Supporting Information

Su *et al.* 10.1073/pnas.0807975106

SI Text

Cell Lines and Culture Conditions. Normal human immortalized prostate epithelial cells P69 and DU-145 and LNCaP prostate carcinoma cells were cultured as described (1). Normal human immortal mammary epithelial cells HBL-100 and MCF-7 and T47D human breast cancer cells were cultured as described (2). Normal human immortal melanocytes FM516-SV and HO-1 and MeWo melanoma cells were cultured as described (3). PHFAs and T98G, U87MG, and H4 human malignant glioma cell lines were cultured as described (4). Normal human pancreatic mesothelial cells LT2 and PANC-1 and BxPC-3 pancreatic cancer cells were cultured as described (5). CREFs and CREFs transformed by H-ras, v-src, HPV-18, and a specific temperature-sensitive mutant of type 5 adenovirus H5hrl (CREF-ras, CREF-src, CREF-HPV, and CREF-H5hrl, respectively) were cultured as described previously (6). CREF cells were transfected with a c-jun expression vector and stable clones were generated by selection with hygromycin. All cultures were maintained at 37 °C in a humidified 5% CO_2/95% air incubator.

Construction of Adenovirus and Plasmids. Ad.SARI was constructed using recombination in bacteria as described previously (2). Ad.vec was used as control. SARI expression plasmid was created by cloning SARI cDNA in pcDNA3.1 with or without Hygro vector. SARI-mt was created by site-directed mutagenesis kit (Stratagene).

Cell Growth and Apoptosis Studies. Cell growth was monitored by viable cell counting using the trypan blue dye exclusion assay. Apoptosis was monitored by Annexin V binding assays and propidium staining followed by flow cytometry as described (1, 3). The data were analyzed by FlowJo software.

Transient Transfection and Luciferase Assay. Cells were seeded at 2×10^5/35-mm tissue culture plates and transfected ≈24 h later with Lipofectamine-2000 transfection reagent (Invitrogen) and 6 μg of plasmid DNA per plate that included 5 μg of AP-1-luc and 1 μg of β-galactosidase-expression plasmid (pSV-β-gal; Promega) according to the manufacturer's instructions. Luciferase assays were performed 48 h after transfection using a Luciferase reporter gene assay kit (Promega) according to the manufacturer's protocol. The β-galactosidase activity was determined using the Galacto-Light Plus kit (Tropix). Luciferase activity was normalized by β-galactosidase activity and the data from triplicate determinations were expressed as mean ± SD.

5′ and 3′ Rapid Amplification of cDNA Ends. 5′ and 3′ rapid amplification of cDNA ends was performed using a GeneRacer kit (Invitrogen) according to the manufacturer's instructions. The PCR products were cloned into a TA-cloning vector (PCR2.1; Invitrogen) and sequenced.

RNA Extraction, Northern Blot Analysis, RT-PCR, Quantitative RT-PCR, and Multiple Tissue Northern Blot. Total cellular RNA was isolated by the guanidinium/phenol extraction method and Northern blotting was performed as described (6). Fifteen micrograms of RNA were denatured and electrophoresed in 1.2% agarose gels with 3% formaldehyde, transferred to nylon membranes, and hybridized with ^{32}P-labeled cDNA probes as described previously (6). Following hybridization, the filters were washed and exposed for autoradiography. The cDNA probes were full-length human SARI and GAPDH. For RT-PCR, 5 μg of total RNA was used for reverse transcription using SuperScript II reverse transcriptase (Invitrogen) according to standard methods. The primers used for PCR were as follows: IL-8 sense, 5′ GGTGCAGAGGGTTGTGGAGAA 3′; IL-8 antisense, 5′ GCAGACTAGGGTTGCCAGATT 3′; GAPDH sense, 5′ ATGGGGAAGGTGAAGGTCGGAGTC 3′; GAPDH antisense, 5′ GCTGATGATCTTGAGGCTGTTGTC 3′. Quantitative RT-PCR was performed using a kit from Stratagene based on SYBR Green I DNA-binding dye. Multiple tissue Northern blot was obtained from Clontech and hybridization was performed according to the manufacturer's instruction.

Preparation of Whole-Cell Lysates, Western Blotting, and Co-Immunoprecipitation. Western blotting was performed as previously described (6). Briefly, cells were harvested in radioimmunoprecipitation assay buffer (1× PBS solution, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) containing protease inhibitor mixture (Roche), 1 mM Na_3VO_4, and 50 mM NaF and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was used as total cell lysate. Thirty micrograms of total cell lysate were used for SDS/PAGE and transferred to a nitrocellulose membrane. The primary antibodies used were anti-HA (Covance), anti-Cyclin A, anti-Cyclin D1, anti-Cyclin E, and anti-EF1α (Upstate). For, co-immunoprecipitation, cell lysates were incubated with 10 μl of 50% Protein A agarose at 4 °C for 1 h to eliminate non-specific interactions. Samples were centrifuged and mixed with anti-HA or anti-c-Jun antibodies overnight at 4 °C. Immune-complexes were precipitated with 25 μl of 50% protein A agarose for 2 h. The immunoprecipitates were washed very gently three times with the immunoprecipitation buffer, resuspended in resolving buffer, and subjected to SDS/PAGE.

EMSA. Nuclear extracts were prepared from 2 to 5×10^8 cells using consensus AP-1 probe (Santa Cruz Biotechnology) as described (6). The double-stranded oligonucleotides were labeled with [γ-^{32}P]ATP (Amersham) and T4 polynucleotide kinase. The labeled probes were then incubated with nuclear extract at room temperature for 30 min. The reaction mixture consisted of ^{32}P-labeled deoxy-oligonucleotides (≥50,000 cpm), 2 μg of poly(dI–dC), and 10 μg of nuclear protein extract with 10 mM Hepes pH 7.5, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, and 12.5% glycerol. After incubation for 30 min at room temperature, the reaction mixtures were electrophoresed on a 5% non-denaturing polyacrylamide gel with 0.5× Tris-borate-EDTA buffer (160 V for 3 h). The gel was dried and autoradiographed. EMSA was also performed with *in vitro* translated proteins generated by TNT-reticulocyte expression system (Promega) using SARI and c-jun expression constructs and AP-1 consensus probe instead of using nuclear extracts.

ChIP Assays. ChIP assays were performed using a commercially available kit from Active Motif. HeLa cells (5×10^7) were fixed with formaldehyde for 10 min at room temperature. Cells were harvested and the nuclei were isolated using a dounce homogenizer. The nuclear pellet containing chromatin was sheared with enzyme shearing mixture solution, the chromatin was pre-cleared with protein G beads and anti-SARI antibody was added to the pre-cleared chromatin. The DNA-protein complex was precipitated using protein G beads and washed thoroughly, and DNA was eluted from the beads. The eluted DNA was treated with RNase A and proteinase K, purified, and used as template for PCR using IL-8 promoter-specific primers:

sense, 5′ ATGTCAGCTCTCGACGAAAA-TAGA 3′; antisense, 5′ GGAGGGATTGCAAGGTTTAGC 3′. The PCR products were analyzed by agarose gel electrophoresis.

Immunofluorescence Analysis. Cells (1×10^5) were grown on two chamber slides (BD Falcon Biosciences) and the next day were infected with 50 pfu/cell of Ad.vec or Ad.SARI. After 24 h, cells were fixed in 4% paraformaldehyde in PBS solution for 30 min and permeabilized by 0.1% Triton X-100 in PBS for 10 min. Cells were rinsed in PBS solution, blocked in 5% BSA in PBS solution for 2 h, and then incubated with anti-HA and anti-c-Jun antibodies overnight. Cells were washed three times for 5 min each in PBS solution and incubated with anti-mouse-FITC and anti-rabbit-rhodamine secondary antibodies (Molecular Probes) for 2 h. Cells were washed three times for 5 min each in PBS solution. Slides were mounted and cells were visualized using a Zeiss LSM 510 fluorescence and a ×100 objective.

Generation of Anti-SARI Antibody. The SARI cDNA was cloned in the pET vector. The His-tagged recombinant protein was generated in *E. coli* and purified using Ni-NTA metal chelate affinity chromatography. The purified protein was used to raise polyclonal antibodies in the rabbits according to the manufacturer's protocol (Invitrogen).

Mammalian Two-Hybrid Assay. Plasmid pCMV-AD was used for cloning the c-jun cDNA to be expressed as a fusion with the activation domain of VP16 (Clontech). Plasmid pCMV-BD was used to clone the SARI cDNA to be expressed as a fusion with DNA-binding domain of GAL4. These constructs were transfected along with a reporter vector that contains five GAL4 binding sites (GAL4UAS) upstream of a minimal TATA box promoter driving the expression of a luciferase reporter gene. Luciferase activity was monitored by a luminometer and normalized by β-galactosidase activity.

Statistical Analysis. All experiments were performed at least three times. The results are expressed as mean ± SD. Statistical comparisons were made using an unpaired two-tailed Student *t* test. A P value <0.05 was considered significant.

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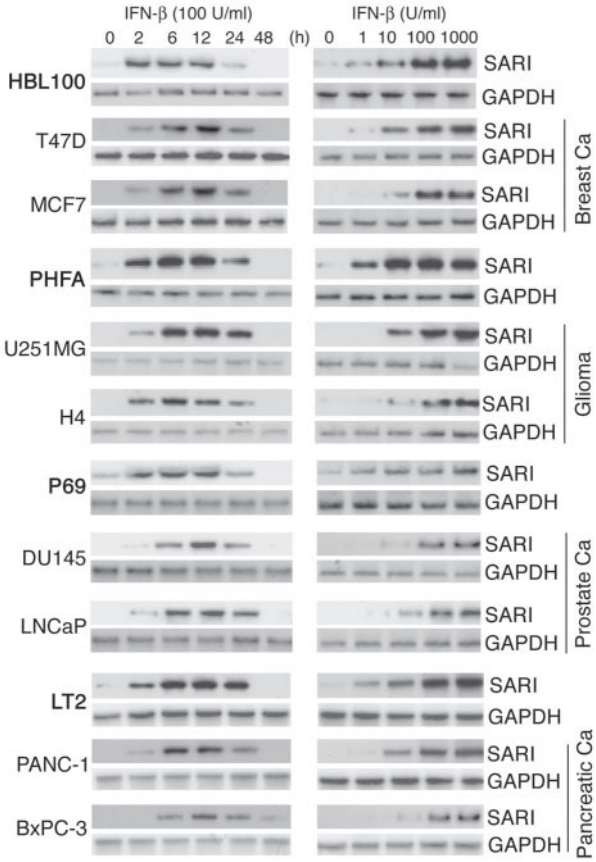

Fig. S1. Dose- and time-dependent induction of SARI mRNA in different cell types. The indicated cells were treated with IFN-β (100 U/ml) for the indicated time points (*Left*) and with IFN-β at the indicated doses for 12 h (*Right*), and SARI and GAPDH expression was analyzed by Northern blot analysis. The normal cells (HBL100, PHFA, P69, and LT2) are in boldface. HBL100 is an immortal breast epithelial cell. P69 is an immortal prostate epithelial cell and LT2 is immortal pancreatic mesothelial cell.