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Protective role of the endoplasmic reticulum protein mitsugumin23 against ultraviolet C-induced cell death

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ABSTRACT

The endoplasmic reticulum (ER) operates in adaptive responses to various stresses, dictating cell fate. Here we show that knockdown of the ER protein mitsugumin23 (MG23) enhances cell death induced by ultraviolet C (UVC), which causes DNA damage. The small heat shock protein αB-crystallin (αBC) is identified as a MG23 binding molecule and its knockdown facilitates death of UVC-exposed cells. Conversely, αBC lowered UVC sensitivity when expressed as an ER-anchored form. Taken together, the results suggest that MG23 plays a protective role against UVC by accumulating αBC in the close vicinity of the ER.

Structured summary of protein interaction:
MG23 physically interacts with **αBC** by anti tag coimmunoprecipitation (View interaction)
MG23 physically interacts with **αBC** by two hybrid (View interaction)

1. Introduction

The ER is a multifunctional organelle. It is involved in the synthesis and processing of protein, lipid synthesis, detoxification of drugs and regulation of Ca^{2+} mobilization as an intracellular storage [1,2]. Recently, considerable attention has been given to roles for the ER in adaptive stress responses collectively called the UPR [3–6]. The UPR is provoked under various pathophysiological circumstances, including hypoxia, hypoglycemia and viral infection. In these contexts, misfolded proteins are accumulated in the ER, followed by signal propagation from this organelle. Although little is known about their molecular nature, signals emanating from the ER elicit diametrically opposite cellular phenomena, namely, survival and death.

Recently, we have demonstrated using whole-body γ irradiation of mice that MG23, a three-transmembrane domain protein localized predominantly to the ER and the nuclear membrane [7,8], is involved in a response to DNA DSBs [9]. The finding prompted us to postulate that MG23 operates in response to other types of DNA lesion, thereby enabling the ER to govern fate determination of DNA-damaged cells. To explore this hypothesis, we investigated if knockdown of MG23 in HEK293T (hereafter referred to as 293T) cells would affect their vulnerability to UVC, which causes DNA lesions such as cyclobutane pyrimidine dimers and 6,4-photoproducts [10–13]. Here, we have revealed that MG23 would be protective against UVC and propose that the ER functions as a signaling platform supporting the DDR as well as the UPR.

2. Materials and methods

2.1. Expression constructs and siRNAs

To generate expression constructs, DNA fragments encoding murine MG23 and human αBC were PCR-generated and cloned in frame into the pcDNA4/myc-His (Invitrogen) or pEGFP-N1 vector (TAKARA Bio, Shiga, Japan). To knock down MG23, two different siRNA duplexes (MG23si and MG23siAlt) as below were individually transfected into 293T cells: MG23si (5′-GCAUGUGUUCAAAGCCAUUTT-3′ and 5′-AAUGGCUUUGAACACAUGCTT-3′) and MG23 siAlt (5′-GACCCAGAUAGGUCGAUCUTT-3′ and 5′-AGAUCGACCUAUCUGGGUCTT-3′). The siRNA duplex against αBC (αBCsi) was comprised of the following siRNAs: (5′-CCAGGGAGUUCCACAGGAATT-3′ and 5′-UUCCUUGGUCCAUUCACAGTT-3′).

Abbreviations: 7-AAD, 7-aminoactinomycin D; DDR, DNA damage response; DSBs, double-strand breaks; EGFP, enhanced green fluorescent protein; Etop, etoposide; HEK293T, human embryonic kidney 293T; Iv, insertless vector; MEFs, murine embryonic fibroblasts; RT-PCR, reverse transcription polymerase chain reaction; siRNA, small interfering RNA; Tg, thapsigargin; Tu, tunicamycin; UPR, unfolded protein response

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2.2. Transfection and pharmacological treatment

293T cells were grown in DMEM (WAKO, Tokyo, Japan) supplemented with 10% fetal calf serum at 37 °C in a 5% CO_2 humidified incubator. The cells were plated 22 h prior to transfection in 24-well plates at 5×10^4 cells per well. The expression constructs and siRNA duplexes were transfected into the cells using X-tremeGENE siRNA transfection reagent (Roche). At 24 h posttransfection, Etop, Tg, and Tu (all from WAKO) were added to the culture medium. The cells were incubated for a further 40 h and then subjected to flow cytometric analysis.

2.3. UVC exposure

At 22 h posttransfection, 293T cells were irradiated with UVC (254 nm) at 15 or 50 J/m^2 using a UV crosslinker CX-2000 (UVP). The cells were incubated for a further 24 h and then subjected to flow cytometric analysis.

2.4. Immunoprecipitation and immunoblotting

Immunoprecipitation and immunoblotting experiments were performed as described previously [14]. The following antibodies were used: actin (C-2) and myc (9E10) from Santa Cruz Biotech, αBC (SPA-223) from Stressgen, and GFP from MBL (Nagoya, Japan). Anti-MG23 antiserum was produced by injecting mice with a key limpet hemocyanin-conjugated peptide corresponding to amino acid residues 186–243 of human MG23.

2.5. Flow cytometry

Flow cytometric analysis was performed as described previously [14].

2.6. Reverse transcription polymerase chain reaction analysis

RT-PCR was performed as described previously [15]. To examine transcript levels of the indicated genes, the following primers were used: 5′-GCATTGCTGACAGGATGCAG-3′ and 5′-CCTGCTTGCTGATCCACATC-3′ for β-actin, 5′-GCTCGAGCACCATG GCAGGCGCAC-3′ and 5′-CGAATTCGCTCTTCTTCCATGCTCC-3′ for MG23, and 5′-CGGCGGCCGCTATGGGGTGGAATATC-3′ and 5′-CGG TCGACTTTCTTGGGGGCTGCG-3′ for αBC.

2.7. Yeast two-hybrid screening

The Matchmaker GAL4 Two-hybrid System 3 (TAKARA Bio.) was used according to the manufacturer's manual. The C-terminal region of murine MG23, corresponding to amino acid residues 201–243, was employed as a bait to survey a cDNA library derived from human skeletal muscle.

2.8. Statistics

Statistical significance in Fig. 1 was evaluated using Wilcoxon rank sum test. For non-parametric all-pairs multiple comparisons in Figs. 2–4, Steel–Dwass test was used.

3. Results

3.1. MG23 knockdown enhanced UVC-induced cell death

We previously demonstrated that thymocytes in MG23-knockout mice are more resistant to γ irradiation compared with their wild-type counterparts [9]. The finding prompted us to hypothesize that MG23 might operate in response to any DNA lesion other than that caused by γ ray. To explore this possibility, we examined whether MG23 knockdown would impinge on a cellular damage response to UVC. MG23 was depleted comparably by two siRNA duplexes (MG23si and MG23siAlt) individually transfected into 293T cells (Suppl. Fig. 1A and B). The cells were then subjected to flow cytometric analysis using Annexin V and 7-AAD, which evaluate phosphatidylserine exposure and membrane permeability, respectively. Early (Annexin V$^+$/7-AAD$^-$) and late (Annexin V$^+$/7-AAD$^+$) apoptotic cells were discriminated (Suppl. Fig. 1C) and Annexin V positivity was quantified according to the flow cytometric results. Twenty-four hours after UVC irradiation, MG23 knockdown cells were ~15% more Annexin V-positive compared with

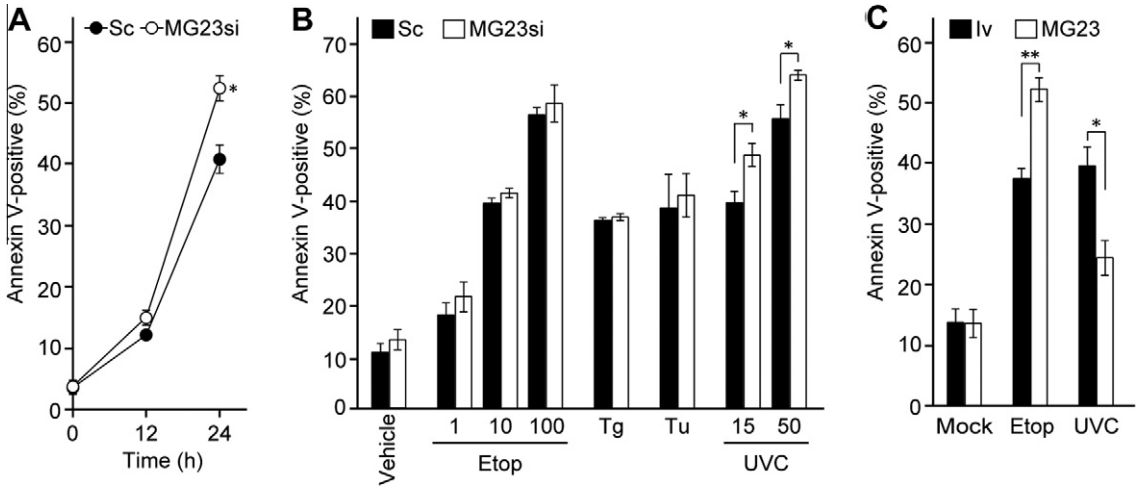

Fig. 1. MG23 knockdown enhanced UVC-induced cell death. (A) 293T cells transfected with either siRNA against MG23 (MG23si) or a scrambled control siRNA (Sc) were exposed to UVC (15 J/m^2) and then analyzed by flow cytometry at the indicated time points after the irradiation. Annexin V positivity indicates the total percentage of two subpopulations (Annexin V$^+$/7-AAD$^-$) and Annexin V$^+$/7-AAD$^+$). The result represents mean ± S.E. of seven separate experiments. Wilcoxon rank sum test was used in (A–C) to test the difference between paired values. Asterisk: $P < 0.01$. (B) Transfected 293T cells were UVC irradiated (15 or 50 J/m^2) or treated with the following agents: Vehicle, 0.1% dimethylsulfoxide; Etop, 1–100 μM; Tg, 2 μM; Tu, 10 μg/ml. Flow cytometric analysis was performed at 24 and 40 h after UVC irradiation and the addition of agents, respectively. The result represents mean ± S.E. of four separate experiments. Asterisk: $P < 0.01$. (C) 293T cells were transfected with either an insertless vector (Iv) or the MG23 expression plasmid. Flow cytometric analysis was performed at 24 and 40 h after UVC irradiation and the addition of Etop (10 μM), respectively. The result represents mean ± S.E. of six separate experiments. $^*P < 0.05$, $^{**}P < 0.01$.