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Mitogen-activated Protein Kinase Phosphatase-1 Represses c-Jun NH_2-terminal Kinase-mediated Apoptosis via NF-κB Regulation*

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The mechanism regulating radiation-induced anti-apoptotic response, a limiting factor in improving cell radiosensitivity, remains elusive. Mitogen-activated protein kinase (MAPK) phosphatase (MKP)-1 is the major member of MKPs that dephosphorylates and inactivates MAPK. Here we provide the evidence that MKP-1 was negatively bridging between NF-κB-mediated prosurvival pathway and c-Jun N-terminal kinase (JNK)-mediated proapoptotic response. MKP-1 was induced by γ-radiation and repressed radiation-induced pro-apoptotic status. NF-κB RelA/p50 heterodimer was recruited to MKP-1 gene promoter to induce MKP-1 transcription. Deletion of the NF-κB-binding site or inactivation of NF-κB by its small interfering RNA significantly decreased the radiation-induced MKP-1 promoter activity. In addition, MKP-1-deficient mouse embryonic fibroblasts exhibited a prolonged activation of JNK but not p38 or extracellular signal-regulated kinase subfamilies of MAPKs. The prolonged activation of JNK was not induced by treatment with tumor necrosis factor α or interleukin-6, and inactivation of JNK but not p38 or ERK abolished radiation-induced proapoptotic status, indicating that JNK is specifically inhibited by radiation-induced MKP-1. Three MKP-1 wild type human tumor cell lines treated with MKP-1 small interfering RNA showed an increased proapoptotic response that can be rescued by overexpression of wild type mouse MKP-1. Together, these results suggest that MKP-1 is a NF-κB-mediated prosurvival effector in attenuating JNK-mediated pro-apoptotic response; NF-κB/MKP-1-mediated negative JNK regulation represents a potential therapeutic target for adjusting cell radiosensitivity.

Radiation continues to be used as the major modality of anticancer treatment because of its strong effect in inducing cell death and apoptosis (1, 2). The efficacy of this treatment may be severely compromised because of radiation-induced radioresistance with adaptive anti-apoptotic response (3–5). Radiation can activate various cellular signal pathways, including MAPKs[2] (6) and NF-κB pathways (7). The interaction between these two pathways have been identified in cells under different stresses, such as treatment of TNFα (8, 9). However, the mechanism balancing the MAPK-mediated pro-apoptotic and NF-κB-mediated anti-apoptotic pathways in response to radiation remains unclear.

MAPKs are major signaling transduction molecules in apoptosis (10). In mammals, MAPKs consist of three subfamilies: c-Jun NH_2-terminal kinases (JNKs) (11), extracellular signal-related kinases (ERKs) (12), and p38 kinases (10). Although γ-radiation can activate JNKs (6, 13), ERK (12), and p38 kinases (14), it is not clear which subfamily of MAPKs plays a specific role in regulation of the γ-radiation-induced pro-apoptotic response. It is generally believed that phosphorylation and dephosphorylation cause activation and inactivation of MAPKs, respectively. Activation of MAPKs can be executed by their upstream dual specific MAPK kinases, which phosphorylate both threonine and tyrosine residues of the T*X*Y motif (15). Inactivation of MAPKs is accomplished through dephosphorylation of the two same residues of the T*X*Y motif by MKPs (16).

MKPs belong to a family of dual specificity protein phosphatases with 13 members with a function of dephosphorylation of both phosphothreonine and phosphotyrosine residues (14, 16, 17). MKP-1, as the first defined member of MKPs (18), can be rapidly induced in mammalian cells in response to an array of stress stimuli, including oxidative stress and heat shock (19), UV light (20), and DNA-damaging anti-cancer drugs (21, 22) through transcriptional (23, 24) and post-transcriptional mechanisms (25, 26). MKP-1 induction protects cells from stress-induced apoptosis predominantly through negative regulation of MAPK activities.

The heterodimeric transcription factor NF-κB inhibits cellular apoptotic response and promotes survival in response to radiation (27–29). In mammals, there are five Rel/NF-κB proteins: RelA (p65), RelB, c-Rel, NF-κB1 (p50 and its precursor p105), and NF-κB2 (p52 and its precursor p100) (30, 31). In

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[2] The abbreviations used are: MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase-1; WT, wild type; MEF, mouse embryonic fibroblast; ERK, extracellular signal-regulated kinase; JNK, c-Jun NH_2-terminal kinase; ChIP, chromatin immunoprecipitation; CHX, cycloheximide; siRNA, small interfering RNA; TNF, tumor necrosis factor; IL, interleukin; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GST, glutathione *S*-transferase; JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′- tetraethylbenzimida-zolyl carbocyanine iodide; Gy, gray(s).

resting cells, NF-κB is retained in the cytoplasm in an inactive form by a tight association with its IκB. Activation of RelA/p50 requires IKKβ-dependent degradation of IκBs, which liberates the RelA·p50 complex from IκBs and subsequently leads its translocation into the nucleus. The IKK complex contains two catalytic subunits, IKKα and IKKβ, and two regulatory subunits, IKKγ and ELKS. The classical manner for NF-κB activation mainly depends on the IKKβ subunit activation (32, 33). However, it is unknown whether MKP-1 is a target of NF-κB to mediate pro-survival signaling upon the genotoxic stress with γ-radiation.

Although all of MAPKs, MKP-1, and NF-κB have been implicated in regulation of apoptotic and anti-apoptotic response in DNA-damaging response, their connections in signaling γ-radiation-induced apoptosis remain elusive. Here we demonstrate that MKP-1 is the target gene of NF-κB upon γ-radiation. MKP-1 activation through NF-κB is specifically required for inhibition of JNK-associated pro-apoptotic response to decrease cell death. Thus, our data reveal a novel anti-apoptosis mechanism by which the NF-κB and MKP-1 pathways crosstalk in response to γ-radiation treatment.

EXPERIMENTAL PROCEDURES

Cell Lines, Cell Culture, and Treatments—*MKP-1*$^{-/-}$ (*MKP-1* knock-out) MEFs and paired *MKP-1* wild type (WT) MEFs were kindly provided by Robert Z. Orlowski at University of North Carolina. WT and *MKP-1*$^{-/-}$ MEFs were maintained in DMEM with 10% FBS. MKP-1$^{-/-}$ MEFs with reconstitution of MKP-1 were established by stable transfection with pcDNA3-MKP-1 together with pCEP-4 vector with hygromycin B resistance gene. The cells were transfected for 48 h and subjected to hygromycin B (50 μg/ml) selection for 7–10 days. The clones surviving from the selection were pooled as stable MKP-1$^{-/-}$/MKP-1 MEFs that were cultured in the selective drug-free medium for at least three passages before used for further experiments. The human skin keratinocytes (HK18) and HK-18/IκB-αM stable transfectants were maintained as described previously (34). The human breast cancer MDA-MB-231 cells and lung cancer A549 cells (ATCC, Manassas, VA) were maintained in RPMI 1640 with 10% FBS. The human breast cancer MCF-7 (ATCC) were maintained in DMEM with 5% FBS and insulin (10 μg/ml). All of the radiation treatments were delivered with γ-radiation-12 irradiator (U.S. Nuclear Co.) equipped with a cobalt-60 (dose rate, 2.3 Gy/min). For treatment with TNFα and IL-1, the cells were incubated with or without 20 ng/ml of TNFα and IL-1 for different intervals of time. After radiation or treatment with TNFα and IL-1, the cells were cultured for different time intervals for further experiments.

Cell Viability—Cells with 60% confluence were treated with or without radiation. At 24 h post-radiation, the cells were trypsinized and diluted in 0.5 ml of complete medium without serum to an approximate concentration of 1×10^5 to 2×10^5 cells/ml and stained by 0.4% trypan blue. The nonviable stained cells were accounted with Nikon microscope (TE 2000-U, Th4–100; Nikon Instruments Inc.).

Assays of Caspase 3/7 Activity—Enzymatic activities of caspase 3/7 were assayed using the Caspase Glo® 3/7 assay kit (Promega, San Luis Obispo, CA). The cells were treated with or without γ-ray (10 Gy) in the presence or absence of different inhibitors. At 24 h post-radiation, the cells were incubated with 100 μl of caspase 3/7 activity reagent at room temperature for 2 h. The luminescence was measured using a plate reader.

Flow Cytometry Analysis—The cells were maintained in DMEM with 0.1% FBS for 24 h and then were cultured in DMEM with 10% FBS for three passages before used for further experiments. Exponentially growing cells were exposed to 10 Gy of γ-ray. At 24 h post-radiation, the cells were trypsinized and fixed with ice-cold 70% (v/v) ethanol. The cell pellets were prepared by centrifugation at 200 g for 5 min, washed with phosphate-buffered saline (pH 7.4), and resuspended in phosphate-buffered saline containing propidium iodide (50 μg/ml), Triton X-100 (0.1%, v/v), and DNase-free RNase (1 μg/ml) for 1 h, and DNA content was determined by flow cytometry analysis using a FACScan flow cytometer (Becton Dickinson, San Jose, CA).

Immunoblotting—Protein samples (20 μg) were separated from cells after different treatments by SDS-PAGE, blotted onto polyvinylidene difluoride membranes, and incubated with specific first antibody, followed by incubation with the horseradish peroxidase-conjugated or alkaline phosphatase-linked secondary antibody, and visualized by the ECL or ECF immunoblot detection system.

Mitochondrial Membrane Potential ($\Delta\Psi_m$) Assays—Cells (10^4) were treated with or without radiation 10 Gy of γ-ray. At 24 h post-radiation, the cells were incubated in culture medium containing 2 μg/ml of JC-1 for 30 min. Cationic dye taken up by mitochondria was detected by formation of red precipitate in cells. In cells with disrupted mitochondria, the dye was excluded and detectable as a green monomer in the cytoplasm. After washing with phosphate-buffered saline (pH 7.4), the fluorescence intensity of the red precipitate (JC-1 red) and green monomer (JC-1 green) was detected using a plate reader of Spectra Max M^{2e} (Molecular Devices Co., Sunnyvale, CA) at the wavelength of excitation 485/emission 595 or excitation 485/emission 525, respectively. The ratio of JC-1 red (595)/JC-1 green (525) was calculated as the indicator of $\Delta\Psi_m$.

Clonogenic Survival Assays—The standard radiation clonogenic survival assay was performed as previously described (34). Survival fraction was assessed by colony formation following exposure to various radiation doses of γ-ray. Each radiation dose was delivered in three duplicated plates, and all of the experiments were repeated three times. The irradiated and control cells were trypsinized and cultured for 14 days, and colonies with more than 50 cells were scored and normalized against the plating efficiency of cells without radiation.

Quantitation of Gene Expression—Total RNA was purified and quantified with semi-quantitative reverse transcription-PCR or real time PCR. The reverse transcription-PCR primer sequences for human MKP-1 were 5′-CCATCTGCCTTGCTTACCTT-3′ (sense) and 5′-AGCACCTGGGACTCAAACTG-3′ (antisense). The internal control human GAPDH primers were 5′-GGACTCATGACCACAGTCCAT-3′ (sense) and 5′-GTTCAGCTCAGGGATGACCTT-3′ (antisense). Real time PCR was performed in a My IQ thermocycler using SYBR Green supermix reagents following the manufac-

turer's protocol (Bio-Rad). The cycle conditions were: one cycle of 3 min at 95 °C and 45 cycles of 30 s at 95 °C, 30 s at the 60 °C, and 30 s at 72 °C. The data were analyzed with MX 3000P software. Each sample was assigned as an expression value based on its threshold cycle (C_T) value, and the data were expressed as arbitrary units by normalization with internal control of GAPDH. The results of relative MKP-1 mRNA induction after radiation were expressed as fold increase over its basal expression without radiation. The PCR primer sequences for mouse MKP-1 were 5′-ACCATCTGCCTTGCTTACCTT-3′ (sense) and 5′-AGCACCTGGGACTCAAACTG-3′ (antisense) and for human MKP-1 were 5′-CCATCTGCCTTGCTTACCTT-3′ (sense) and 5′-AGCACCTGGGACTCAAACTG-3′ (antisense). The internal control PCR primers for mouse β-actin were 5′-GAAGAGCTATGAGCTGCCTGA-3′ (sense) and 5′-CAGCACTGTGTTGGCATAGAG-3′ (antisense) and for human GAPDH were 5′-GGACTCATGACCACAGTCCAT-3′ (sense) and 5′-GTTCAGCTCAGGGATGACCTT-3′ (antisense).

Luciferase Reporter Assays—Cells (1×10^4) were transfected with 0.1 μg of reporter constructs and 2 ng of pRL-TK (Promega) using Lipofectamine™ LTX and PLUS™ reagent (Invitrogen). Transfected cells were treated with different chemicals or γ-ray as indicated in each figure legend. Both firefly and *Renilla* luciferase activities were measured using a dual luciferase reporter assay system in a Turner TD20/20 luminometer (Promega). Firefly luciferase activity was normalized by *Renilla* luciferase activity, which was detected by co-transfection with pRL-TK in all reporter experiments. The luciferase activity was further normalized with the luciferase activity of cells transfected with control vector of pGL3-E. The results of MKP-1 relative luciferase activities were expressed as fold increase over pGL3-E transfected cells.

Chromatin Immunoprecipitation (ChIP) Assays—Exponentially growing HK-18 cells were treated with or without radiation and cross-linked with 1% formaldehyde at 37 °C for 10 min. The cell extracts were prepared and sonicated to obtain DNA fragments with sizes between 0.2 and 0.7 kb. Protein-DNA complexes were immunoprecipitated using p65, p50, and c-Rel antibodies (5 μg each reaction) or IgG control. DNAs were purified and used for PCR with primers specific for the gene promoter region encompassing NF-κB-binding sites. The MKP-1 primers were 5′-GAGGAAACCGCAGAATGTTCCTGA-3′ (sense) and 5′-GGCCGTTATAGGCCGAAAGCAAAA-3′ (antisense); primers for upstream sequence of NF-κB-binding site in the MKP-1 promoter were 5′-AACCATTCTGCTGGGAAGGGGAAA-3′ (sense) and 5′-TGTCATTTTGCTGCTGGATGGTCA-3′ (antisense); primers for NF-κB-binding site in the IκB-α promoter were 5′-TGTAGCACCCATTAGAAACACTTC-3′ (sense) and 5′-TTCTTGTTCACTGACTTCCCAATA-3′ (antisense); GAPDH primers were 5′-GGACTCATGACCACAGTCCAT-3′ (sense) and 5′-GTTCAGCTCAGGGATGACCTT-3′ (antisense).

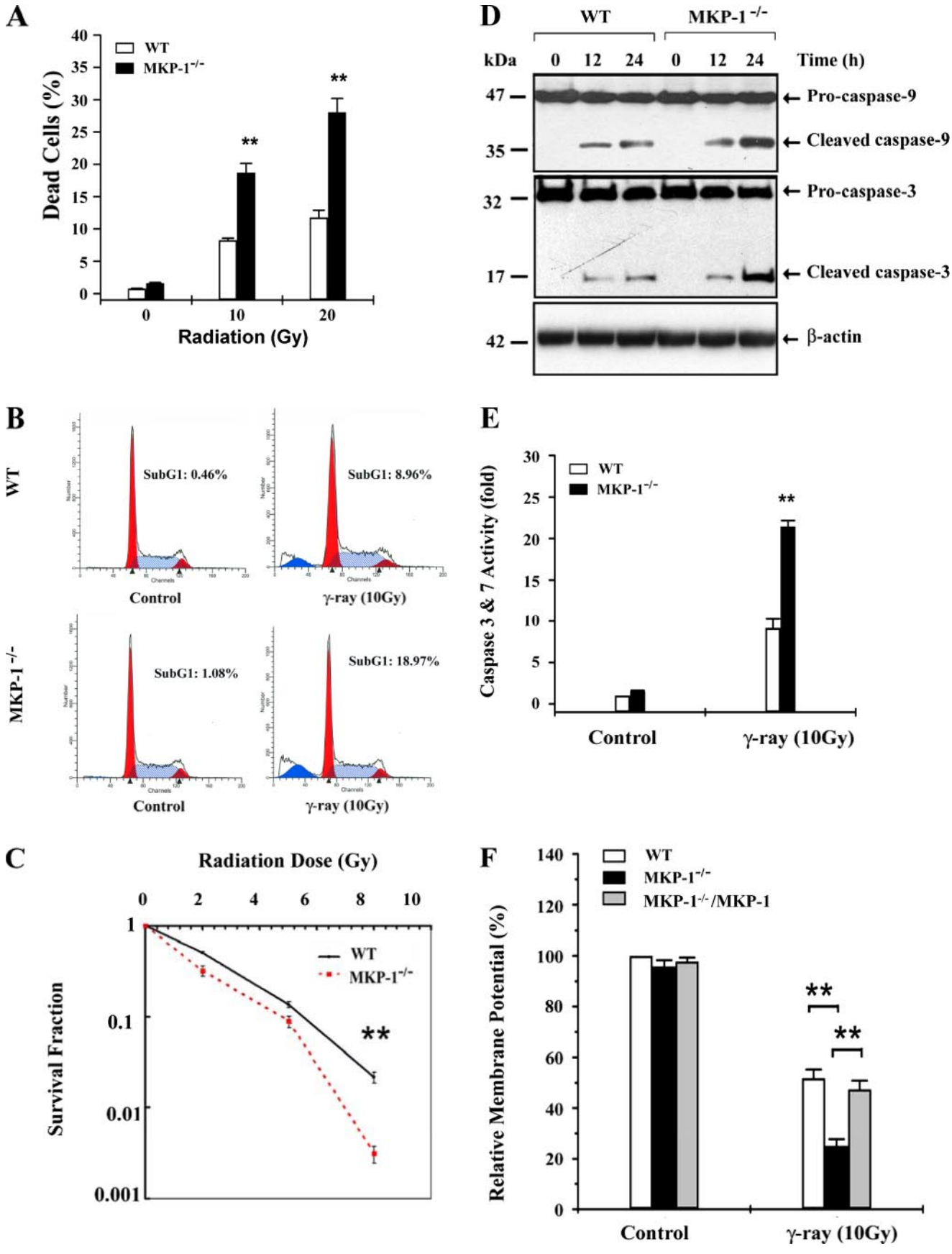
JNK Kinase Assays—Cells were lysed in IP buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1% Triton X-100, 2 mM EDTA, 20 mM EGTA, 1 mM Na_3VO_4, 50 mM NaF, 5 mM β-glycerophosphate, and protease inhibitors). For JNK immunoprecipitation, 500 μg of cell lysate in 500 μl was incubated with 2 μg of anti-JNK antibodies for 60 min at 4 °C and then with 50 μl of protein A beads for an additional 60-min incubation. Precipitated protein complexes were washed three times with 1 ml of IP buffer, washed twice with kinase buffer containing 5 mM EGTA, and then incubated with 1 μg of GST-c-Jun in kinase buffer containing 100 μM [γ-^{32}P]ATP for 60 min at 30 °C. The samples were analyzed by SDS-PAGE and autoradiography. The JNK activity was correlated with phosphorylated GST-c-Jun levels.

siRNA Synthesis and Transfection—siRNA duplex oligonucleotides were designed and synthesized with a Silencer siRNA construction kit (Ambion, Austin, TX). The cells were seeded to achieve 30–50% confluence, and siRNA was transfected for 48 h with Lipofectamine™ RNAiMAX reagent (Invitrogen). Scramble RNA Duplex (Ambion) was included in each transfection as controls. The primers used to synthesize the siRNAs for mouse Jnk-1 were: 5′-AAGAGACGTTGATCAAGGCAGCCTGTCTC-3′ (sense) and 5′-AACTGCCTTGATCAACGTCTCCCTGTCTC-3′ (antisense); mouse Jnk-2 primers were 5′-AAGAGACGTTGATCAAGGCAGCCTGTCTC-3′ (sense) and 5′-AACTGCCTTGATCAACGTCTCCCTGTCTC-3′ (antisense); and human MKP-1 primers were 5′-AAGAGACGTTGATCAAGGCAGCCTGTCTC-3′ (sense) and 5′-AACTGCCTTGATCAACGTCTCCCTGTCTC-3′ (antisense).

Reagents and Antibodies—Antibodies: JNK1, p-JNK, ERK2, p-ERK, p38, MKP-1, p65, c-Rel, β-actin, and rabbit IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA); p50 and caspase 3 were purchased from Upstate Biotechnology Inc. (Lake Placid, NY); p-p38 was purchased from Promega. JNK inhibitor SP600125 was purchased from Calbiochem (Gibbstown, NJ); ERK inhibitor U0126 was purchased from VWR International (West Chester, PA); protease inhibitors, p38 inhibitor SB 202190, IKK-β inhibitor IMD-0354, the protein synthesis inhibitor cycloheximide (CHX), and trypan blue solution were purchased from Sigma; proteasome inhibitor was purchased from Peptides International Inc. (Louisville, KY); caspase 9 and GST-c-Jun were purchased from Cell Signaling (Danvers, MA); [γ-^{32}P]ATP (6000 Ci/mmol) was purchased from Amersham Biosciences; the JC-1 was purchased from Invitrogen; the TNFα and IL-1 were from PeproTech Inc. (Rocky Hill, NJ); and the caspase inhibitor Ac-Val-Ala-Asp-CMK was purchased form Anaspec Inc. (San Jose, CA).

Plasmids—pcDNA3-MKP-1 (35) was kindly provided by Robert Z. Orlowski at the University of North Carolina. The pGL3-E (Enhancer)-mouse-MKP-1 plasmid was kindly provided by Eisuke Nishida at Kyoto University (36). The plasmid of pGL3-E-human-MKP-1-ΔNF-κB with the deletion of the NF-κB-binding site (GGGTCTTCCC, −1210 to −1200) was constructed with *Pfu* Turbo DNA polymerase (Stratagene, La Jolla, CA) with the primer sequences: 5′-GAGATCGCTGGGCGGGGCGGTGTTGCGATCCAGGTCCTGG-3′ (sense) and 5′-CCAGGACCTGGATCGCAACACCGCCCCGCCCAGCGATCTC-3′ (antisense). A plasmid of pGL3-E-human-MKP-1-NF-κB was used as a template (24).

Statistic Analyses—The data are presented as the means ± S.E. Statistical significance among groups was determined by using paired, two-tailed Student's *t* tests with SAS software (version 9). The findings were considered significant at $p < 0.05$.


A
Dead Cells (%)
WT
MKP-1 -/-
**
**
Radiation (Gy)
D
WT
MKP-1 -/-
kDa
Time (h)
Pro-caspase-9
Cleaved caspase-9
Pro-caspase-3
Cleaved caspase-3
β-actin
B
WT
MKP-1 -/-
SubG1: 0.46%
SubG1: 8.96%
SubG1: 1.08%
SubG1: 18.97%
Control
γ-ray (10Gy)
E
Caspase 3 & 7 Activity (fold)
WT
MKP-1 -/-
**
Control
γ-ray (10Gy)
C
Radiation Dose (Gy)
Survival Fraction
WT
MKP-1 -/-
**
F
Relative Membrane Potential (%)
WT
MKP-1 -/-
MKP-1 -/- /MKP-1
**
**
Control
γ-ray (10Gy)

RESULTS

Radiation-induced Apoptosis Was Repressed by MKP-1—To determine whether MKP-1 plays a role in regulation of γ-radiation-induced apoptosis, we tested radiosensitivity and apoptotic response induced by radiation with γ-ray in MKP-$1^{-/-}$ *versus* WT MEFs. A considerably increased cell death was induced by radiation in MKP-$1^{-/-}$ MEFs compared with WT MEFs (Fig. 1*A*), suggesting that MKP-1 status plays a role in anti-radiation function. To confirm that MKP-1 negatively mediates the radiation-induced apoptotic response, we measured apoptosis in WT and MKP-$1^{-/-}$ MEFs by sorting SubG1 DNA content with flow cytometry analysis and clonogenic radiosensitivity of WT and MKP-$1^{-/-}$ MEFs. The result showed that radiation significantly increased SubG1 subdiploid apoptotic nuclei (SubG1 fraction) population that contains the apoptotic cell fraction in MKP-$1^{-/-}$ MEFs compared with WT MEFs (Fig. 1*B*). Consistent with the enhanced cell death, clonogenicity was significant reduced detected in irradiated MKP-$1^{-/-}$ cells, especially by irradiation with 8 Gy of γ-ray (Fig. 1*C*). Although MKP-1 is able to suppress apoptosis induced by various stimuli (20), the present data provide the first evidence that cell radiosensitivity is increased because of a lack of MKP-1 activity.

To investigate whether mitochondria-dependent apoptotic pathway is involved in the MKP-1-associated anti-apoptosis, we compared the cleavage of caspase 9, an indicator of mitochondria-dependent apoptosis, with the cleavage of caspase 3, an executor of apoptosis, in MKP-$1^{-/-}$ and WT MEFs. Contrasted with WT cells, the cleavage of both caspase 9 and caspase 3 was significantly increased in MKP-$1^{-/-}$ MEFs at 24 h post-radiation (Fig. 1*D*). Consistent with the increased cleavage of caspase 3, radiation-induced caspase 3 and 7 activities in MKP-$1^{-/-}$ MEFs (~22%) were doubled over their levels in WT MEFs (~9%) 24 h after 10 Gy of γ-ray treatment (Fig. 1*E*).

The mitochondrial inner membrane potential ($\Delta\Psi_m$) plays an important role in the apoptotic cascades, and it can be affected by radiation-induced reactive oxygen species (37). Because MKP-1 is also detected in mitochondria (38), we addressed the question of whether MKP-1 modulates the $\Delta\Psi_m$ upon exposure to radiation. Our data showed that although radiation significantly decreased $\Delta\Psi_m$ in both WT and MKP-$1^{-/-}$ MEFs, the reduction of $\Delta\Psi_m$ was more profound in MKP-$1^{-/-}$ MEFs (75%) than WT MEFs (50%). Importantly, when MKP-1 was reconstituted to MKP-$1^{-/-}$ MEFs (MKP-$1^{-/-}$/MKP-1), the $\Delta\Psi_m$ was restored to a level similar to WT MEFs (Fig. 1*F*). These results strongly indicate that the loss of MKP-1 function increases the cell radiosensitivity caused by enhanced apoptosis.

MKP-1 Expression Was Induced by Radiation—To test whether MKP-1 is activated by radiation in normal cells, we measured MKP-1 expression levels in WT MEFs irradiated with 10 Gy of γ-rays. The MKP-1 mRNA levels were increased as early as 1 h after radiation and reached the maximum at 4 h post-radiation (Fig. 2*A*). Consistent with the radiation-induced MKP-1 mRNA, a substantial amount of MKP-1 protein was also induced by radiation (Fig. 2*B*). In contrast, no MKP-1 was induced by radiation in MKP-$1^{-/-}$ MEFs, confirming the deficiency of MKP-1 in MKP-$1^{-/-}$ cells (Fig. 2*B*). These results clearly indicate the inducibility of MKP-1 gene expression under the stress of ionizing radiation. We then studied whether radiation induces MKP-1 expression because of transactivation of MKP-1 gene promoter. We thus constructed and tested mouse MKP-1 promoter luciferase reporter activity. Strikingly, radiation significantly activated the MKP-1 promoter with a similar pattern of time point and level to the induction of MKP-1 mRNA (Fig. 2*C*).

We further studied whether radiation also up-regulates MKP-1 expression in human cells. Similarly to the data obtained from mouse cells, both MKP-1 mRNA and protein levels were significantly induced by radiation in immortalized normal human keratinocytes (Fig. 2, *D* and *E*). In addition, we constructed and tested human MKP-1 promoter activity that was also enhanced with a similar pattern as mouse cells by radiation (Fig. 2*F*). These results suggest that radiation-induced MKP-1 expression is a common feature in mammalian cells, and MKP-1 may function to increase cell survival by inhibition of apoptosis.

NF-κB Was Required for Radiation-induced MKP-1 Gene Expression—Because NF-κB is a well documented transcription factor signaling radiation response (28, 39), we studied whether NF-κB is responsible for radiation-induced MKP-1 expression. Using TFSEARCH program (version 1.3), we found a NF-κB consensus sequence (−1210/−1200) located in the MKP-1 promoter. We next generated a construct with a deletion of the NF-κB-binding site (pGL3-E-MKP-1-ΔNF-κB) (Fig. 3*A*) and tested the MKP-1 promoter activity. Because H_2O_2 is known to be a MKP-1 inducer by radiation (24), we used it as a positive control. Our results showed that the radiation-induced and H_2O_2-induced MKP-1 promoter activities were significantly reduced in the absence of the NF-κB-binding site (Fig. 3*B*), indicating that NF-κB is required for radiation-induced MKP-1 promoter activity.

To confirm that NF-κB is required for radiation-induced MKP-1 gene activation, we tested MKP-1 expression in NF-κB inhibited HK-18/IκB-αM cells, IKK-β inhibitor (IMD-0354), and siRNA transfection against NF-κB subunit p65. Different inhibition levels of MKP-1 were detected with a maximal inhi-

FIGURE 1. **Radiation-induced apoptosis was increased in MKP-$1^{-/-}$ MEFs.** *A*, cell viability was detected by trypan blue exclusion assay in WT and MKP-$1^{-/-}$ MEFs at 24 h post-radiation with 0, 10, or 20 Gy of γ-ray (at least 1000 cells were counted for each group). *B*, flow cytometric analysis of radiation-induced apoptosis (SubG1 fraction) in WT and MKP-$1^{-/-}$ MEFs at 24 h post-radiation with 10 Gy of γ-ray. *C*, increased clonogenic radiosensitivity in MKP-$1^{-/-}$ MEFs determined in WT and MKP-$1^{-/-}$ MEFs after radiation with different doses. *D*, enhanced caspases 9/3 activation in MKP-$1^{-/-}$ MEFs. The cleavage of caspases 9 and 3 was detected by immunoblotting analysis in WT and MKP-$1^{-/-}$ MEFs at indicated times after radiation with 10 Gy of γ-ray. *E*, enhanced caspase 3/7 activity in irradiated MKP-$1^{-/-}$ MEFs. Caspase 3/7 activity was determined using the Caspase Glo® 3/7 assay kit (Promega) at 24 h post-radiation with 10 Gy of γ-rays (normalized with caspase 3/7 activities of control cells without radiation). *F*, reconstitution of MKP-1 expression in MKP-$1^{-/-}$ MEFs improved the $\Delta\Psi_m$ in MKP-$1^{-/-}$ MEFs. $\Delta\Psi_m$ was assayed in WT, MKP-$1^{-/-}$, and MKP-$1^{-/-}$/MKP-1 MEFs at 24 h post-radiation with 10 Gy of γ-ray (data were normalized with WT MEFs without radiation; means ± S.E., $n = 3$; **, $p < 0.01$ compared with WT MEFs).

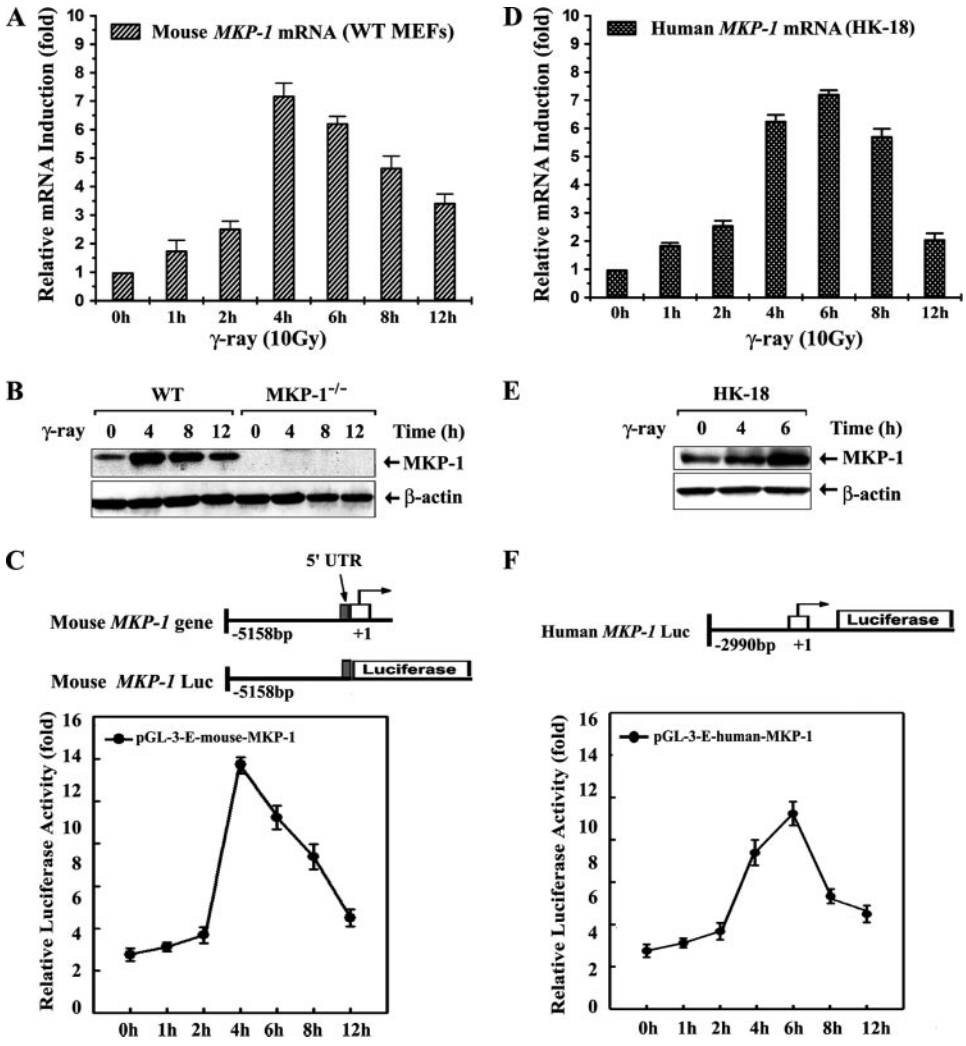


FIGURE 2. **MKP-1 mRNA and protein levels were increased by radiation.** *A*, MKP-1 mRNA level after radiation with 10 Gy of γ-ray was estimated by real time PCR. The data are presented as fold increase over basal MKP-1 mRNA level in WT MEFs without radiation and normalized with β-actin expression. *B*, immunoblotting analysis of MKP-1 protein levels in WT and MKP-$1^{-/-}$ MEFs after radiation with 10 Gy of γ-ray. *C*, MKP-1 promoter activity was enhanced by radiation. *Upper panel*, diagrammatic depiction of pGL3-E-mouse-MKP-1 luciferase reporter; *Lower panel*, luciferase activity was determined in WT MEFs transfected with pGL3-E-mouse-MKP-1 or control vector pGL3-E together with pRL-TK after radiation with 10 Gy of γ-ray (normalized with the luciferase activity in cells transfected with vectors pGL3-E). Radiation-enhanced MKP-1 mRNA (*D*) and protein (*E*) levels in MKP-1 WT human keratinocytes HK18 cells were detected by real time PCR and immunoblotting. *F*, the human MKP-1 luciferase reporter containing the NF-κB-binding site was transfected in HK-18 cells, and luciferase activity was determined with normalization by pRL-TK activity and compared with that in cells transfected with the vector (means ± S.E., $n = 3$).

bition by p65 siRNA transfection in both MKP-1 luciferase activity (Fig. 3*C*) and MKP-1 gene expression (Fig. 3*D*). These results indicate that NF-κB p65 subunit plays a key role in radiation-induced MKP-1 gene expression.

The NF-κB-mediated MKP-1 promoter activity was then analyzed by ChIP assay. As shown in Fig. 3*E*, the MKP-1 promoter region (−1362/−1125) encompassing the NF-κB-binding site (−1210/−1200) was clearly immunoprecipitated in radiation-treated HK18 cells with both anti-p65 and p50 antibodies. In contrast, same ChIP setting did not detect the binding by an anti-c-Rel antibody, neither by species-matched and nor by isotype-matched control antibodies. The failure of binding to the MKP-1 promoter region for c-Rel was not due to inefficient ChIP analysis, because all three NF-κB subunits antibodies were immunoprecipitated with a IκBα promoter region, which was included as a positive control. In addition, control fragments of an upstream sequence on the MKP-1 promoter region (−2100/−1800) and the promoter region of GAPDH were not immunoprecipitated. Therefore the highly selective binding of the NF-κB p65/p50 heterodimer to the MKP-1 promoter suggests that a classical IKKβ-dependent NF-κB activation regulates radiation-induced MKP-1 activation. These results demonstrate that NF-κB is specifically recruited to the MKP-1 promoter to stimulate MKP-1 gene expression and thus confirms a new NF-κB target gene in radiation response.

MKP-1 Selectively Inhibited Radiation-induced Activation of JNK—Because MAPKs are involved in signaling apoptosis and can be inhibited by MKP-1 (16), we investigated whether MAPKs are inactivated by MKP-1 upon radiation by measurement of phosphorylation JNK, p38, and ERK in WT and MKP-$1^{-/-}$ MEFs with or without radiation. In Fig. 4*A*, phosphorylation of all three subfamilies of MAPKs was increased to the similar extent between WT and MKP-$1^{-/-}$ MEFs at 4 h post-radiation, and phosphorylation of p38 and ERK gradually decreased afterward. In contrast, the increased JNK phosphorylation was maintained at elevated levels in MKP-$1^{-/-}$ MEFs at 8 and 12 h post-radiation compared with MKP-1 WT cells at the same time points. Thus, although radiation is able to activate all three MAPKs, the deficiency of MKP-1 appears specifically to contribute to prolonged activation of JNK in MKP-$1^{-/-}$ cells. Therefore, it is possible that radiation-induced MKP-1 inhibits JNK-mediated apoptosis by inactivation of JNK.

It is well documented that ionizing radiation induces cellular redox (oxidation/reduction) alterations (40) that may drastically affect MKP-1 function. MKP-1 belongs to protein-tyrosine phosphatases containing the protein-tyrosine phosphatase signature motif. A key feature of this motif is that the catalytic cysteine is highly sensitive to oxidation because of its low pK_a (41). To insights of MKP-1 induction, we asked whether basal levels of MKP-1 without radiation could inactivate JNK by CHX to block *de novo* synthesis of MKP-1. As shown in Fig. 4*B*, CHX only marginally activated JNK in WT MEFs at 12 h after CHX treatment, consistent with previously reported results

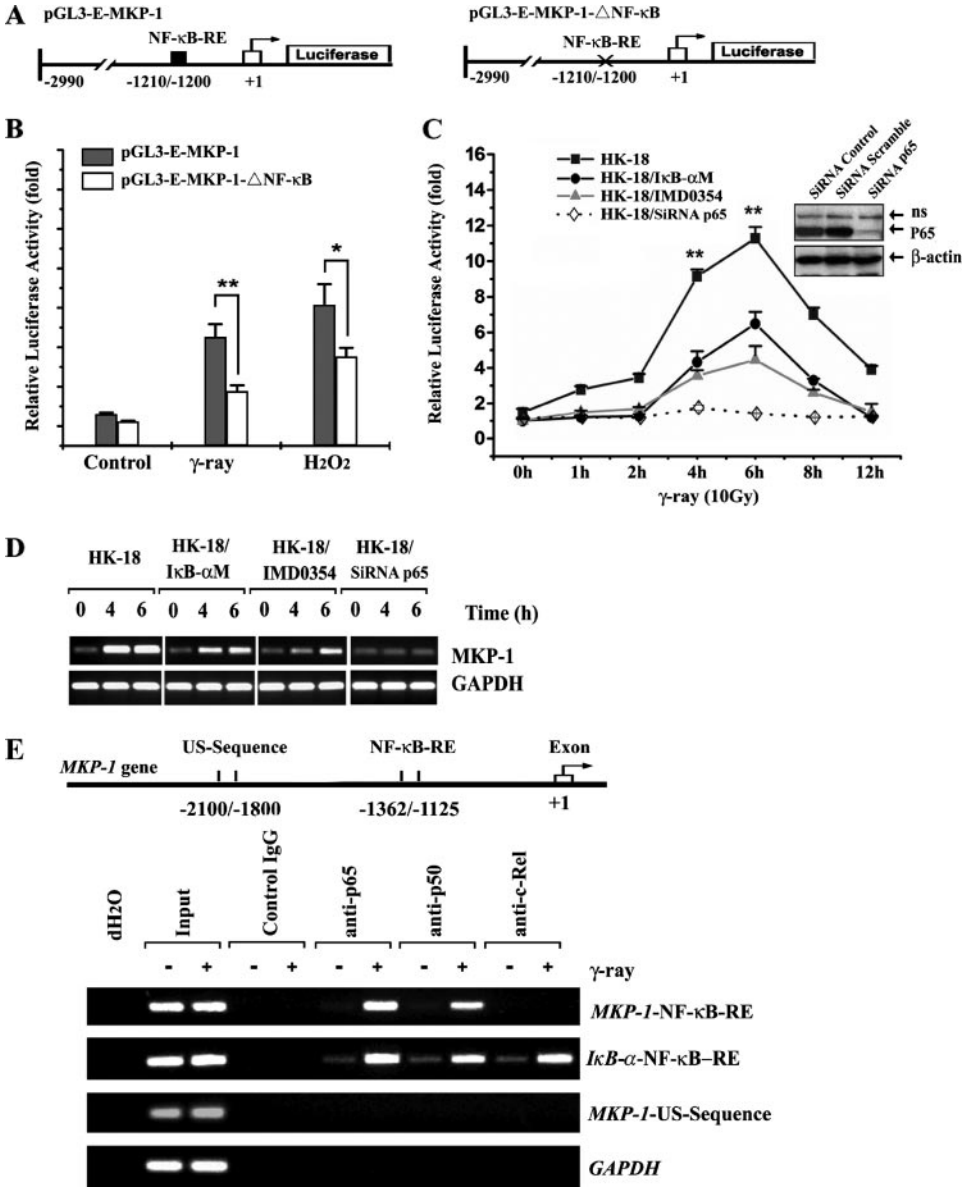


FIGURE 3. **NF-κB was required for radiation-induced MKP-1 expression.** *A*, diagrammatic depictions of human MKP-1 luciferase reporter (*left panel*) and deletion of the NF-κB-binding site (pGL3-E-MKP-1-ΔNF-κB) (*right panel*). *B*, HK-18 cells transfected with pGL3-E-MKP-1, pGL3-E-MKP-1-ΔNF-κB, or pGL3-E together with pRL-TK were irradiated with 10 Gy of γ-ray or treated with H_2O_2 (400 μM, as a control). Luciferase activity was determined 6 h after treatment. *C*, inhibition of NF-κB blocked radiation-induced MKP-1 transactivation. pGL3-E-MKP-1 luciferase reporter activity was measured in HK-18/IκB-αM cells and HK-18 cells treated with IKKβ inhibitor IMD-0354 (2 μM) or p65 siRNA. Luciferase activity was determined at 6 h post-radiation with 10 Gy of γ-ray. *Inset*, inhibition of p65 expression by p65 siRNA transfection. *D*, blocking NF-κB activation inhibited radiation-induced MKP-1 gene expression. MKP-1 mRNA in HK-18/IκB-αM and HK-18 cells treated similarly as in *C* was assessed by semi-quantitative reverse transcription-PCR. GAPDH served as an internal control. *E*, ChIP assay for NF-κB recruitment to the MKP-1 promoter. Two fragments shown as NF-κB-RE (−1362/−1125, encompassing the NF-κB-binding site) and US sequence (−2100/−1800, located 0.8 kb upstream of the NF-κB-RE region served as a negative control) were used in the ChIP assay. Chromatin was immunoprecipitated with antibodies of p65, p50, or c-Rel (an irrelevant antibody as control). Total chromatin before immunoprecipitation (total input) was included as positive control for PCR. The PCR amplification of the IκB-α promoter region (−1134/−902) or the GAPDH was also included as positive and negative controls, respectively (means ± S.E., $n = 3$; *, $p < 0.05$; **, $p < 0.01$).

(42). In contrast, CHX treatment induced a substantially high level of JNK activity in MKP-$1^{-/-}$ cells that reached a maximum at 4 h and remained elevated 12 h after CHX treatment. These results indicate that under basal conditions without radiation treatment, the presence of MKP-1 can attenuate CHX-induced JNK activation as observed in WT MEFs. To further determine whether the increased JNK activity at 12 h post-CHX treatment in WT MEFs was due to degradation of MKP-1, we treated WT MEFs with protease inhibitor MG115 followed by CHX to estimate the MKP-1 degradation rate. The half-life of MKP-1 protein in WT MEFs was ~6 h (Fig. 4*C*), suggesting that the increased JNK activation after CHX treatment is related to MKP-1 protein degradation.

Based these observations, we tested whether newly synthesized MKP-1 induced by radiation in WT MEFs plays a key role in attenuation of JNK phosphorylation. WT and MKP-$1^{-/-}$ MEFs were irradiated with 10 Gy of γ-ray in the presence of CHX. Contrasted to the significant difference in radiation-induced JNK activation between MKP-$1^{-/-}$ and WT MEFs observed in Fig. 4*A*, there was no obvious difference in JNK phosphorylation between WT and MKP-$1^{-/-}$ MEFs (Fig. 4*D*), suggesting that the *de novo* synthesized MKP-1 induced by radiation effectively inhibits radiation-induced JNK activity.

Several lines of evidence indicate that caspase-dependent pathways contribute to the prolonged JNK activation induced by treatment with TNFα (8, 41, 43). We thus hypothesized that the prolonged JNK activation in MKP-$1^{-/-}$ cells by radiation (Fig. 4*A*) was dependent on the caspase pathway. The WT and MKP-$1^{-/-}$ MEFs were treated with or without 10 Gy of γ-ray in the presence of irreversible caspase inhibitor, Ac-Val-Ala-Asp-CMK (Ac-VAD-CMK). Fig. 4*E* demonstrates that caspase inhibitor did not affect the prolonged activation of JNK in MKP-$1^{-/-}$ MEFs, nor did it in the induction of MKP-1 in WT MEFs. Thus, these data demonstrated that inducible expressed MKP-1 contributed to inactivation of JNK, and the radiation-prolonged activation of JNK in MKP-$1^{-/-}$ MEFs was independent on the caspase pathway.

MKP-1 Was Not Involved in Attenuation of JNK Activation Induced by TNFα or IL-1—Because TNFα and IL-1 are well established inducers of NF-κB, we further studied whether TNFα or IL-1 could induce MKP-1 expression and whether

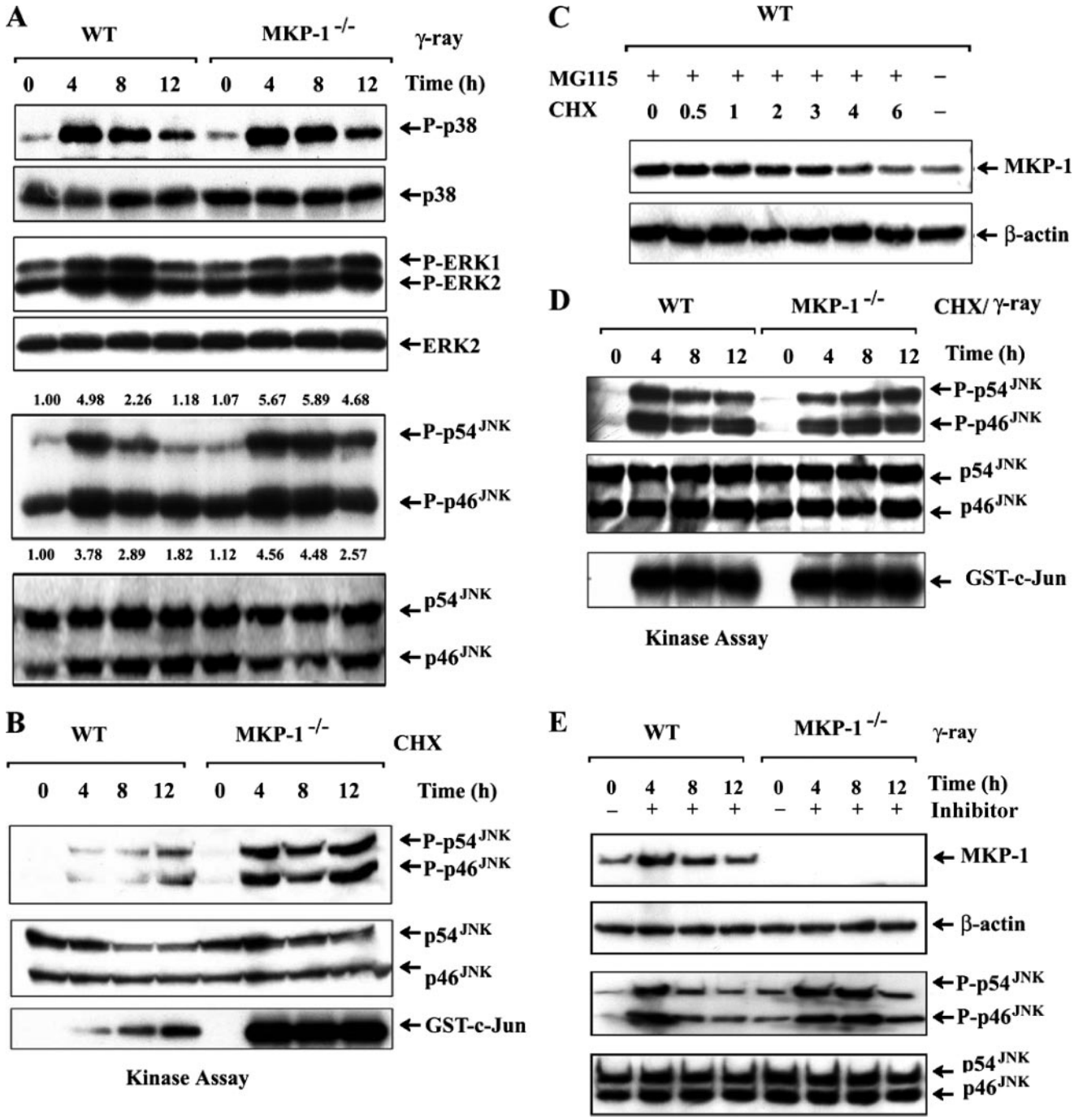


FIGURE 4. **Radiation-induced MKP-1 inhibited JNK activation.** *A*, prolonged JNK activation by radiation in MKP-1$^{-/-}$ MEFs. Total protein and phosphorylation of p38, ERK and JNK were measured at indicated times by immunoblotting in WT and MKP-1$^{-/-}$ MEFs irradiated with 10 Gy of γ-ray. *B*, MKP-1 was required for inactivation of CHX-induced JNK activation. JNK phosphorylation (*top panel*) and total protein levels (*middle panel*) were measured by immunoblotting in WT and MKP-1$^{-/-}$ MEFs treated with CHX (10 μg/ml) for indicated time points. JNK kinase assay (*bottom panel*) was performed with the same treatment using GST-c-Jun as substrate. *C*, WT MEFs were pretreated with proteasome inhibitor MG115 (10 μM) for 12 h and then incubated with CHX (10 μg/ml) for indicated time points, and the MKP-1 protein levels were determined by immunoblotting (the relative MKP-1 level was estimated by densitometry and normalized by b-actin expression). *D*, CHX abolished the difference in radiation-induced JNK activity between WT and MKP-1$^{-/-}$ MEFs. JNK phosphorylation (*top panel*) and total protein levels (*middle panel*) were measured by immunoblotting after treatment with 10 Gy of γ-ray in the presence of CHX (10 μg/ml). JNK kinase assay (*bottom panel*) was performed using GST-c-Jun as substrate. *E*, radiation-induced JNK activation is independent on caspase pathway. WT and MKP-1$^{-/-}$ MEFs were pretreated with or without caspase inhibitor Ac-Val-Ala-Asp-CMK (Ac-VAD-CMK) (50 μM) for 3 h and then exposed to 0 or 10 Gy of γ-ray at indicated time points. The MKP-1, total, and phospho-JNK levels were determined by immunoblotting.

MKP-1 is also involved in attenuation of JNK activation induced by TNFα or IL-1. Our results showed that MKP-1 protein levels were not changed by treatment with either TNFα (Fig. 5*A*) or IL-1 (Fig. 5*B*) in WT MEFs. In addition, both TNFα- and IL-1-induced JNK activation patterns were identical between WT and MKP-1$^{-/-}$ MEFs (Fig. 5). Therefore, MKP-1-mediated JNK inhibition appears to be dependent on the nature of the stimuli.

JNK-mediated Changes in $\Delta\Psi_m$ Were Regulated by MKP-1—Radiation-induced JNK and p38 activities have been linked with pro-apoptosis, whereas activation of ERK is associ-

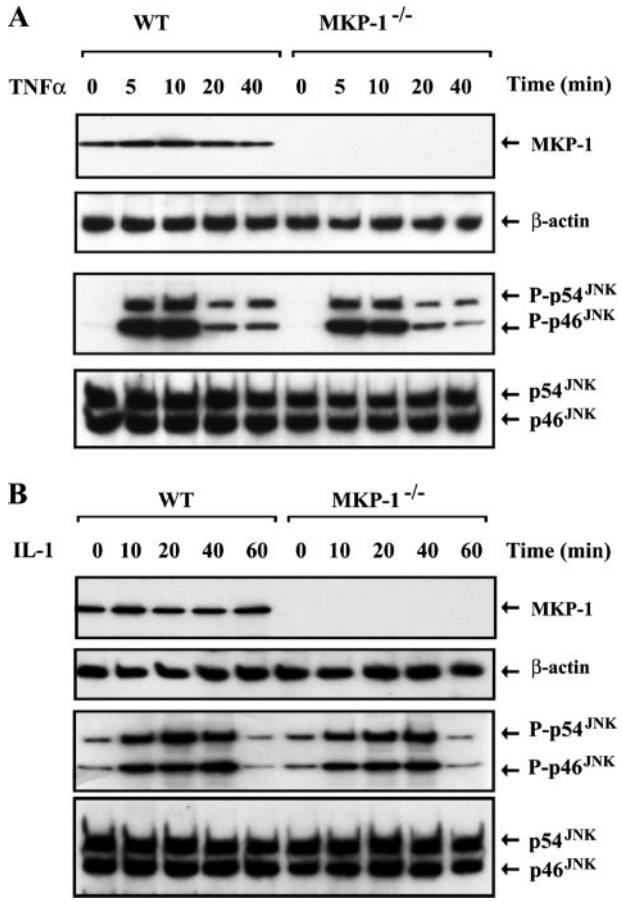


FIGURE 5. **MKP-1 was not required for inhibition of JNK activation induced by TNFα or IL-1.** WT and MKP-1$^{-/-}$ MEFs were incubated with 20 ng/ml of TNFα (*A*), or 20 ng/ml of IL-1 (*B*) for indicated times, and the MKP-1, β-actin, total, and phospho-JNK levels were analyzed by immunoblotting 24 h after treatment.

ated with cell proliferation (6). We thus determined whether MKP-1 specifically targets JNK to inhibit radiation-induced apoptosis. To block kinase activities without affecting expression of individual kinases, we treated WT and MKP-1$^{-/-}$ MEFs with different MAPK inhibitors (ERK inhibitor U0126, p38 inhibitor SB202190, JNK inhibitor SP600125, and Me_2SO as solvent control) followed by radiation with 10 Gy of γ-ray (Fig. 6*A*). Interestingly, blocking the activity of p38 or ERK did not rescue the radiation-decreased $\Delta\Psi_m$. In contrast, blocking JNK activity significantly reversed the radiation-induced decrease in $\Delta\Psi_m$ in MKP-1$^{-/-}$ MEFs to a level similar to that in WT MEFs (Fig. 6*B*), suggesting that MKP-1 specifically regulates JNK-mediated pro-apoptotic response in response to radiation.

To further confirm the involvement of MKP-1 in JNK-mediated pro-apoptotic response, we inhibited the expression of JNK1/2 in WT and MKP-1$^{-/-}$ MEFs by transfection with siRNA against JNK1 and JNK2, and the $\Delta\Psi_m$ was determined after exposure to 10 Gy of γ-ray. As shown in Fig. 6*C*, siRNA against JNK1/2 substantially inhibited expression of JNK1/2 in both WT and MKP-1$^{-/-}$ MEFs. JNK1/2 inhibition by siRNA rescued the radiation-induced decrease in $\Delta\Psi_m$ in MKP-1$^{-/-}$ MEFs (Fig. 6*D*). The results clearly demonstrate that MKP-1 negatively regulates JNK-mediated pro-apoptosis in response to radiation.

Inhibition of MKP-1 Enhanced Proapoptotic Response Induced by Radiation—MKP-1 has been linked with tumor development, progression, and resistance to anticancer drugs (32–34). To determine whether MKP-1 is an effective target to enhance radiation-induced apoptosis, we designed and tested MKP-1 siRNA on enhancement of radiation-induced proapoptotic response in three human cancer cell lines (lung cancer A549 cells, breast cancer MCF-7 cells, and MDA-MB-231 cells, all of which are MKP-1 wild type; data not shown). Radiation significantly increased MKP-1 mRNA and protein levels in all three cancer cell lines (Fig. 7, *A* and *B*), and MKP-1 siRNA significantly reduced the radiation-induced MKP-1 expression (Fig. 7*C*). The caspase 3/7 activity, an index of proapoptotic response, was significantly increased in tumor cells transfected with MKP-1 siRNA, indicating that reduced MKP-1 expression is correlated with radiation-induced apoptosis (Fig. 7*D*).

MKP-1 homologous genes are significantly conserved in evolution; the degree of sequence similarity between MKP-1 proteins of mouse and human is 98%. The siRNA we designed was against human MKP-1 and cannot interfere with mouse MKP-1 mRNA because of differences in three nucleic acids between human and mouse MKP-1 mRNA. Therefore, to further confirm specificity of the effect of MKP-1 inhibition by siRNA on radiation-induced apoptosis in three human tumor cells, we co-transfected human MKP-1 siRNA with mouse MKP-1 (pcDNA3-MKP-1). As shown in Fig. 7*D*, overexpressing the nontargeted mouse MKP-1 completely reversed the increased pro-apoptotic effect of human MKP-1 siRNA. These results provide the evidence that direct inhibition of MKP-1 is capable of enhancing radiation-induced proapoptotic status in cancer cells.

DISCUSSION

This study tested the hypothesis that γ-radiation-induced MKP-1 lessens cell radiosensitivity by inhibition of pro-apoptotic response. Our results demonstrate that radiation-induced MKP-1 expression is regulated by NF-κB causing the attenuation of JNK-mediated pro-apoptotic response. The negative regulation of JNK activity by NF-κB-regulated MKP-1 expression represents a potential therapeutic target to adjust cell radiosensitivity.

MKP-1 Bridged Negative Cross-talk between NF-κB and JNK Pathways—The fate of an irradiated cell is believed to depend on a balance of anti-apoptotic and pro-apoptotic pathways induced by different genotoxic conditions including ionizing radiation. Both anti-apoptotic NF-κB and pro-apoptotic JNK pathways are sensitive to the oxidative stress and are well documented to play essential roles in regulation of radiation-induced anti- and pro-apoptotic responses (29, 30, 44, 45). NF-κB is a well defined stress-sensitive heterodimeric transcription factor that is able to inhibit apoptosis and promote cell survival in response to a variety of genotoxic stress including γ-radiation (28, 46, 47). After long being speculated, the exact cross-talk between radiation-induced pro-survival NF-κB pathway and pro-apoptotic JNK pathway remains elusive. The results of the present study provide evidence indicating that MKP-1 is a new effector gene for NF-κB-regulated anti-apoptotic response (Figs. 2 and 3).

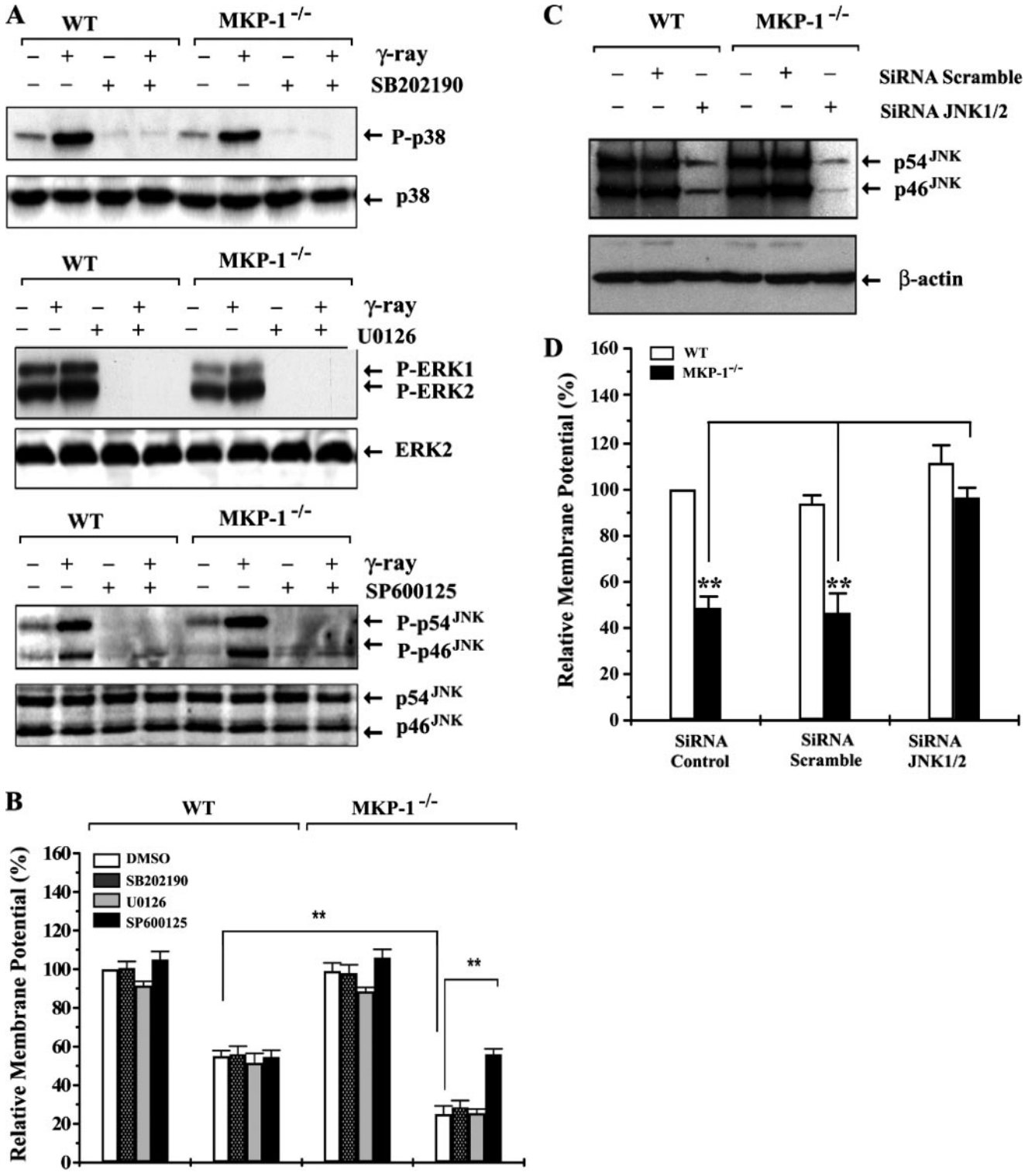


FIGURE 6. **Inhibition of JNK, but not p38 and ERK, inhibited radiation-induced $\Delta\Psi_m$ decrease.** WT and MKP-1$^{-/-}$ MEFs were treated with or without JNK inhibitor SP600125 (2 μM), ERK inhibitor U0126 (20 μM), p38 inhibitor SB202190 (1 μM), or Me_2SO (solvent negative control) for 60 min followed by radiation with 0 or 10 Gy of γ-ray. Protein activity (*A*) and $\Delta\Psi_m$ (*B*) were determined at 24 h post-radiation (the basal $\Delta\Psi_m$ of WT without radiation in the presence of Me_2SO was set as 1; means ± S.E., $n = 3$; **, $p < 0.01$). WT and MKP-1$^{-/-}$ MEFs were transfected with 100 nM of mouse JNK1/2 siRNA for 24 h and JNK protein levels (*C*) and $\Delta\Psi_m$ (*D*) were measured at 24 h post-radiation with 10 Gy of γ-ray (means ± S.E., $n = 3$; **, $p < 0.01$).

NF-κB-mediated MKP-1 activation directly accounts for negative regulation of JNK activation and inhibition of the JNK-mediated pro-apoptotic status.

MKP-1 Selectively Inactivated JNK upon γ-Radiation—Three genes encode JNKs; the *Jnk1* and *Jnk2* genes are ubiquitously expressed, whereas *Jnk3* is expressed in limited tissues. An interesting finding of the current study is that JNK was specifically targeted by radiation-induced MKP-1. Although MKP-1 is shown to be able to inactivate three MAPKs, its substrate selectivity varies because of the nature of different stimuli

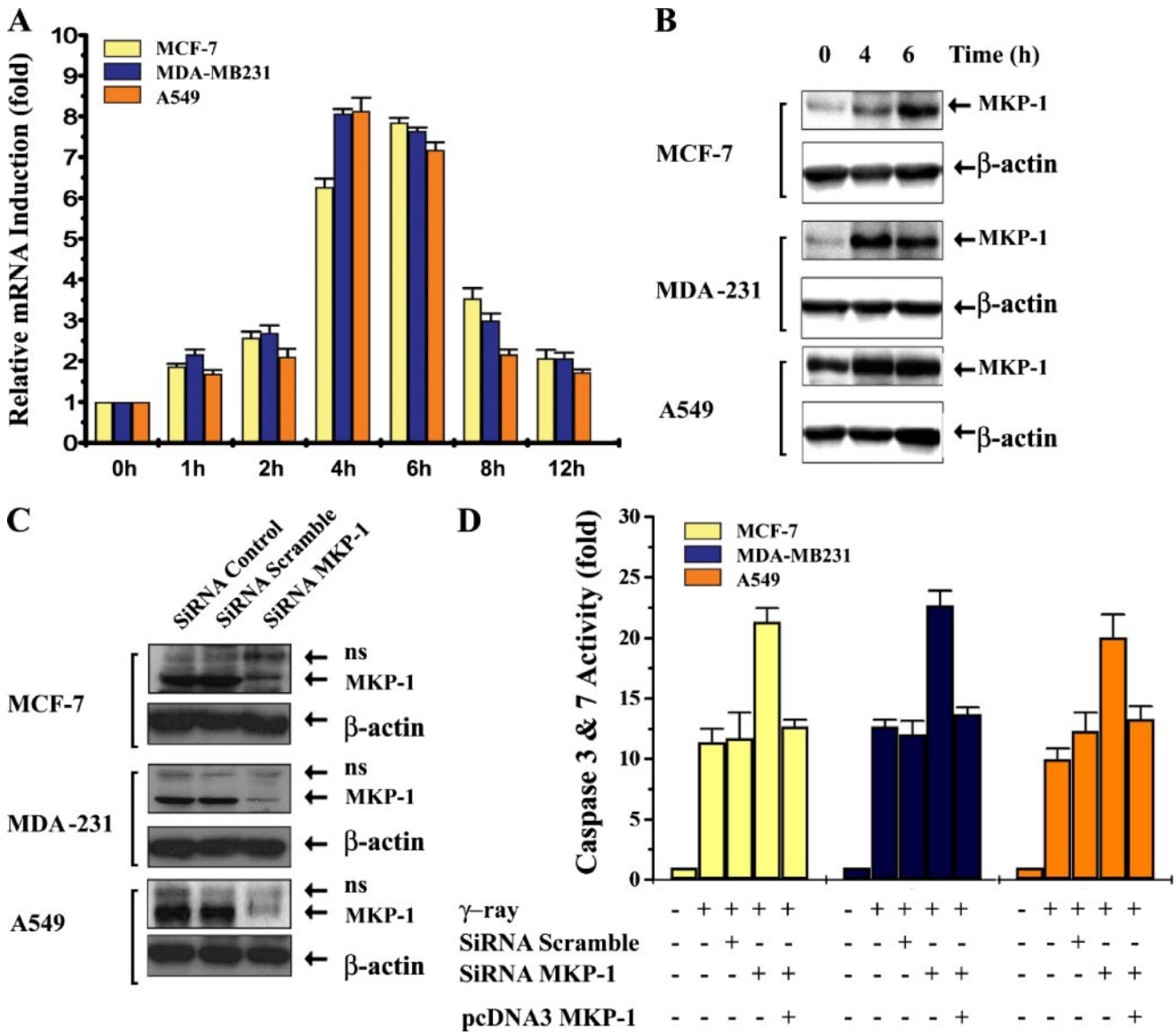


FIGURE 7. **Inhibition of MKP-1 enhanced radiation-induced apoptosis in tumor cells.** MKP-1 mRNA (*A*) or MKP-1 protein (*B*) was measured by real time PCR or immunoblotting in three MKP-1 wild type human tumor cell lines (MCF-7, MDA-MB231, and A549) at indicated times after radiation with 10 Gy of γ-ray. *C*, tumor cells were transfected with the human MKP-1 siRNA (50 nM) and scrambled siRNA as described under "Experimental Procedures" for 48 h, and MKP-1 inhibition was detected by immunoblotting (siRNA control = cells without transfection). *D*, caspase 3/7 activity was analyzed in tumor cells at 24 h post-radiation with 10 Gy of γ-ray following transfection with scrambled or MKP-1 siRNA (50 nM) or transfection with MKP-1 siRNA (50 nM) together with overexpressing MKP-1 by transfection with pcDNA3-MKP-1 for 48 h (means ± S.E.; $n = 3$).

(48). We found that radiation with 10 Gy of γ-ray indeed activated all three members of MAPKs in both WT and MKP-$1^{-/-}$ cells, but only JNK exhibited a prolonged activation in MKP-$1^{-/-}$ cells (Fig. 4*A*). This prolonged JNK activation was not detected by the treatment of TNFα and IL-6 (Fig. 5), suggesting that JNK is the preferred substrate of MKP-1 upon γ-radiation. Because both TNFα and IL-6 are well known inducers for NF-κB activation, which is required to up-regulate MKP-1, the exact mechanisms underlying the specifically targeting of MAPKs by MKP-1 between radiation and other stimulations remain to be elucidated.

The capability of basal level MKP-1 to inactivate JNK varies with different types of cellular stimuli. Interestingly, our results suggested that the basal level of MKP-1 did not significantly suppress the radiation-induced JNK activation (Fig. 4*D*), whereas it almost totally blocked CHX-induced JNK activation (Fig. 4*B*). These results together with the reported data (49) suggest that inactivation of JNK by basal level MKP-1 depends on the distinct nature of stimulus. The low levels of basal MKP-1 protein could be oxidized by reactive oxygen species generated during γ-radiation, which, could result in a dysfunction in the basal MKP-1 function for JNK inhibition. In contrast, this dysfunction of MKP-1 was not detected in CHX-treated cells as reported by others (39). In addition, we found that both MKP-1 induction and MKP-1-mediated attenuation of JNK activation were independent from caspase pathway (Fig. 4*E*). Thus, induction of *de novo* MKP-1 protein synthesis appears to be a specific event required for inhibiting the JNK-mediated apoptosis by radiation.

MKP-1 Induction Was Correlated Negatively with JNK Inactivation upon γ-Radiation—Our data showed a direct link between MKP-1 induction and inhibition of JNK-mediated pro-apoptosis (Fig. 6). In agreement with the prolonged JNK activation in MKP-1 null cells, an elevated apoptosis was induced in MKP-1 null but not in WT MEFs, indicating that prolonged activation of JNK is due to the loss of MKP-1. In addition, blockade of JNK activity by its inhibitor or siRNA abrogated the enhanced apoptosis in MKP-$1^{-/-}$ cells, suggesting that the elevated apoptosis induced by γ-radiation in MKP-$1^{-/-}$ cells is dependent on the JNK activation. Although the JNK inhibitor SP600125 may affect other kinases at high concentrations, it has been broadly used for inactivating JNK (48) as a powerful JNK inhibitor (50). Thus, an acute response of MKP-1 induction is specifically required for inhibiting JNK-mediated pro-apoptosis by radiation.

Inhibition of MKP-1 Sensitized Radiation-induced Proapoptotic Status in Tumor Cells—Previous reports indicate that activation of MKP-1 can cause resistance to chemotherapy (35, 51). MKP-$1^{-/-}$ cells showed higher sensitivity to γ-radiation-induced cell death and lower level of clonogenic survival (Fig. 1), and inhibition of MKP-1 by siRNA enhanced radiation-induced proapoptotic status with enhanced caspase 3 and 7 activity in MKP-1 wild type human cancer cell lines (Fig. 7). These results further highlight a crucial function of MKP-1 in signaling radiation-induced adaptive resistance. In addition, our study revealed a previously unrecognized function of MKP-1 as a key effector protein of NF-κB-mediated pro-survival pathway. Abrogating NF-κB activity has been extensively studied for enhancing anti-cancer therapy, and identification of specific NF-κB effectors is essential for new drug development. The present study indicates that MKP-1 has a therapeutic potential for sensitization of tumor cells to radiotherapy.

We conclude that γ-irradiation-induced MKP-1 plays an anti-apoptosis function via the inhibition of JNK-mediated pro-apoptosis. NF-κB controls the *de novo* synthesized MKP-1 expression that is specifically required for inactivation of JNK, thereby attenuating radiation-induced proapoptotic response and increasing cell survival. siRNA-mediated inhibition of MKP-1 in tumor cells significantly enhanced proapoptotic response. These findings provide the mechanistic insight of a cross-talk between anti-apoptotic NF-κB pathway and pro-apoptotic JNK pathway in irradiated cells.

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