 72 h in a 37 °C incubator. The cytotoxicity in cisplatin-treated cells was indicated as the percentage of cell survival in relation to the control cells. The experiments were performed in duplicate, with each concentration tested in triplicate.

2.3. Transfection and constructs

The cells were transfected with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). The WRN-GFP construct was a kind gift from Dr. Vilhelm A. Bohr, National Institute of Health. WRN-specific shRNA constructs were purchased from Sigma–Aldrich (Mission shRNA). SIRT1-specific siRNAs were purchased from Santa Cruz (USA). For the SIRT1 down-regulation experiment, the cells were transfected with siRNA to SIRT1 using Lipofectamine RNAiMAX reagent (Invitrogen, USA) following the manufacturer's instructions with a final siRNA oligonucleotide concentration of 25 pmol.

2.4. Comet assay

The single-cell gel electrophoresis assay (Comet assay) was performed under alkaline conditions. The cells (1×10^4) were mixed with LMP agarose and overlaid on top of an agarose-coated slide. The slides were immersed into a pre-chilled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris base, 1% Triton X-100, and 10% DMSO) and incubated for 60 min. The slides were placed into an alkaline solution (300 mM NaOH and 1 mM EDTA, pH 12.1) for 20 min and voltage was subsequently applied for 30 min. The slides were stained with SYBR® Gold nucleic acid gel stain (Invitrogen, Carlsbad, CA, USA) and analyzed by fluorescence microscopy. DNA damage was evaluated by the tail moment as the percentage of DNA in the tail × tail length using the Cometscore software (TriTek Corp.).

2.5. Immunofluorescence

The cells were seeded onto duplicated sterile coverslips in a 6-well tissue culture plate at a density of 10,000 cells per cm^2. The cells were treated with 5 μM cisplatin for 6 h and then washed and supplied with fresh medium for an additional 24 h. The cells were fixed in 3% paraformaldehyde or in 1:1 methanol:acetone. The fixed cells were blocked with IF buffer containing 3% bovine serum albumin. The samples were then incubated for 3 h at 37 °C in a humidified chamber with primary antibodies in a dilution of 1:500. Polyclonal antibody against WRN (200 μg/ml, Santa Cruz, USA) and polyclonal antibody against SIRT1 (200 μg/ml, Santa Cruz, USA) were used. After intensive washing in IF buffer, Alexa Fluor dye-coupled secondary antibodies (2 m/ml, Invitrogen, USA) in a 1:2000 dilution were incubated for 1 h at room temperature. The nuclei were stained with 0.5 μg/mL DAPI (4′,6-diamidino-2-phenylindole). The cells were rinsed with PBS and mounted using Vectashield (Vector Laboratories, USA). Cells were examined using a Carl Zeiss fluorescence microscope equipped with epifluorescence filters. At least 100–200 cells were analyzed in each sample in two to three independent experiments.

2.6. Immunoprecipitation, western blot analysis, and antibodies

The cells were lysed in IP buffer (50 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% NP-40, and 0.25% Na-deoxycholate) containing protease inhibitor cocktail [Calbiochem® (0.5 mM pefa-bloc, 150 nM aprotinin, 1 μM E-64 protease inhibitor, and 1 μM leupeptin)], phosphatase inhibitors (10 mM sodium orthovanadate (Na_3VO_4) and 10 mM sodium fluoride (NaF). The lysates were incubated with 2 μg of primary antibody and subsequently incubated with 50 μl of protein A/G agarose beads (Upstate, Lake Placid, NY, USA) for 3 h at 4 °C. The beads were washed with washing buffer (50 mM Tris, 150 mM NaCl, 1 mM EDTA, and 0.1% NP-40; pH 7.5), and the immunoprecipitates were boiled in SDS-sample buffer and analyzed using western blot analysis.

Western blot analysis was performed using polyclonal antibodies against WRN (Santa Cruz, Santa Cruz, CA, USA), α-tubulin (BD Transduction Laboratories, San Jose, CA, USA), γ-H2AX (Millipore, Temecula, CA, USA), and SIRT1 (Cell signaling Technology, Inc., Danvers, MA, USA).