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Deletion of NAD(P)H:quinone oxidoreductase 1 represses Mre11-Rad50-Nbs1 complex protein expression in cisplatin-induced nephrotoxicity

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HIGHLIGHTS

- MRN complex proteins and NQO1 were induced by cisplatin nephrotoxicity.
- Cisplatin-induced MRN complex protein expression decreased in NQO1-knockdown cells.
- Severe renal damage by cisplatin was followed by reduced MRN complex in NQO1$^{-/-}$ mice.
- Cisplatin-injected NQO1$^{-/-}$ mice showed disrupted DNA damage repair-related proteins.
- Reduced MRN complex might be related to the aggravated renal damage in NQO1$^{-/-}$ mice.

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ABSTRACT

The Mre11, Rad50, and Nbs1 (MRN) complex is a DNA double-strand break sensor involved in DNA damage repair. Herein, we explored whether deletion of NAD(P)H:quinone oxidoreductase 1 (NQO1), a cytoprotective gene, affected MRN complex expression in the kidney after cisplatin-induced acute kidney injury (AKI). *In vitro*, cisplatin increased the expression of MRN complex proteins and NQO1 in NQO1-expressing ACHN cells in a time- and concentration-dependent manner. The expression of MRN complex proteins was relatively inhibited in NQO1-knockdown cells. *In vivo*, increased expression of renal MRN complex proteins was accompanied by upregulation of γ-H2A histone member X, a DNA damage marker, in cisplatin-treated wild-type mice. Although the NQO1-knockout (NQO1$^{-/-}$) mice showed more severe cisplatin-induced renal damage, the renal expression of MRN complex proteins was lower than in NQO1-expressing mice; expression of poly[ADP-ribose] polymerase 1, which promotes MRN complex accumulation, was also lower in these animals. In addition, cisplatin-induced expression of DNA damage repair-related proteins, ataxia telangiectasia mutated and sirtuin1, markedly decreased in the NQO1$^{-/-}$ group, relative to the NQO1-expressing mice. These findings suggest that NQO1 deletion might be associated with decreased MRN complex expression, which might be partially responsible for the exacerbation of cisplatin-induced AKI in the absence of NQO1.

1. Introduction

The kidneys play a crucial role in homeostasis by repetitive reabsorption and filtration of the blood, activities that render them prone to cytotoxic drug exposure. Cisplatin (cis-diamminedichloroplatinum II) is a potent platinum-containing anticancer agent; however, its dose-related nephrotoxicity limits long-term therapy. About one-fifth of patients receiving high-dose cisplatin develop severe renal injury (Yao et al., 2007). In kidney cells, the platinum

Abbreviations: AKI, acute kidney injury; ATM, ataxia telangiectasia mutated; DSBs, double-strand breaks; MRN, Mre11, Rad50, and Nbs1; NQO1, NAD(P)H:quinone oxidoreductase 1; NAD, nicotinamide adenine dinucleotide; NQO1, NAD(P)H:quinone oxidoreductase 1; PARP1, poly[ADP-ribose] polymerase 1; ROS, reactive oxygen species; Sirt1, sirtuin1.

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ion of cisplatin binds to DNA and forms cisplatin-DNA adducts, which induce DNA damage (Fuertes et al., 2003).

DNA damage can include either DNA base modification or DNA strand breaks; double-stranded breaks (DSBs) are one of the most detrimental DNA lesions arising from exposure to genotoxic chemicals (Ánchez-Pérez, 2006). The recognition of DNA DSBs initiates a damage response that activates DNA repair protein kinases and cell cycle regulatory proteins. The Mre11, Rad50, and Nbs1 (MRN) multi-protein complex is known to assemble at the site of DNA damage and participate in the damage repair process (Dinkelmann et al., 2009; Zha et al., 2009). Mre11, which has binding sites for Rad50 and Nbs1, is the first component of the complex. It has both endo- and exo-nuclease activities, allowing it to participate in intrinsic DNA binding and DNA end resection activity (de Jager et al., 2001). Rad50 is the second component, and contains globular and coiled-coil domains that bind with Mre11. The last component of the MRN complex, Nbs1, is responsible for the translocation of the complex to the nucleus; Nbs1 is known to interact with ataxia telangiectasia mutated (ATM) kinase (Hopfner et al., 2001; Williams et al., 2007). Expression of the MRN complex was previously reported in the kidney in association with hypertonicity induced DNA damage (Sheen et al., 2006).

NAD(P)H:quinone oxidoreductase 1 (NQO1) is a well-characterized antioxidant enzyme that catalyzes the reduction of quinone using NAD(P)H as the hydride donor (Dinkova-Kostova and Talalay, 2010). NQO1 exerts its direct antioxidant effects by scavenging superoxide, although to a lesser extent than superoxide dismutase (Siegel et al., 2004). In addition, the reduced endogenous quinones produced by NQO1 have been reported to participate in cell membrane protection against oxidative damage (Ross et al., 2000). In addition to its antioxidant properties, NQO1 is also known to have an antiproliferative effect in various cancer cell lines (Iskander et al., 2008; Zhang and Go, 2009). NQO1, along with other detoxifying enzyme genes, can be induced in response to external stimuli such as xenobiotics, electrophiles, and radiation (Venugopal and Jaiswal, 1998). A recent study reported that NQO1 was induced to counter cisplatin nephrotoxicity; NQO1 deletion increased the levels of reactive oxygen species (ROS) and oxidative stress, which aggravated tissue damage and led to further loss of renal function (Gang et al., 2013). During cisplatin nephrotoxicity, cisplatin-induced DNA damage triggers ROS production, leading to irreversible death via an apoptotic pathway (Huang et al., 2003). In a previous study, cisplatin treatment was found to induce the expression of various DNA damage repair-related proteins, including the MRN complex proteins (Kim et al., 2015). In this context, the present study aimed to explore whether the absence of NQO1 affected the expression of the MRN complex in the kidney during cisplatin-induced acute kidney injury (AKI).

2. Materials and methods

2.1. Cisplatin treatment in ACHN cells

ACHN cells (ATCC 1611), a human kidney adenocarcinoma cell line, were maintained as a monolayer culture in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 mg/ml) in humidified air with 5% CO_2 at 37 °C. The cells were seeded at a density of 1.5×10^5 cells/ml in 6-well plates with regular growth medium. The following day, the cells were treated with cisplatin (0, 5, 10, or 20 μM) and harvested after 3 and 6 h for western blot. For cell viability assays, ACHN cells were seeded in 96-well plates (1×10^4 cells/ml). MTT and crystal violet assays were performed the next day. The EZ-Cytox cell viability assay kit (Daeil, Seoul, Korea) was used for the MTT assay. The crystal violet assay was carried out based on a previous study (Clément et al., 1998).

2.2. Cisplatin treatment in mice

Male C57BL/6 N mice (7- to 8-week-old; Samtako, Korea) were housed under a 12-h light/dark cycle at 23 ± 2 °C in a pathogen-free facility at Chungnam National University, with free access to a standard diet and water. After 1 week of acclimation, the mice (n = 5/group) were euthanized by carbon dioxide 12 and 72 h after cisplatin treatment (20 mg/kg, IP). Serum was separated by centrifuging at 800 g for 15 min and subjected to serum creatinine (CRE) and blood urea nitrogen (BUN) analysis using a Fuji Dry-Chem analyzer (Fujifilm, Tokyo, Japan). The kidneys were removed and subjected to western blot. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Chungnam National University and performed in accordance with the Guidelines for Care and Use of Laboratory Animals (NIH publication, 8th edition, 2011).

2.3. Cisplatin treatment in ACHN cells after NQO1 silencing

Small interfering RNA (siRNA) was used to knockdown NQO1 expression in ACHN cells. The cells were seeded in 6-well plates (1.5×10^5 cells/ml) and incubated with 40 nM siNQO1 (5′-AAACCAGCCUUUCAGAAUGGCUGGC-3′; Invitrogen, San Diego, CA, USA) together with lipofectamine RNAi max (Life Technologies, Carlsbad, CA, USA). After 32 h of si-scrambled (control) or siNQO1 treatment, the cells were incubated with cisplatin (20 μM) for 3, 6, 9, and 18 h.

2.4. NQO1-knockout animal study

Male C57BL/6N-knockout mice (7- to 8-week-old) were kindly donated by Dr. C. H. Lee (Korea Research Institute of Bioscience and Biotechnology, Korea) and non-transgenic, age-matched male mice (Samtako, Korea) were used as controls. All mice were housed in a pathogen-free facility at Chungnam National University, in the same conditions mentioned above. After acclimation, mice were assigned to 4 groups (n = 5/group); $NQO1^{+/+}$ (wild-type, control), $NQO1^{-/-}$ (NQO1 knockout), $NQO1^{+/+}$ + cis (wild-type, treated with cisplatin), and $NQO1^{-/-}$ + cis (NQO1 knockout, treated with cisplatin). Three days after an intraperitoneal injection of cisplatin (20 mg/kg), all mice were sacrificed by cardiac puncture under carbon dioxide anesthesia. Their serum was subjected to BUN and CRE analysis. One kidney was quickly removed for histopathological and immunohistochemical (IHC) studies, while the other was removed and stored at −70 °C prior to the western blot assay. The animal experiments were approved by the Institutional Animal Care and Use Committee of Chungnam National University.

2.5. Western blot assay

The appropriate cells and tissues were homogenized and lysed in lysis buffer (20 mM Tris–HCl pH 8, 150 mM NaCl, and protease inhibitor cocktail). After protein assay using bovine serum albumin, 30 μg protein was separated using 10–12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins were transferred onto a nitrocellulose membrane using a wet transfer system (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% (w/v) dry milk in 1 × Tris buffered saline with 20% Tween-20 and incubated with antibodies against NQO1 (1:1000, Santa Cruz, CA, USA), Mre11 (1:500, Abcam, MA, USA), Rad50 (1:500, Abcam), Nbs1 (1:1000, Abcam), sirtuin1 (Sirt1; 1:3000, Abcam), ATM (1:2000, Abcam), γ-H2A histone member X (H2AX;