T-LAK Cell-originated Protein Kinase (TOPK) Phosphorylation of Prx1 at Ser-32 Prevents UVB-induced Apoptosis in RPMI7951 Melanoma Cells through the Regulation of Prx1 Peroxidase Activity

Tatyana A. Zykova, Feng Zhu, Tatyana I. Vakorina, Jishuai Zhang, Lee Ann Higgins, Darya V. Urusova, Ann M. Bode, and Zigang Dong

Protein kinases are potential targets for the prevention and control of UV-induced skin cancer. T-cell-originated protein kinase (TOPK) is highly expressed in skin cancer cells, but its specific function is still unknown. We investigated the role of TOPK in UVB-induced apoptosis in RPMI7951 human melanoma cells. Liquid chromatography-tandem mass spectrometry analysis was used to identify proteins that bind with TOPK. Immunofluorescence, Western blot, and flow cytometry were used to assess the effect of UVB on TOPK, peroxiredoxin 1 (Prx1), and apoptosis in RPMI7951 cells. TOPK binds with Prx1 and its phosphorylation of Prx1 at Ser-32 is important for regulation of H2O2-mediated signal transduction. Analysis of the CD spectra of Prx1 and mutant Prx1 (S32A) proteins showed that the secondary structure of Prx1 was significantly altered by phosphorylation of Prx1 at Ser-32. UVB irradiation induced phosphorylation of TOPK in RPMI7951 human melanoma cells and phosphorylated TOPK co-localized with Prx1 in the nucleus. UVB induced the peroxidase activity of Prx1 in vitro and ex vivo. Following treatment with UVB, H2O2 levels and apoptosis were increased in RPMI7951 cells stably expressing TOPK siRNA or stably mutant Prx1 (S32A). Phosphorylation of Prx1 (Ser-32) by TOPK prevents UVB-induced apoptosis in RPMI7951 melanoma cells through regulation of Prx1 peroxidase activity and blockade of intracellular H2O2 accumulation.

PDZ-binding kinase/T-LAK cell-originated protein kinase (PBK/TOPK)3 is a novel MEK3/6-related MAPKK family member that phosphorylates p38 and is involved in H-Ras signaling (1–3). During mitosis, TOPK is phosphorylated at Thr-9 by Cdk1/cyclin B (4, 5), and phosphorylation is required for its kinase activity (6). TOPK expression is regulated by cell cycle-specific transcription factors E2F and CREB/ATF (7). TOPK is found in activated T-LAK cells, lymphoid tumor cells, and normal testicular tissue (1). It is highly expressed in hematologic tumors such as leukemia (8), lymphoma (9), and myeloma (10); and its expression corresponds with malignant potential of these tumors. TOPK is also overexpressed in breast cancer (11) and human colorectal cancer and colorectal cancer cell lines (12). Recent studies suggest a role for TOPK in DNA damage sensing and repair through phosphorylation of histone H2AX (2, 13, 14). Earlier we showed that TOPK is involved in preventing apoptosis in melanoma cells (13) and is a positive regulator of c-Jun-NH2-kinase 1 (JNK1) signaling and H-Ras-induced cell transformation (15). We reported that a positive feedback loop between TOPK and ERK2 increases the carcinogenic properties of HCT116 colorectal cancer cells, and therefore TOPK-regulated signaling might be a potential therapeutic target in colorectal cancer (12).

The 25-kDa protein peroxiredoxin 1 (Prx1) is an antioxidant enzyme that participates in the regulation of hydrogen peroxide-mediated signal transduction and is also implicated in the immune response, cell proliferation, differentiation, and apoptosis (16–20). Prx1 plays a negative role in apoptosis signal-regulating kinase 1 (ASK1)-induced apoptosis (21), and its overexpression in cancer cells could enhance survival. Prx1 is a dual function protein because its phosphorylation at Thr-90 by Cdc2 either decreases its peroxidase activity or its function as a molecular chaperone (22, 23). However, the role of Prx1 in cancer development is not clear.

We report that Prx1 is newly discovered direct target of TOPK. Our results demonstrate that TOPK phosphorylation of Prx1 at Ser-32 inhibits UVB-induced apoptosis in RPMI7951 melanoma cells by increasing Prx1 peroxidase activity and decreasing the intracellular accumulation of H2O2.

MATERIALS AND METHODS

Reagents, Antibodies, and Cell Culture—SK-MEL-5, SK-Mel-28, and RPMI7951 human malignant melanoma epithelioid-like cell lines were from the American Type Culture Collection (Manassas, VA). RPMI7951 cells stably expressing control siRNA or TOPK siRNA were prepared as described (13). Cells were cultured in MEM supplemented with 10% FBS, 2 mM...
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1-glutamine, 25 µg/ml gentamicin, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, and 200 µg/ml G418. Active PBK/TOPK kinase and antibodies that detect cleaved caspase-3, PBK/TOPK and phospho-PBK/TOPK (Thr-9) were from Cell Signaling Technology, Inc. (Beverly, MA). Active TOPK was from Signal Chem (Richmond, BC, Canada). β-Actin was from Sigma/Aldrich and antibodies to detect Prx1, Prx2, and Prx3 were from Upstate (Lake Placid, NY). Anti-peroxiredoxin-SO₃ was from Abcam Inc. (Cambridge, MA).

Identification of Proteins Binding with TOPK (24)—Liquid chromatography–tandem mass spectrometry analysis to determine TOPK-binding partners was performed as described (13). Product ion spectra were correlated with theoretical peptides and proteins using Protein Pilot v.2.0.1 (ABI) and the Paragon algorithm (25).

UVB and Western Blotting—Cells (7 × 10⁵) were treated with UVB (4 kJ/m²) as described (13, 26). Protein concentration was determined by the Bradford method (27). Antibody-bound proteins were detected by chemiluminescence (ECL, Amer sham Biosciences, Piscataway, NJ) and analyzed using the Storm 840 Scanner (Molecular Dynamics, Sunnyvale, CA). Untreated cells were used as negative controls.

Construction of Expression Vectors and Stable Cells—RPMI7951 cells stably expressing control siRNA or TOPK siRNA were prepared and cultured as described (13). The sense siRNA template sequence for TOPK was 5'-GATCCGAGGTTTGGTCATCTCCTCCTCAAGAGAGGAAAATGACAA- AACCCTCTTTTTGGAAA-3' and the antisense siRNA template sequence was 5'-AGCTTTTCCAAAAAAAGGGTTTGTCATTCTCCTCCTCTTGAAACATGAGA CAAACCTCG-3'. The sense and antisense oligonucleotides were annealed and cloned into the pSilencer 3.1-H1 neo vector (Ambion, TX) at the BamHI and HindIII sites followed by transcription. For purification of His-Prx1 or His-Prx3 fusion proteins, the pCMV6-XL5 plasmid (OriGene Technologies, Rockville, MD) was amplified by PCR using 5'-CTGTAAGGACGGTTGCTCC-3' as the sense primer and 5'-GAGGAGAAGGCCCCGCTgacctcgtgagagacaaagaggt-3' and antisense: 5'-GAGGAGAAGGCCCCGCttaattctttgctc-3'. For the C-terminal Prx1 deletion mutant (D128-199, Cdel), sense: 5'-GAGGAGCAGACAAGattctgtcctcattgata-3' and antisense: 5'-GAGGAGAAGGCCCCGTTaagagagacaaagaggt-3'. For Δ-Prx1 deletion mutant (D57-127, Ddel), sense: 5'-GAGGAGCAGACAAGattctgtcctcattgata-3' and antisense: 5'-GAGGAGAAGGCCCCGTTaagagagacaaagaggt-3'. The capitalized bases are sequences required for pET-46 LIC cloning.

Bacterial Expression and Purification of His-Prx1 or Mutant Prx1 or Prx3 or Prx1 Deletion Fusion Proteins—His-Prx1-Wt (Wt), His-Prx1-S32A (S32A), His Prx1-S126A (S126A), His-Prx3 fusion proteins or His-Prx1-deletion mutant proteins (Ndel, Cdel or Ddel) were expressed in BL21 (DE3) bacteria (Novagen). Bacteria were grown at 37 °C to an absorbance of 0.9–1.0 at 600 nm and induced with 0.5 mM IPTG overnight at 15 °C and harvested by centrifugation. Cell pellets were suspended in 50 mM Tris-HCl (pH 8.0) lysis buffer containing 200 mM NaCl, 10 mM imidazole, and 10 mM mercaptoethanol. After sonication and centrifugation, the supernatant fraction was incubated with Ni-NTA-agarose beads (Qiagen, Valencia, CA) overnight at 4 °C. Beads were washed with lysis buffer and PBS and His-proteins eluted with 100 mM imidazole. His-Prx1-Wt, His-Prx1-S32A or His-Prx1-S126A was purified by FPLC on a HiLoad 16/60 system using Superdex-75 gel filtration chromatography (GE Healthcare, Piscataway, NJ) in buffer (20 mM Tris-HCl, 100 mM NaCl). Proteins were concentrated using centrifugal filter tubes with Biomax 5K NWMV membranes (Millipore Corp., Billerica, MA).

Pulldown Assay—The supernatant fraction prepared from RPMI7951 melanoma cells (500 µg of protein) was incubated with TOPK-Ni-NTA-agarose beads (Qiagen, Valencia, CA) overnight at 4 °C. Bacterial beads were washed with lysis buffer and PBS and His-proteins eluted with 100 mM imidazole. His-Prx1-Wt, His-Prx1-S32A or His-Prx1-S126A was purified by FPLC on a HiLoad 16/60 system using Superdex-75 gel filtration chromatography (GE Healthcare, Piscataway, NJ) in buffer (20 mM Tris-HCl, 100 mM NaCl). Proteins were concentrated using centrifugal filter tubes with Biomax 5K NWMV membranes (Millipore Corp., Billerica, MA).

Construction of the GST-TOPK Plasmid and Preparation of the GST-TOPK Protein Beads—A cDNA encoding TOPK was generated by PCR and subcloned into the BamHI/XhoI sites of the pGEX-5X-1 vector (Amer sham Biosciences) to produce the GST-TOPK plasmid. Bacteria were grown at 15 °C and harvested by centrifugation. Cell pellets were sonicated. After centrifugation for 30 min at 15,000 rpm at 26 °C, the supernatant fraction was added to GST-agarose beads.
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FIGURE 1. TOPK interacts with Prx1 in vitro and ex vivo. A, tandem mass spectrum for the doubly charged ion from the peptide sequence LVQAFQFTDK (theoretical, monoisotopic neutral MW of 1195.62 Da), labeled with select experimental product ions, corresponding m/z values, and select ammonium ions (Im). The precursor mass error is 40 ppm. The experimentally determined diagnostic b- and y-type product ions with S/N > 3 are shown on the peptide sequence above the spectrum (S/n = signal to noise). B, TOPK binding with Prx1 ex vivo. Lane 1: input, whole-cell lysate from RPMI7951 human melanoma cells; lane 2: negative control, whole-cell lysate from RPMI7951 cells precipitated with Ni-NTA-agarose beads; lane 3: whole-cell lysate from RPMI7951 cells precipitated with TOPK-Ni-NTA-agarose beads. TOPK and Prx1 binding was confirmed by immunoblotting with anti-Prx1. C and D, the endogenous TOPK-Prx1 complex was immunoprecipitated from RPMI7951 human melanoma cells with a TOPK (C) or a Prx1 (D) antibody, and TOPK or Prx1 were detected by Western blotting with a TOPK or Prx1 antibody. Precipitation with normal IgG served as a negative control. E, schematic diagrams for construction of Prx1 deletion mutants. Ndel (D1–56), Cdel (D128–199), or A del (D57–127) were each introduced into the pET46 Ek/LIC His fusion vector. F, TOPK interaction with Prx1 in vitro and ex vivo. To determine peroxidase activity, we used the Amplex Red Hydrogen Peroxide/Peroxidase assay kit, and proteins visualized by autoradiography or Western blot with anti-TOPK or anti-Prx1 loading control.
siRNA or TOPK siRNA in 100 μl of Krebs-Ringer phosphate buffer (KRPB: 145 mM NaCl, 5.7 mM sodium phosphate, 4.86 mM KCl, 0.54 mM CaCl2, 1.22 mM MgSO4, 5.5 mM glucose, pH 7.35). Cells were harvested after UVB at 0 (control), 0.25, 0.5, and 24 h at 37 °C as described above.

Measurement of H2O2—RPMI7951 cells (2 × 10^4 in 100 μl KRPB) stably expressing mock, Prx1-Wt or Prx1-S32A were incubated at 37 °C for various times after UVB. Measurement of H2O2 released from cells was determined and nonphosphorylated Prx1-Wt and Prx1-S32A were incubated and high salt/PBS (2 × 10^4 in 100 μl reaction) in 10× kinase buffer containing 50 μmol/liter of unlabeled ATP and the reaction was incubated at 30 °C for 60 min. Phosphorylated and nonphosphorylated Prx1-Wt and Prx1-S32A were used for far UV CD spectrometry. CD spectra were recorded (in triplicate) at room temperature with a Jasco 500Aspec spectrophotometer (Jasco Co., Tokyo, Japan). The wavelength range measured was from 250 to 190 nm at a scan rate of 20 nm/min. The CD spectra are expressed in terms of ellipticity, [θ] (degrees × cm^2 × decimol^-1), which is related to the mean residue molecular weight of 110 Da. The following formula was used to generate as [θ] = [θ]observed × S × 110/10 × C × 1, where [θ]observed is expressed in degrees; S = sensitivity of spectropolarimeter; C = concentration of protein, mg/ml; and 1 = path length, mm. Calibration of the spectrometer was prepared with 0.06% ammonium salt of d-camphor-10-sulfonic acid (Katayama Chemical, Japan). The CDPro software package was used for calculation of elements of secondary structure (28, 29).

Flow Cytometry Analysis—UVB-induced apoptosis was determined using the Annexin V-FITC Apoptosis Detection kit (MBL International Corp., Watertown, MA). Cells were harvested with 0.025% trypsin in PBS/5 mM EDTA. After washing with MEM containing serum, cells were incubated 5 min at room temperature with Annexin V-FITC plus propidium iodide according to the protocol provided by the manufacturer.

a CFI Plan Flur 40× oil objective. The channels were collected individually to avoid bleed-through between the 2 channels, and to “balance” the fluorescence intensity in each channel. Confocal Z-sections of 0.6 μm thickness were imaged.

FIGURE 2. TOPK phosphorylates Prx1 in vitro and UVB induces phosphorylation of TOPK in RPMI7951 melanoma cells. A, His-Prx1 and His-Prx3 fusion proteins were purified from BL21 bacteria using Ni-NTA-agarose beads and purity was confirmed by Coomassie Blue R-250 staining. B, in vitro kinase assay to determine whether TOPK phosphorylates Prx1 or Prx3. Results are visualized by autoradiography with [γ-32P]ATP. C, different human melanoma cell lines were screened by Western blot to determine total protein abundance of endogenous TOPK, Prx1, or Prx2. β-Actin verified equal protein loading. D, time-dependent UVB-induced phosphorylation of TOPK (Thr-9). RPMI7951 cells were harvested at various times after UVB (4 kJ/m2) treatment. Phosphorylation of TOPK (Thr-9) or nonphosphorylated TOPK was detected. Data for C and D are representative of three independent experiments.

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Apoptosis was analyzed on a Becton Dickinson FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ).

**Statistical Analysis**—Comparisons were analyzed by one-way ANOVA, and a p value of <0.05 was considered statistically significant.

**RESULTS**

**Prx1 Binds TOPK**—We previously identified 26 proteins that could bind with TOPK (13). Human peroxiredoxin was identified by tandem mass spectrometry as potential binding partner for TOPK. The protein was identified from 15 distinct peptide assignments (54% coverage by amino acid sequence), of which 8 (33% coverage) showed >95% confidence in peptide identification, according to the Paragon scoring algorithm. All tandem mass spectra were validated by visual inspection. A fragment composed of 10 amino acids, LVQAFQFTDK, was identified with a Pro ID confidence of 99% as a peptide from the typical 2-Cys peroxiredoxin family (17, 19, 20) (Fig. 1A). The gene sequence of this peptide was 100% homologous to the sequence of Prx1, which indicated that TOPK could bind with Prx1. Thus, we focused on elucidating the function and physiologic significance of the interaction of TOPK and Prx1.

**TOPK Interacts and Co-localizes with Prx1**—To confirm the interaction of TOPK and Prx1, RPMI7951 melanoma cell lysates were incubated with Ni-NTA-agarose beads (negative control) or with His-TOPK-Ni-NTA-agarose beads at 4 °C overnight. The binding interaction was analyzed by Western blot, and results confirmed that TOPK binds with Prx1 (Fig. 1B). To determine whether the interaction of TOPK with Prx1 occurs endogenously, we used RPMI7951 cell lysates to perform immunoprecipitation experiments with anti-TOPK or anti-Prx1. The results shown that TOPK binds with Prx1 in vivo (Fig. 1, C and D).

To determine the specific binding region of Prx1 that binds with TOPK, we first generated three Prx1 deletion constructs: N-terminal deletion (D1–56) as Ndel; C-terminal deletion (D128–199) as Cdel; and -deletion (D57–127) as del (Fig. 1E).

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**TABLE 1**

**Identification of phosphorylation sites on Prx1**

| Amino acid | Sequence | NetPhos 2.0 Score |
|-----------|----------|------------------|
| Ser-2     | MSSGNA   | 0.014            |
| Ser-3     | MSSGNAK  | 0.002            |
| Ser-30    | FKDISLDY | 0.970            |
| Ser-32    | DISLDYVK | 0.959            |
| Ser-60    | IIASFDKAE| 0.930            |
| Ser-77    | VIGASVDSH| 0.152            |
| Ser-80    | ASVDSSHFC| 0.575            |
| Ser-106   | IPLVSDFKR| 0.006            |
| Ser-126   | DEGISFRGL| 0.736            |
| Ser-152   | PVGRSVDET| 0.942            |
| Ser-181   | WKPQGSDTIK| 0.056            |
| Ser-191   | DVQKSKYE| 0.954            |
| Ser-196   | KEFSKQK  | 0.290            |
| Thr-18    | NFKATAVM| 0.133            |
| Thr-49    | PLDFTVCP | 0.026            |
| Thr-54    | FVCPTXH  | 0.013            |
| Thr-90    | AWYNTPKQ | 0.966            |
| Thr-111   | DPKRTIAQD| 0.287            |
| Thr-143   | LRQITVNDL| 0.404            |
| Thr-156   | SVTDLRV  | 0.790            |
| Thr-166   | AFQFTDKG | 0.123            |
| Thr-183   | PGSDTIKD | 0.077            |

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**FIGURE 3. TOPK phosphorylates Prx1 (Ser-32).** A, comparison of amino acid sequences of Prx1 and Prx3. Amino acids Ser-32 and Ser-126 in Prx1 correspond with Asp-32 and Ala-126 in Prx3, respectively. B, in vitro kinase assay using [-32P]ATP to determine whether TOPK phosphorylates Wt or mutant (S126A or S32A) Prx1. Proteins were visualized by autoradiography. C, TOPK binds with phosphorylated Prx1. TOPK-phosphorylated or nonphosphorylated Prx1 (Wt) or Prx1 mutant (S32A) protein (in vitro kinase assay) were used for binding with GST-TOPK beads. TOPK and Prx1 binding was confirmed by immunoblotting with anti-Prx1. D and E, CD spectra in the far UV range were prepared to determine changes in the secondary structure of Prx1 induced by phosphorylation. D, Prx1-Wt (solid line, 1) or phosphorylated Prx1-Wt (dashed line, 2). E, Prx1-S32A (solid line) or phosphorylated Prx1-S32A (dashed line).
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Prx1-Wt and all deletion mutant proteins were used for assessment of binding with GST-TOPK beads. Results indicated that the N\(_{\text{del}}\) and Δ\(_{\text{del}}\) mutant proteins interacted efficiently with TOPK (Fig. 1F). The results indicated that, deletion of the C-terminal amino acids (D128–199) of Prx1 abrogated the binding of TOPK and Prx1. The crystal structure of Prx1 (30) shows that the C-terminal region contains a potential TOPK-binding site that is located close to Ser-32, which may be important for Prx1 phosphorylation.

Immunocytofluorescence analysis was used to examine the localization and interaction of TOPK and Prx1. Results indicated that following UVB treatment, most phosphorylated TOPK (red) and Prx1 (green) co-localized in the nucleus (supplemental Fig. S1; Merge, bottom right). Overall, the data show that TOPK interacts with Prx1.

**TOPK Phosphorylates Prx1 in Vitro**—The gene sequence of the LVQAFQFTDK peptide is also 60% homologous to the sequence of Prx3. Prx1 is located in the cytosol, whereas Prx3 is only found in the mitochondria (31, 32). To test the specificity of TOPK to phosphorylate Prx1, we determined whether TOPK can phosphorylate Prx3. The Prx proteins were purified from BL21 bacteria using Ni-NTA-agarose beads, and purity was confirmed by Coomassie Blue R-250 staining (Fig. 2A). These proteins were used as a substrate for active TOPK. Prx phosphorylation was visualized by autoradiography in the presence of [γ-\(^{32}\)P]ATP. Results strongly indicated that TOPK phosphorylates Prx1 and not Prx3 (Fig. 2B).

**TOPK and Prx1 Are Expressed in Cancer Tissues and Human Melanoma Cells**—Our earlier study showed that TOPK is highly expressed in human colorectal cancer tissues and cell lines and plays an important role in colorectal cancer (12). Furthermore, melanoma cells expressing high levels of TOPK were more resistant to arsenite-induced apoptosis (13). These results suggested that the TOPK protein level might be higher in malignant tissues compared with normal tissues. Results of a human cancer tissue array analysis indicated that the abundance of TOPK and Prx1 proteins was greater in malignant melanoma tissues (supplemental Fig. S2). We also examined the levels of TOPK and Prx1 in different melanoma cell lines. Results indicated that the expression of TOPK was highest in SK-MEL-28 or RPMI7951 cell lines compared with SK-MEL-5 cells (Fig. 2C). The abundance of Prx1 was similar in the three cell lines. Human Prx1 and Prx2 share >90% homology in their amino acid sequences (19), but our results suggest that Prx2 abundance is very low in RPMI7951 cells (Fig. 2C). Based on these results, we chose the RPMI7951 melanoma cell line to study the function and relationship of TOPK and Prx1.

**UVB Induces Phosphorylation of TOPK in RPMI7951 Melanoma Cells**—UVB is an important etiological factor in human skin cancer (33). The effect of UVB irradiation on phosphorylation of TOPK (Thr-9) in RPMI7951 melanoma cells was examined by Western blot. Results indicated that UVB (4 kJ/m\(^2\)) induced a strong time-dependent phosphorylation of TOPK at Thr-9 (Fig. 2D).

**TOPK Phosphorylates Prx1 at Ser-32**—Because TOPK is a serine/threonine kinase, we examined potential phosphorylation of serine and threonine residues in Prx1 using NetPhos 2.0 (34) (Table 1). We compared sequences (Fig. 3A) of Prx1 and Prx3 (negative control) because TOPK phosphorylated Prx1 but not Prx3. We found that only Ser-32 and Ser-126 are potential sites for phosphorylation by TOPK. To confirm the possibility that TOPK phosphorylates these serine residues, we constructed and expressed His-Prx1-Wt or His-Prx1-S32A or His-Prx1-S126A mutant fusion proteins in BL21 bacteria. Proteins were purified by FPLC and purification of proteins was confirmed by Coomassie Blue R-250 staining (supplemental Fig. S3A). Purified protein fraction 3 for each protein was used...
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for an in vitro kinase assay (Fig. 3B). The results showed that mutation of Ser-32 to Ala (S32A) abrogated the phosphorylation of Prx1, whereas mutation of Ser-126 to Ala (S126A) had no effect on phosphorylation of Prx1. This result indicated that TOPK phosphorylated Prx1 at Ser-32 only.

Phosphorylation Prx1 at Ser-32 by TOPK Mediates Changes in the Secondary Structure of Prx1—TOPK-phosphorylated or nonphosphorylated wild-type or mutant (S32A) Prx1 was used to assess binding with GST-TOPK beads to determine whether phosphorylation of Prx1 is necessary for the interaction. The data indicated that TOPK bound to nonphosphorylated Prx1 but not to phosphorylated Prx1 (Fig. 4A). This result was confirmed by the calculation of the elements of secondary structure (supplemental Table S1).

TOPK Regulates the Level of H2O2 through Its Phosphorylation of Prx1—Prx1 can be inactivated by oxidation of the active Cys-52 site to sulfenic acid (Cys-SO) or sulfonic acid (Cys-SO3). The significance of this inactivation as a means to regulate intracellular H2O2 levels has generated interest in peroxiredoxin as a mediator of H2O2-regulated signaling pathways. The sulfenic or sulfonic forms of Prx1 lose their anti-peroxidase activity, which allows cells to accumulate H2O2 for signaling or pathogenesis in inflammation or cancer (35–37). The anti-peroxidase activity of Prx1 decreases and H2O2 level was accumulating.

The phosphorylation of Prx1 by TOPK on Prx1 peroxidase activity ex vivo, we compared RPMI7951 cells stably expressing TOPK siRNA or control siRNA. Cells were treated with UVB and peroxidase activity was determined at 37 °C at various times after UVB. At 24 h, cells expressing TOPK siRNA exhibited about a 2-fold decrease in peroxidase activity compared with cells expressing control siRNA (Fig. 4B). Overall, these results show that phosphorylation of Prx1 (Ser-32) by TOPK increases the peroxidase activity of Prx1 and suggests that this phosphorylation is important for optimal function of Prx1.

Phosphorylation of Prx1 (Ser-32) Increases Peroxidase Activity—To investigate the effect of phosphorylation at Ser-32 on the peroxidase activity of Prx1 in vitro, we first performed a kinase assay following phosphorylation, Prx1 peroxidase activity was assessed. The peroxidase activity of Prx1-Wt after phosphorylation by TOPK was increased more than 2-fold compared with the nonphosphorylated Prx1-Wt (Fig. 4A). In contrast, TOPK had no effect on peroxidase activity of the mutant Prx1-S32A protein. To investigate the effect of secondary structure of phosphorylated Prx1, only the β-strand structure decreased by 12% (supplemental Table S1). The CD spectrum results indicated that TOPK binds with Prx1 and phosphorylation of Prx1 by TOPK leads to significant changes in the secondary structure of Prx1 that affects the CD spectrum in the far UV CD (Fig. 3D, line 2). This result was confirmed by the calculation of the elements of secondary structure (supplemental Table S1).
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compared with mock or Prx1-Wt cells (Fig. 4D), indicating that TOPK suppresses H$_2$O$_2$ accumulation and the phosphorylation of Prx1 (Ser-32) is important for this function.

**TOPK Inhibits UVB-induced Apoptosis in RPMI7951 Melanoma Cells**—We previously reported that high levels of TOPK in different melanoma cells were associated with resistance to arsenite-induced apoptosis (13). RPMI7951 melanoma cells treated with UVB show an approximate 12% increase in early apoptosis (Fig. 5A). At 24 h after UVB, a significant ($p < 0.0001$) increase in early apoptosis was observed in TOPK siRNA cells compared with control siRNA-transfected cells (Fig. 5B). This suggested that the absence of TOPK sensitizes cells to UVB-induced apoptosis. Cells overexpressing Prx1 (Wt) exhibit a significant ($p < 0.001$) decrease in UVB-induced early apoptosis compared with cells stably expressing mock or mutant Prx1-S32A (S32A) (Fig. 5C). This result was further confirmed with the observation of markedly decreased levels of cleaved caspase-3 in Prx1-Wt cells compared with mock or mutant Prx1-S32A cells (Fig. 5D). Taken together, these results indicated that TOPK plays an important role in suppressing UVB-induced apoptosis that is mediated through its phosphorylation of Prx1 at Ser-32.

**DISCUSSION**

UV irradiation activates various signaling pathways that are oncogenic or protective or both (33). Progress in understanding the mechanisms of UV-induced signal transduction could lead to the use of protein kinases as specific targets for the prevention and control of skin cancer. The mitogen-activated protein (MAP) kinase pathway is activated after UVB treatment. Previous studies indicated that TOPK is heavily involved in MAP kinase signaling. Yeast two-hybrid screening analysis suggested a possible association between Raf and TOPK (41); and p38 MAP kinase and c-Myc are potential substrates of TOPK (1, 8). TOPK appears to play an important role in melanoma. We found that TOPK was more abundant in malignant melanoma tissues compared with squamous and basal carcinoma tissues and that UVB increased the phosphorylation of TOPK in RPMI7951 melanoma cells. We previously identified 26 individual proteins that could bind with TOPK (13), including peroxiredoxin 1. Prx1 is a member of the redox-regulating peroxiredoxin protein family and has been shown to be elevated in several cancers. Its ability to enhance the survival and progression of cancer cells has been suggested (42–45). Prx1 plays a role in regulating hydrogen peroxide levels, which might be important in melanoma. The RPMI7951 cells express high levels of Prx1, but not Prx2, which makes these cells a good model to study Prx1 function.

Our results clearly indicate that TOPK phosphorylates Prx1 at Ser-32 and increases its peroxidase activity. Phosphorylation of Prx1 at Thr-90 was reported to decrease Prx1 activity and the resulting intracellular accumulation of H$_2$O$_2$ was suggested to be important for progression of the cell cycle in HeLa cells (22). A mutant Prx1-T90D (i.e. a mimic form of phosphorylated-Prx1) showed higher chaperone activity (23, 46). In the present study, we showed that UVB induced phosphorylation of TOPK at Thr-9. However, TOPK did not phosphorylate Prx1 at Thr-90, but instead phosphorylated Prx1 at Ser-32, resulting in increased peroxidase activity of Prx1 and reduced accumulation of H$_2$O$_2$ in vitro and ex vivo. No change was observed in chaperone activity (data not shown). Prx1 is reportedly involved in ASK1-mediated apoptosis induced by H$_2$O$_2$ and negatively regulates ASK1-induced apoptosis in human embryonic kidney 293 and HeLa cells (21). Our results suggest that phosphorylation of Prx1 (Ser-32) by TOPK is associated with an inhibition of UVB-induced apoptosis in RPMI7951 melanoma cells that is mediated by increased Prx1 peroxidase activity associated with a decreased level of H$_2$O$_2$. Based on our results, increased TOPK and Prx1 in melanoma might play a critical role in providing resistance against UVB-induced apoptosis. Further studies on the mechanisms of the regulation of TOPK activity and the development of TOPK- or Prx1-targeting drugs might contribute to treatment of melanoma.

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