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Human rpS3 is involved in DNA repair and cell cycle control

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In the cellular response to DNA damaging agents, cells undergo cell cycle arrest or apoptosis against irrepairable DNA damage. RpS3 is known to function as UV DNA repair endonuclease III and ribosomal protein S3. In this study, we used normal and rpS3-overexpressed 293T cells to examine the role of rpS3 in response to DNA damaging agents. When 293T cells transfected with rpS3 were irradiated with UV, the pattern of cell cycle was dramatically changed in comparison with un-transfected 293T cells. We also found that the expression of rpS3 in normal cells was increased by treatment with DNA damaging agents. By means of Western and Northern blot analyses in rat tissues, we showed the expression pattern of rpS3 protein and its mRNA. These data suggest that DNA repair and cell cycle arrest are inter-related to each other through rpS3, and the increased expression of rpS3 seems to regulate the cell cycle arrest by DNA damaging agents.

key words: Cell cycle, rpS3, UV, repair, DNA damage

INTRODUCTION

When mammalian cells are damaged by intracellular or extracelluar genotoxic stimuli, several defense mechanisms are triggered to protect organisms. Cells with DNA damage either repair the DNA damage or commit suicide. Initially, mammalian cells are arrested in G_1, S or G_2/M phase of the cell cycle, depending on the phase in which the damage is sensed, meanwhile the cells repair their damaged DNA. However, if DNA damage is too severe to repair, cells initiate the apoptotic machinery to avoid cancer developments [1,2,3].

An anthracycline antibiotic, Doxorubicin is widely used for the treatment of cancer [4]. Doxorubicin damages DNA by intercalation of the anthracycline portion or by generation of free radicals. Doxorubicin has also been shown to inhibit DNA topoisomerase II which is critical to DNA function [5,6].

UV endonuclease III, 27 kDa protein, is an enzyme to repair the DNA damage by UV irradiation [7,8,9,10]. This enzyme cleaves UV irradiated DNA and AP (apyrimidin/apurinie) DNA, by cleaving the phosphodiester bond within a cyclobutane pyrimidine dimer and 3' of AP site via a lyase mechanism [7,10,11,12]. Interestingly, it was also reported that this enzyme serves as rpS3 and is known to be involved in the initiation of translation, as well as the cross-links between rpS3 and eukaryotic initiation factors, eIF-2 [13] and eIF-3 [14,15]. In addition, yeast RPS3 protein showing 78% amino acid similarity with human rpS3 was indicated to have an endonuclease activity on AP DNA [9]. Cells from XPD (Xeroderma pigmentosum group D) patients who have high incidence of skin cancers show abnormal expression and altered AP endonuclease I activity of this enzyme [7,16]. Since the column profile of the endonuclease activity of rpS3 appears to differ in XPD cells compared to normal cells [7], there is a probability that rpS3 is connected with xeroderma pigmentosum disease. RpS3 proteins are also conserved at several motifs: the nuclear localization signal-KKRK, the KH motif-RNA binding motif, and the S3-C domain are well conserved among the eukaryotes.

Recently, by using yeast two hybrid system and biochemical analysis we showed that rpS3 is directly associated with apoptosis and that rpS3 binds directly to several proteins which are important for decision of either cell death or survival. Mammalian cells respond to DNA damage signals by activating cell cycle checkpoints that arrest cell cycle, and activating DNA repair system, or inducing apoptosis [1,2,17]. It is well known that there must be a checkpoint regulator for DNA repair or apoptosis during the DNA damage response [18,19,20]. To elucidate the role of rpS3 in the decision of DNA repair on cell cycle arrest, we tested the change of rpS3 cell cycle and expression patterns by FACS and Western blotting respectively.

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MATERIALS AND METHODS

Plasmids

A full length of human rpS3 gene was cloned in-frame with a sequence coding for green fluorescent protein (pEGFP-C1, CLONTECH, Palo Alto, CA).

Materials

Lipofectamine reagent was obtained from GIBCO-BRL. PI (propidium iodide) was obtained from CALBIOCHEM. Monoclonal antibody to Actin was obtained from Santa-Cruz Biotechnology Inc., and rabbit polyclonal anti-rpS3 antibody was raised against a polypeptide corresponding to C-terminal 146 residues of rpS3.

Cell culture and Transfections

Human embryonic kidney epithelial 293T cells and skin fibroblast F-65 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat inactivated fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. These cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Transfections were performed with Superfect (Quiagen Co.) as instructed by the manufacturer.

Cell cycle analysis

Transfectants were grown in 60 mm dishes with 75 J/m^2 of UVC irradiation and incubated at various intervals. Transfectants were harvested using 0.25% trypsin, washed in PBS and suspended in 70% ethanol at 4°C overnight. The cells were then treated with propidium iodide solution (50 μg/ml) containing RNase A (100 μg/ml) for 30 min at 37°C in the dark. Green GFP fluorescence was collected after a 530/30-nm bandpass filter. In addition to FL2 (orange fluorescence) area, PI fluorescence was collected using linear amplification. The PI fluorescence of each cell was analyzed with a FACScan flow cytometer (Beckton Dickinson)

Western blots

F-65 skin fibroblast cells were harvested in resuspension buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 2 mM PMSF, 1 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin A) and lysed by sonication. Supernatants were collected by centrifugation at 12,000×g for 10 min at 4°C and boiled in SDS/PAGE sample buffer. The lysates were separated by SDS/PAGE, transferred to PVDF membranes, probed with antibodies as indicated, and illuminated with the enhanced chemiluminescence (ECL) system.

RESULTS AND DISCUSSION

To determine whether the expression of rpS3 is induced by genotoxic agents or not, several cell lines were treated with doxorubicin and UV. Interestingly, the expression of rpS3 is induced by doxorubicin only in normal skin fibroblast F-65 cells. The level of rpS3 protein was increased slightly at 30 min after addition of 1.2 μg/ml doxorubicin, and sustained until 4-5hr (Figure 1).

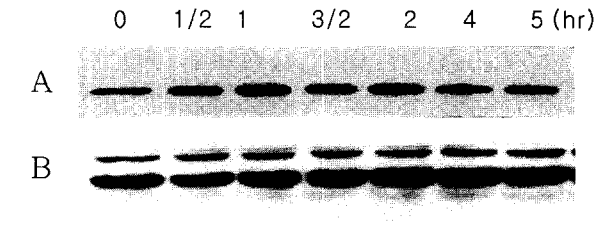

Figure 1. Time courses of rpS3 protein expression after treatment of F-65 cells with doxorubicin. The cells were lysed at the indicated time points and analyzed by immunoblotting with specific antibodies against rpS3 (A) and actin (B).

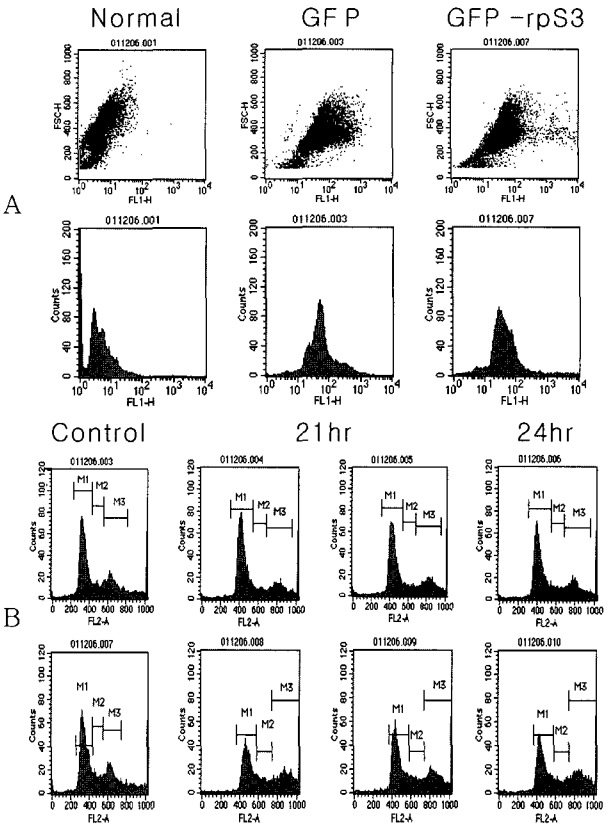

Figure 2. UV-induced G2/M arrest in ectopic expression of rpS3. A) Untransfected and GFP- or GFP-rpS3-transfected 293T cells are analyzed by FL1. B) After irradiation with UVC 75 J/m^2, GFP (up) or GFP-S3 (down) transfected 293T cells were incubated for indicated time duration. The cells were stained with PI.
GFP: Green fluorescent protein
M1: $G_{0/1}$ phase, M2: S phase, M3: G_2/m phase

UV-induced DNA damage results in either cell cycle arrest to facilitate DNA repair, or elimination of cells with irreparable DNA damage. Previous data showed that rpS3 is an enzyme to repair the DNA damage by UV irradiation [1].

Thus, to examine the roles of rpS3 in UV-induced cell cycle

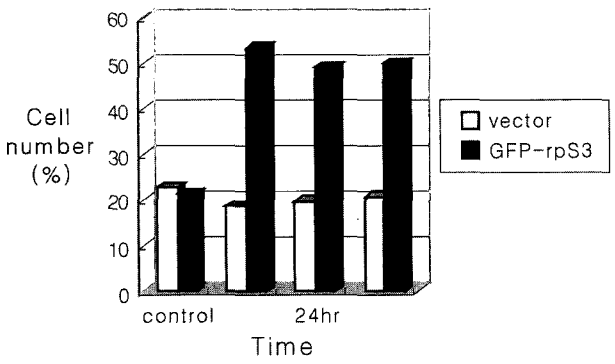

Figure 3. G2/m arrest via rpS3 under UV-irradiation. After irradiation with UVC 75 J/m^2, GFP- and GFP-S3-transfected 293T cells were incubated for indicated time duration. The cells were stained with PI. The results were normalized to the percentage of G2/M cells that were induced by rpS3 under UV-irradiation.

arrest, we made rpS3-transfected 293T cells. And to exclude non-transfected cells, we have gated in high GFP-positive cells in contrast with histogram of un-transfected cells (Figure 2 A). Following transient transfections with GFP-encoded plasmids, the percentage of cells expressing GFP ranged between 23% and 40% (data not shown). To investigate the effect of rpS3 expression in cells treated with DNA damaging agents, the transfected cells were irradiated with 75 J/m^2 of UVC, and harvested at desired times.

The cell cycle profiles of UV-irradiated GFP expressed cells were compared to each other by the representative experiment in Figure 2. The percentage of rpS3-transfected population in G1 phase was less than what was observed for the vector-transfected population, whereas the percentage of cells in G2/M phase was higher in the rpS3-transfected population than the vector-transfected population (about 2.5 fold) (Figure 2 B, Figure 3). The increase was sustained until 27 hours.

These data suggest that rpS3 in UV-irradiated cells has repaired the specific DNA damages, and the pool of unrepaired DNA damage has yielded the signal, which might activate cell cycle checkpoint. Here we showed that UV-induced G_2/M arrest of 293T cells is increased by the ectopic expression of rpS3. And we have found that the expression of rpS3 in normal skin fibroblasts exposed to doxorubicin was increased, but UV-irradiated 293T cells did not show the increased expression of rpS3. However, when 293T cells transfected with rpS3 were irradiated by UV, the pattern of cell cycle was dramatically altered comparing to untransfected 293T cells.

Therefore, it can be hypothesized that endonuclease III/rpS3 is closely related with cell cycle in DNA damaged cells. To confirm the hypothesis, the expression pattern of cell cycle regulators will be studied.

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