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Advances in Brief

Activation of the Transcription Factor Oct-1 in Response to DNA Damage

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Abstract

Mammalian cells exhibit complex cellular responses to genotoxic stress, including cell cycle checkpoint, DNA repair, and apoptosis. Inactivation of these important biological events will result in genomic instability and cell transformation. It has been demonstrated that gene activation is a critical initial step during the cellular response to DNA damage. A number of investigations have shown that transcription factors are involved in the regulation of stress-inducible genes. These transcription factors include p53, c-Myc, and AP-1 (c-fos and c-jun). However, the role for the octamer-binding transcription factor Oct-1 in the DNA damage-activated response is unknown. In this report, we have presented the novel observation that the transcription factor Oct-1 is induced after cells are exposed to multiple DNA-damaging agents and therapeutic agents, including UV radiation, methylmethane sulfonate, ionizing radiation, etoposide, cisplatin, and camptothecin. The induction of the Oct-1 protein is mediated through a posttranscriptional mechanism and does not require the normal cellular function of the tumor suppressor p53, indicating that the Oct-1 protein, as a transcription factor, may play a role in p53-independent gene activation. In addition to increased protein level, the activity of Oct-1 DNA binding to its specific consensus sequence is also enhanced by DNA damage. Therefore, these results have implicated that the transcription factor Oct-1 might participate in cellular response to DNA damage, particularly in p53-independent gene activation.

Introduction

It has been well accepted that exposure to DNA damage contributes to the development of many human tumors. Therefore, much effort has been focused on understanding how cells respond to DNA damage and maintain both genomic integrity and chromatin structure. Several important biological events, such as cell cycle growth arrest (1, 2), apoptosis (3), and DNA repair (4, 5), are thought to be critical when cells are exposed to DNA damaging agents such as IR,[2] UV radiation, and alkylating agents. Inactivation of these biological events may result in genomic instability and malignant cell transformation.

Throughout their lives, cells suffer from both endogenous and exogenous sources of DNA-damaging agents. For example, free radicals and peroxides generated during the normal physiological processes or inflammation constitute a source of endogenous DNA-damaging agents. Exposure to numerous chemical and physical agents from the environment is the exogenous source. To a great extent, cellular responses after DNA damage will initially include the induction of the stress-inducible genes. However, gene induction, as a result of exposure to adverse conditions, is dependent on the nature of the stress. Oxidative stress, heat stress, and DNA damage induce different sets of genes that are particular for each type of stress, but some of these genes can also be induced by more than one agent (6, 7). In both bacteria and eukaryotes, induction of genes involved in cell growth delay or cell cycle checkpoint is a common response to DNA damage (2).

Transcription factor genes are of particular interest in cellular response to DNA damage (8, 9). Activation of transcription factors by DNA damage will directly enhance the transcription of their downstream genes, which may exert biological functions in cellular response. Among these transcription factors, p53, c-Myc, and AP-1 (c-jun and c-fos) are well characterized as playing important roles in the control of cell cycle checkpoint, apoptosis, and signaling pathways (2, 10–13). In the case of p53, it is induced by a variety of DNA-damaging agents through a posttranscriptional mechanism (1). The activated p53 will in turn transactivate its targeted genes such as *p21/WAF1* (14), *GADD45* (2, 15), and *BAX* (16, 17). Those p53-targeted genes are thought to mediate the biological roles of p53 as a tumor suppressor. Cells lacking normal cellular p53 function usually display deficient cellular responses to DNA damage, including abrogated cell cycle checkpoint, impaired DNA repair, and deregulated apoptosis.

The transcription factor Oct-1 is a member of the POU homeodomain family and ubiquitously expressed. This protein binds to the specific-octamer sequence (ATGCAAAT) and plays an important role in activating the transcription of various genes that contain an Oct-1 binding motif (18–21). These genes include the histone H2B (22, 23), immunoglobulin genes in B cells (24, 25), small nuclear RNA gene (26), *TIF2* gene (27), and *GnRH* gene (28). Oct-1 can also negatively regulate certain genes, such as the von Willebrand factor (29) and vascular cell adhesion molecule (30). In addition to its direct binding to the specific motif, in some cases, *Oct-1* gene regulation requires cofactors that interact with the DNA binding (POU) domain. For example, MAT1, a subunit of cyclin-dependent kinase activating kinase, directly binds to the POU domain of Oct-1 and, consequently, enhances its phosphorylation by cyclin-dependent kinase activating kinase (31). It has been shown that Oct-1 DNA-binding specificity is regulated by protein kinase A, protein kinase C, and casein kinase 2 (32). The phosphorylation of the Oct-1 protein appears to be cell cycle regulated (33). However, little is known about the roles of the transcription factor Oct-1 in the cellular response to genotoxic stress. In the present study, we have reported that Oct-1 protein is induced in a p53-independent manner after cells are treated with multiple DNA-damaging agents and therapeutic agents. Importantly, the Oct-1 DNA binding activity, as demonstrated by the EMSA, is significantly induced by DNA-damaging agents. These results indicate that Oct-1 may be an important player in the cellular responses to genotoxic stress.

Materials and Methods

Cell Culture and Treatment. The human lung carcinoma cell line H1299, colorectal carcinoma line HCT116, breast carcinoma line MCF-7, and cervical carcinoma line HeLa were grown in F-12 medium supplemented with 10% fetal bovine serum. For MMS treatment, cells were exposed in medium to MMS (Aldrich) at 100 μg/ml for 4 h, and then the medium was replaced with fresh medium. Cells were then collected at the indicated time points. For UV radiation, cells in 100-mm dishes were rinsed with PBS and irradiated to a dose

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[2] The abbreviations used are: IR, ionizing radiation; EMSA, electrophoretic mobility shift assay; MMS, methylmethane sulfonate; RT-PCR, reverse transcription-PCR; dNTP, deoxynucleotide triphosphate; oligo, oligonucleotide.

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of 10 Jm^{-2}. For IR, cells were γ-irradiated with a ^{137}Cs source at 5 Gy/min. In the case of therapeutic agents, cells were treated with 0.4 μM etoposide, 4 μM cisplatin, 4 mM hydroxyurea, and 1 μM camptothecin for 8 h, and the medium was replaced with fresh medium lacking those agents.

Cellular Protein Preparation and Western Blotting Assay. After treatment with DNA-damaging agents, cells were rinsed with PBS and lysed in PBS containing100 μg/ml phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, 2 μg/ml leupeptin, and 1% NP40 (lysis buffer). Lysates were collected by scraping and cleared by centrifugation at 4°C. For Western blotting analysis, 100 μg of cellular lysates were loaded onto 8% SDS-PAGE gels and transferred to Immobilon membranes. Membranes were blocked for 1 h at room temperature in 5% milk, washed with PBST (PBS with 0.1% Tween), and incubated with anti-Oct-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 2 h. Membranes were washed four times in PBST, and horseradish peroxidase-conjugated antirabbit antibody was added at 1:4000 in 5% milk. After 1 h, membranes were washed and detected by ECL (Amersham, Arlington Heights, IL) and exposed to X-ray film (34). The estimated bands were scanned using an ImageQuant analyzer (Molecular Dynamics, Sunnyvale, CA) for the measurement of density.

RT-PCR. Total RNA was isolated using RNeasy Mini kit (Qiagen) according to the manufacturer's protocol. RT-PCR was done using the RNA PCR Core kit (Perkin Elmer Corp.). Total RNA (0.5 μg) in 1 μl of RNase-free water was used in 20 μl of RT mix containing 4 μl of 25 mM $MgCl_2$, 2 μl of 10× PCR buffer, 2 μl of diethyl pyrocarbonate water, 8 μl of dNTP mix (2.5 mM each of dATP, dCTP, dGTP, and dTTP), 1 μl of RNase inhibitor (20 units/μl), 1 μl of Random Hexamers (50 μM), and 1 μl of murine leukemia virus reverse transcriptase (50 units/μl). The mixture was subjected to cDNA synthesis using the GeneAmp PCR System 9600 (Perkin Elmer Corp.). Ten μl of cDNA product were added to 40 μl of PCR mix containing 2 μl of 25 mM $MgCl_2$, 4 μl of 10× PCR buffer, 3 μl of dNTP mix (2.5 mM each of dATP, dCTP, dGTP, and dTTP), 28.5 μl of sterile distilled water, 0.5 μl of Taq DNA polymerase (5 units/μl), and 2 μl of 1:1 primer mix (30 μM each of upstream and downstream primers). The mixture was subjected to DNA amplification using the GeneAmp PCR System 9600 (Perkin Elmer Corp.). Finally, 30 μl of PCR products were loaded on 1% agarose gel for analysis. The primers used to amplify Oct-1, Gadd45, and β-actin were designed as follows: Oct-1, 5′ primer GCAACACAGGCACACAAACC and 3′ primer TTGGCTTTGCTGAGGTAGTT; Gadd45, 5′ primer GGAATTCCATATGGGGCGACCTGCAGTTTGC and 3′ primer TAGCGCACATATGCAATTTGGTTCAGTTATT; and β-actin, 5′ primer GCGGGAAATCGTGCGTGACATT.

EMSA. Nuclear extracts were prepared, and an EMSA was carried out as described previously (35). DNA binding reactions were performed for 15 min at room temperature in a binding buffer containing 20 mM HEPES (pH 7.8), 150 mM NaCl, 1 mM DTT, 1 μg of poly(deoxyinosinic-deoxycytidylic acid), 10% glycerol, 20 μg of nuclear protein, and 4×10^4 dpm of labeled probe. The probe was a 30-mer double-stranded synthetic oligo containing the intact or mutated Oct-1 consensus sequence. Each strand was labeled separately, and the strands were annealed and then purified using a G-25 column. The samples were analyzed on a 4% nondenaturing acrylamide gel (35).

Results

Induction of Oct-1 Protein after DNA Damage. A number of observations have implicated that many transcription factors play important roles in the important biological events after cells are exposed to DNA-damaging agents. To investigate whether the transcription factor Oct-1 participates in the cellular response to genotoxic response, a panel of human cell lines including H1299 (lung carcinoma), HeLa (cervical carcinoma), HCT116 (colorectal carcinoma), and MCF-7 (breast carcinoma) were treated with the DNA base-damaging agent MMS. Cells were collected at 4 and 8 h after treatment and analyzed for the expression of Oct-1 protein. As shown in Fig. 1*A*, after exposure to 100 μg of MMS, the levels of Oct-1 protein were substantially elevated in all cell lines tested. In contrast to the untreated cells, induction of Oct-1 protein was seen between 5–8-fold among the cell lines. As a control, detection of the actin protein was included, and it showed no evident change. Obviously, the MMS induction of Oct-1 protein does not require normal cellular function of

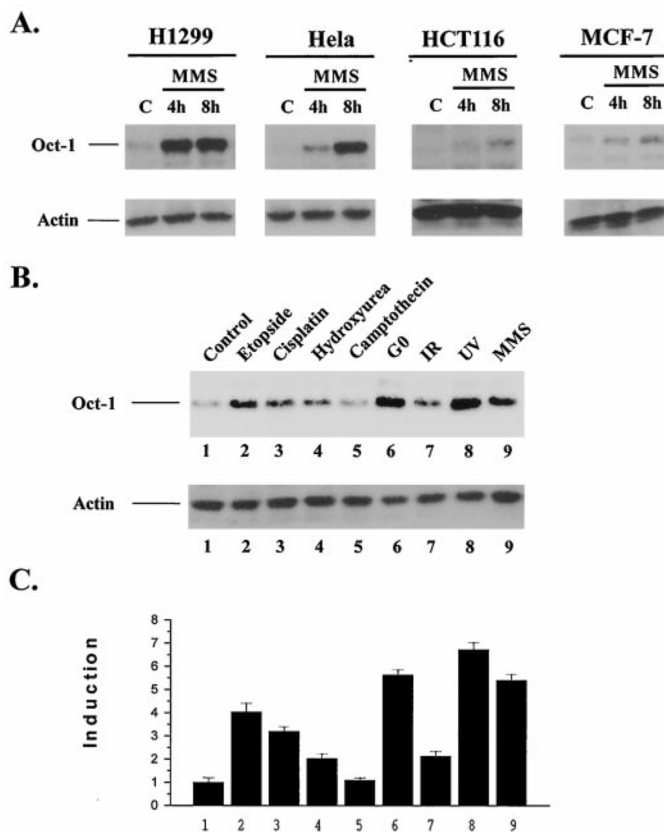

Fig. 1. Induction of Oct-1 protein in human cells after DNA-damaging agents and therapeutic agents. *A*, human cell lines were exposed to 100 μg/ml MMS. Cells were harvested at 4 and 8 h after treatment, and cellular proteins were prepared as described in "Materials and Methods." One hundred μg of total cell protein were loaded onto 8% SDS polyacrylamide gels. After electrophoresis, the proteins were transferred to Immobilon membranes. Membranes were then blocked for 1 h in 5% milk at room temperature. Measurement of Oct-1 protein was performed with anti-Oct-1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA). Immunoreaction was revealed using chemiluminescence detection procedure. As a loading control (*C*), detection of actin protein was included. Only visualized bands are shown; their estimated sizes were M_r 97,000 for Oct-1 and M_r 43,000 for actin. *B*, human H1299 cells were treated with different DNA-damaging agents or medium starvation (*G0*), including MMS (100 μg/ml), UV radiation (10 J/m^{-2}), IR (20 Gy), camptothecin (1 μM), hydroxyurea (4 mM), cisplatin (4 μM), and etoposide (0.4 μM). Eight h later, cells were harvested for Oct-1 detection as in *A*. *C*, quantitative results of Oct-1 protein expression are from *B*. All experiments were performed at least three times. The results of the representative experiment are shown; *bars,* SE.

the tumor suppressor p53, because the induction was seen in the cell lines with disrupted p53, such as H1299, where *p53* gene is deleted, and HeLa that contains HPVE6 protein, an inhibitor of p53.

Next, we examined the induction of Oct-1 protein in cells treated with different genotoxic stresses, including IR (20 Gy), UV radiation (10 Jm^{-2}), medium starvation (G_0) ,and therapeutic agents (1 μM camptothecin, 4 μM cisplatin, and 0.4 μM etoposide). As shown in Fig. 1, *B* and *C*, most of the genotoxic agents were shown to induce the expression of Oct-1 protein except for camptothecin. Among the DNA-damaging agents tested, DNA base-damaging agents, UV radiation, and MMS generated a more appreciable induction of Oct-1 protein. In contrast, ionizing radiation that produces DNA stand breaks induced Oct-1 weakly. Interestingly, medium starvation (G_0), which does not produce typical DNA damage, also exhibited evident induction for this protein. Taken together, these results indicate that the transcription factor Oct-1 is induced in a p53-independent manner after cell exposure to genotoxic stress.

The duration of Oct-1 induction was next measured in both H1299 and HCT116 cell lines after treatment with 100 μg/ml of MMS (Fig. 2, *A* and *B*). Induction was rapid and transient. The induced level of

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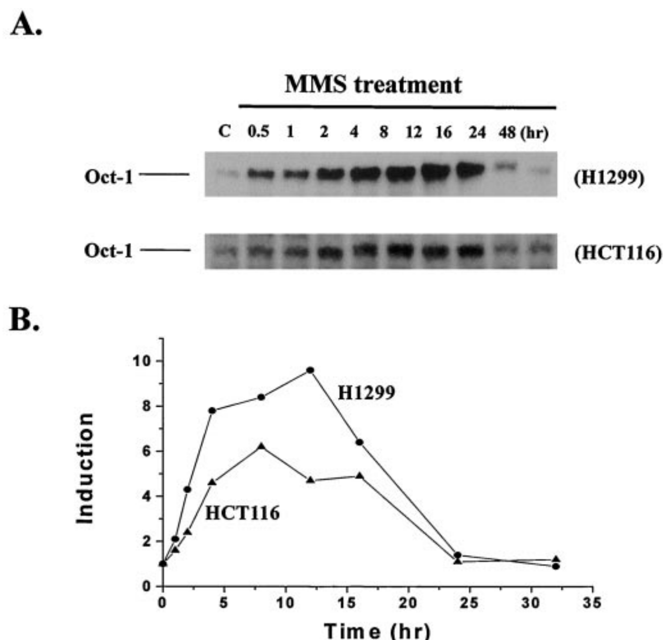

Fig. 2. Time course of Oct-1 protein expression in human H1299 and HCT116 cells after MMS treatment. Cells were treated with 100 μg/ml MMS for 4 h and then collected at the indicated time points. Whole-cell protein was prepared and analyzed for Oct-1 expression as described in Fig. 1. *C,* control. The results of a representative experiment are shown in *A,* and quantitative analysis for the results of three separate experiments is shown in *B*. The results in *B* are normalized to that of the control sample.

Oct-1 protein was seen 30 min after treatment. The induction peaked at 8 h (HCT116) or 12 h (H1299) after cell exposure to MMS and returned to normal level by 24 h. In addition, an analysis was carried out to examine the Oct-1 induction by different MMS doses. An appreciable increase in Oct-1 protein level was seen with the dose as low as 20 μg/ml, which produces little lethality. The magnitude of Oct-1 induction was approximately proportional to the dose and reached maximal induction with 100 μg/ml of MMS (results not shown). These results demonstrate that Oct-1 induction after DNA damage is a rapid, sensitive, and transient response, suggesting that Oct-1 may be well involved in some cellular response to genotoxic stress.

Induction of Oct-1 Protein Is through a Posttranscriptional Mechanism. Additional experiments were performed to determine the mechanism by which DNA-damaging agents activate Oct-1 protein expression. To do this, the levels of *OCT-1* transcripts after DNA damage were measured using an RT-PCR approach. Surprisingly, *OCT-1* mRNA was not seen to be elevated after DNA damage. As shown in Fig. 3*A*, H1299 cells were exposed to MMS and collected at the indicated time for RT-PCR analysis. Expression of *OCT-1* mRNA at all time points remained similar after MMS treatment. As a positive control, we included the analysis of *GADD45* expression in the same experiments. Consistent with our previous reports (11, 36), *GADD45* mRNA was observed to increase >3-fold. To ensure that equal amounts of mRNA were used and equal amounts of PCR products were loaded, β-actin was included in the experiments. These results indicated that induction of Oct-1 protein might be through a posttranscriptional mechanism. We next analyzed Oct-1 protein expression in the presence of actinomycin D, an inhibitor of RNA synthesis. In this experiment, actinomycin D was added at a concentration of 1 μg/ml into tissue culture, whereas cells were exposed to MMS. In Fig. 3*B*, addition of actinomycin D did not reduce the expression of Oct-1 protein, suggesting that induction of Oct-1 protein not require new RNA synthesis. In contrast, actinomycin D was shown to significantly abrogate induction of Gadd45 protein. Taken together with the results in Fig. 3*A*, it can be concluded that induction of Oct-1 is mediated via the posttranscriptional mechanism.

DNA Damage Activates Oct-1 DNA Binding Affinity. Oct-1 is a DNA-binding transcription factor with a specific DNA binding motif (18, 19). To examine whether DNA damage enhances the Oct-1 DNA-binding ability, the EMSA was used. A 30-bp, double-stranded oligo DNA that harbors a typical Oct-1 consensus sequence (ATGCAAAT) was synthesized and labeled with [γ-^{32}P]ATP. The labeled oligos were incubated with the nuclear extracts from H1299 cells treated with MMS. In Fig. 4, *A* and *B*, we detected several DNA-protein complexes by EMSA. A prominent slowly migrating band was seen, but it disappeared when the Oct-1 motif was mutated, indicating that this DNA-protein complex is associated with the Oct-1 consensus sequence. In agreement with the evidence that MMS strongly induces the Oct-1 protein expression, the Oct-1 binding affinity was dramatically enhanced by MMS treatment. The enhanced DNA binding of Oct-1 was observed with the nuclear extracts from MMS-treated H1299 cells isolated at 1, 4, 8, 12, 16, and 24 h after treatment. At 32 h after treatment, the increased binding affinity was reduced to the basal level. To further demonstrate that this protein-DNA complex was specific for Oct-1, a competition experiment with an unlabeled oligo containing an Oct-1 binding site was conducted. In Fig. 4*C*, this prominent band was effectively competed by unlabeled self sequence

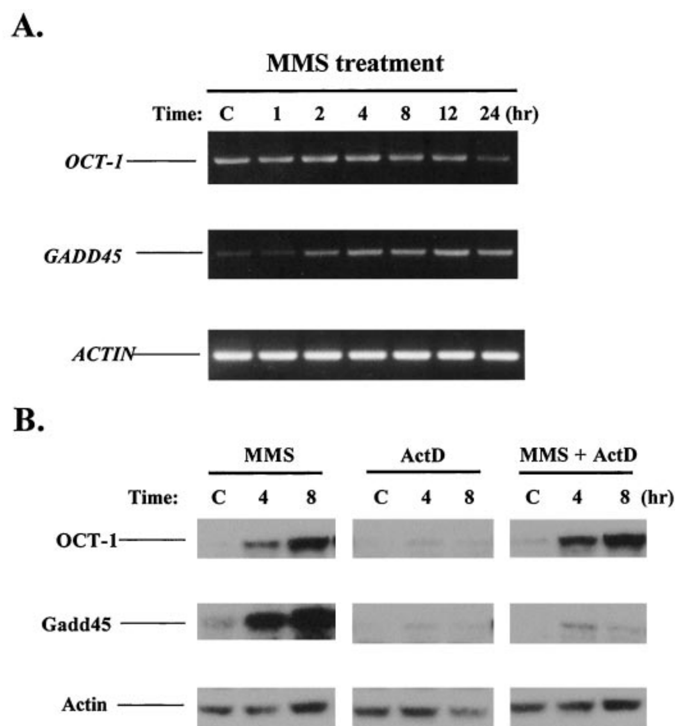

Fig. 3. Induction of Oct-1 protein is mediated through a posttranscription mechanism. *A*, 0.5 μg of total RNA from cells treated with MMS was used in 20 μl of reverse transcriptase mixture containing 4 μl of 25 mM $MgCl_2$, 2 μl of 10× PCR buffer, 2 μl of diethyl pyrocarbonate water, 8 μl of dNTP, 1 μl of RNase inhibitor (20 units/μl), 1 μl of Random Hexamers (50 μM), and 1 μl of murine leukemia virus reverse transcriptase (50 units/μl). After reverse transcription, 10 μl of cDNA products were added to 40 μl of PCR mixture containing 2 μl of 25 mM $MgCl_2$, 4 μl of 10× PCR Buffer, 3 μl of dNTP mix, 0.5 μl of Taq DNA polymerase, and 2 μl of 1:1 primer mix (30 μM each of upstream and downstream primer). The mixture was subjected to DNA amplification using GeneAmp PCR System 9600. Finally, 30 μl of PCR products were loaded on 1% agarose gel for analysis. *C,* control. *B*, human colorectal carcinoma HCT116 cells were treated with MMS (100 μg/ml). Meanwhile, actinomycin D (*ActD*) was added to the cell culture medium at a final concentration of 1 μg/ml. Cells were collected at the indicated time for analysis of Oct-1 protein expression. Each experiment was repeated more than three times, and only the representative one was shown in this figure. The variability of results from each independent experiment was seen <25%. *C,* control.

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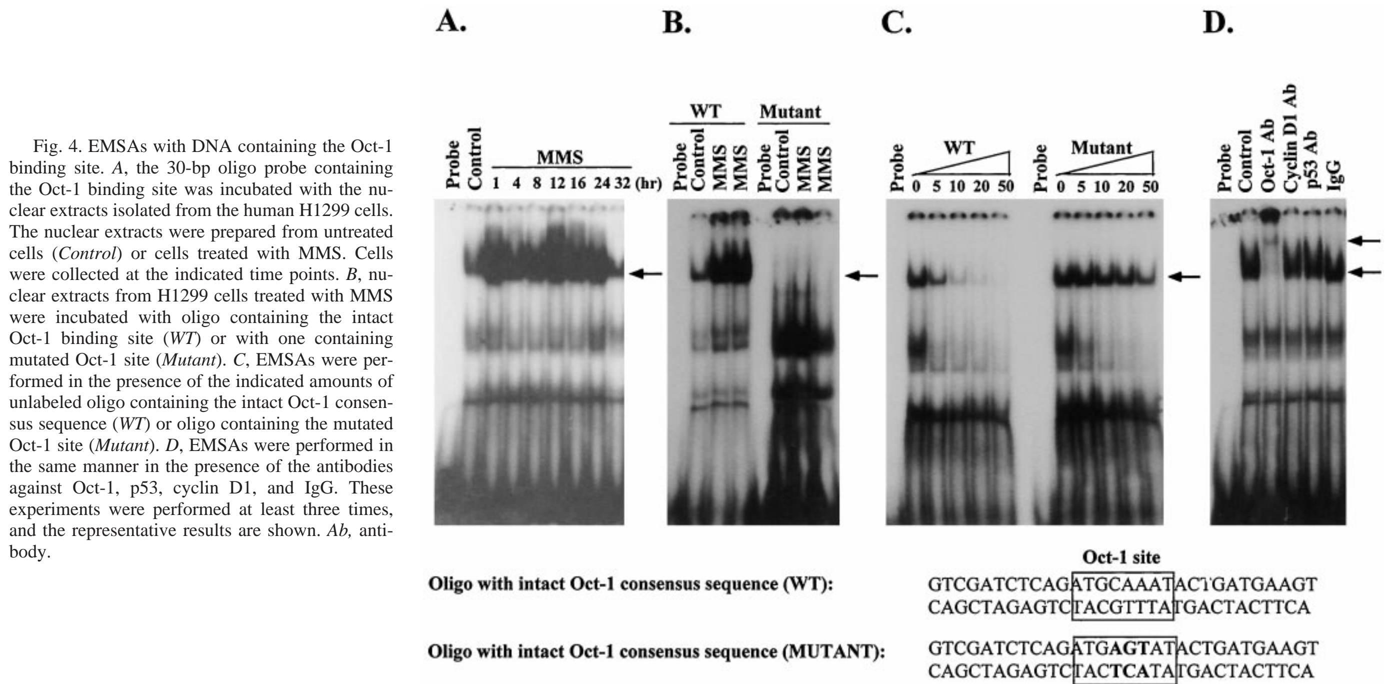

Fig. 4. EMSAs with DNA containing the Oct-1 binding site. *A*, the 30-bp oligo probe containing the Oct-1 binding site was incubated with the nuclear extracts isolated from the human H1299 cells. The nuclear extracts were prepared from untreated cells (*Control*) or cells treated with MMS. Cells were collected at the indicated time points. *B*, nuclear extracts from H1299 cells treated with MMS were incubated with oligo containing the intact Oct-1 binding site (*WT*) or with one containing mutated Oct-1 site (*Mutant*). *C*, EMSAs were performed in the presence of the indicated amounts of unlabeled oligo containing the intact Oct-1 consensus sequence (*WT*) or oligo containing the mutated Oct-1 site (*Mutant*). *D*, EMSAs were performed in the same manner in the presence of the antibodies against Oct-1, p53, cyclin D1, and IgG. These experiments were performed at least three times, and the representative results are shown. *Ab,* antibody.

(WT), whereas the oligo with mutated Oct-1 site did not compete this complex. To examine whether the Oct-1 is directly involved in this DNA-protein complex, we performed supershift experiments. In the presence of the antibody against Oct-1, a higher supershifted band was observed (Fig. 4*D*, *top arrow*). In contrast, nonspecific antibodies (IgG, p53 antibody, and cyclin D1 antibody) did not generate the supershift band. These results demonstrate that the DNA damage-inducible complex that specifically binds to the Oct-1 motif contains Oct-1 protein, and that the Oct-1 DNA-binding activity is enhanced after DNA damage.

Discussion

In summary, we have presented novel observations that the transcription factor Oct-1 may participate in cellular response to genotoxic stress. After DNA damage treatment, levels of Oct-1 protein are elevated in multiple human cell lines. Evident induction of Oct-1 protein can be detected as early as 0.5 h after treatment. The maximal level of Oct-1 was observed at 8 or 12 h and returned to normal at 24 h. The induction of Oct-1 protein does not require the tumor suppressor p53, because cells with disrupted p53 still exhibited a strong induction of Oct-1. Interestingly, induction of Oct-1 protein is mediated through a posttranscriptional mechanism because there was no induction of *OCT-1* mRNA in response to DNA damage, and addition of actinomycin D, an inhibitor of RNA synthesis, had no effect on the induction of Oct-1 protein. More importantly, the affinity of Oct-1 binding to its consensus sequence (ATGCAAAT) was highly enhanced after DNA damage. These results have implicated that the activation of Oct-1, as manifested by both induction of protein level and enhanced DNA binding affinity, may play roles in cellular responses to genotoxic stress.

Mammalian cells demonstrate complex cellular responses to DNA damage, including activation of genes involved in cell cycle arrest (2), DNA repair (37, 38), and apoptosis (3). It is important to determine how the transcription factors are modified in DNA-damaged cells and whether these modifications are necessary intermediates in the DNA damage-induced activation of genes. The results presented in this report demonstrate evidence that the octamer transcription factor Oct-1 is activated and might be one of the important players in gene activation by DNA damage. As discussed earlier, Oct-1 protein is ubiquitously expressed and involved in the regulation of various genes, which function in the development of multiple organs and tissues (27, 33), the control of cell cycle progression (33), and the regulation of signaling pathways as well (39, 40). However, despite its demonstrated biological functions, the role of Oct-1 protein in the regulation of stress-inducible genes after DNA damage remains unclear. It can be expected that after genotoxic stress, activated Oct-1 protein would be able to bind to the octamer-containing promoters of DNA damage-responsive genes and in turn exert its regulatory function. Graunke *et al.* (41) has reported that a DNA-protein interaction at the octamer binding motif is identified in the promoter of the *GADD45* gene, using *in vivo* DNase I hypersensitivity analysis. The *GADD45* gene is transcriptionally up-regulated after DNA-damaging agents (IR, UV radiation, and alkylating agents) and has been suggested to coordinate cell cycle regulation and DNA repair (34, 42, 43). Interestingly, we have found recently that the octamer-binding motif is involved in the regulation of *GADD45* induction in response to certain DNA-damaging agents. Disruption of the Oct-1 binding site located at the *GADD45* promoter attenuated the induction of *GADD45* by DNA damage.[3] Therefore, the finding that the transcription factor Oct-1 is activated by DNA damage has extended the physiological roles of Oct-1 protein to the cellular response after genotoxic stress.

The tumor suppressor p53 plays an important role in cellular response to DNA damage through the regulation of its downstream genes like p21 and *GADD45* (2, 14). In the case of the *GADD45* gene, p53 is required for its IR induction (15), but the induction of *GADD45* by UV or MMS does not require normal cellular p53 function, although p53 can contribute to these responses (36). Most likely, Oct-1 may play a role in the p53-independent cellular response to DNA damage. Therefore, it can be assumed that after DNA damage, Oct-1 protein may delicately coordinate with some other gene regulators to ensure the establishment of cellular defense network under the circumstance of genotoxic stress. However, future studies are

[3] Submitted for publication.

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required to investigate how Oct-1 regulates its targeted genes that are activated when cells are exposed to DNA damage. In fact, in addition to its induction of both protein level and binding activity, Oct-1 may also need to interact with cofactors or posttranslational modification such as phosphorylation or acetylation. In conclusion, we have found that the transcription factor Oct-1 is strongly induced after multiple DNA-damaging agents and therapeutic agents in a p53-independent manner. In agreement with the protein induction, Oct-1 DNA binding activity is also enhanced by DNA damage. These findings provide insight into the biological roles of Oct-1 in cellular response to genotoxic stress, particularly in the p53-independent gene activation.

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