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Constitutive Activation of Transcription Factor AP-2 Is Associated with Decreased MnSOD Expression in Transformed Human Lung Fibroblasts

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ABSTRACT

Activator protein-2 (AP-2) is a transcription factor with transactivating and transrepressing potential in different promoter contexts. AP-2 contains seven cysteines, and its *in vitro* DNA binding activity is redox-sensitive. Superoxide dismutase-2 (SOD2), which encodes the antioxidant enzyme manganese superoxide dismutase (MnSOD), is a putative tumor suppressor gene whose loss of expression is associated with the malignant phenotype. SOD2 promoter mutations that generate new AP-2 sites are associated with loss of MnSOD expression in cancer cells. In the current study, we have identified an inverse expression pattern between AP-2 and MnSOD in normal versus transformed human cells. MRC5 cells are a normal human lung fibroblast cell strain that is mortal and senesces after a certain number of passages *in vitro*. MRC5-VA is a simian virus transformed variant of MRC5. We determined the levels of expression of MnSOD and AP-2 in these two cell types at the levels of mRNA, protein, and activity. Our results indicated that MnSOD expression was significantly decreased in MRC5-VA cells compared with MRC5 cells at each level of investigation, whereas AP-2 showed an opposing pattern of expression and DNA binding activity. These results suggest that AP-2 may participate in the mechanism(s) underlying decreased expression of SOD2 in transformed cells. Antioxid. Redox Signal. 3, 387–395.

INTRODUCTION

ACTIVATOR PROTEIN-2 (AP-2) is a family of cell type-specific, developmentally regulated transcription factors that have been implicated as critical regulators of gene expression during vertebrate development, embryogenesis, and carcinogenesis (3, 25, 26, 29). Although AP-2 is generally considered to be a transcriptional activator, it has also been shown to negatively regulate the transcription of several genes, including type I collagen (23), acetylcholinesterase (8), hepatocyte growth factor (16), retinal fatty acid binding protein (2), and C/EBPα (CAAT enhancer binding protein-α) (17), to name a few. In most of these cases, it has been proposed that AP-2 functions as a repressor by displacing or competing with a positive transcription factor(s) that has a binding site that overlaps, or is adjacent to, the AP-2 recognition site.

SOD2 (superoxide dismutase-2) is a nuclear gene that encodes the mitochondrially targeted antioxidant enzyme manganese superoxide dismutase (MnSOD; EC 1.15.1.1) (6). MnSOD catalyzes the conversion of superoxide, produced as a by-product of oxidative phosphorylation, to hydrogen peroxide (10). SOD2 is

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also a putative tumor suppressor gene whose loss of expression is associated with maintenance of the malignant phenotype (4). However, with the exceptions of malignant melanoma and glioma (20, 24), loss of constitutive MnSOD expression is seldom accompanied by deletion of the SOD2 gene. Thus, the mechanism(s) responsible for down-regulated expression of MnSOD in some cancers remains to be determined. Our previous work suggested that epigenetic alterations involving aberrant methylation near a transcriptional enhancing *cis*-element in the second intron of the human SOD2 gene might contribute, at least in part, to its decreased expression in transformed human cells (14). In addition, mutations in the SOD2 promoter that generate new AP-2 binding sites have been associated with loss of MnSOD expression in human cancer cells (31). Although the mechanism(s) for loss of SOD2 expression in cancer is currently unknown, it is clear that enforced overexpression of MnSOD in cancer cells leads to at least partial reversion of their malignant phenotype (7, 19, 21, 33, 34).

In the current study, we have established an inverse correlation between AP-2 activity and MnSOD expression in a matched pair of normal and transformed human lung fibroblasts. MRC5 cells are a normal human lung fibroblast cell strain that is mortal and senesces after a certain number of passages *in vitro* (15). MRC5 cells lack detectable AP-2 activity (12) and have abundant MnSOD expression. In contrast, MRC5-VA is a simian virus 40 (SV40) immortalized variant of MRC5 (5, 9) that has gained constitutive AP-2 activity (12) and concomitantly lost MnSOD expression. This work establishes a new model system for studying the mechanism(s) of transcriptional repression of the SOD2 gene in transformed cells compared with their normal cell counterparts.

To examine the superoxide-scavenging activity of these cells, we measured the levels of expression and activity of MnSOD in these two cell types at the levels of mRNA, protein, and enzyme activity. Our results indicated striking differences in the SOD2 gene expression and activity profiles in these two cell types that were opposite of the pattern of AP-2 activity. This model system should be useful for future studies investigating the mechanism(s) underlying differential expression of SOD2 in virally transformed cells specifically and malignantly transformed cells in general.

MATERIALS AND METHODS

Cell culture

MRC5 cells were obtained from ATCC (American Type Culture Collection). SV40 transformed MRC5 cells, MRC5-VA, were a kind gift from Dr. Peter Karran. Cells were routinely maintained in Dulbecco's modified Eagle's medium (Life Technologies/BRL) supplemented with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin. Cells were grown at 37°C in humidified air with 5% CO_2.

Northern blot analysis

Total cellular RNA was isolated with Trizol RNA isolating reagent according to the protocol specified by the supplier (Life Technologies/BRL). Fifteen micrograms of total cellular RNA was electrophoresed on an agarose gel containing formaldehyde and transferred to nylon membrane (DuPont). The membrane then was incubated in the prehybridization solution (50% formamide, 10× Denhardt's solution, 10% dextran sulfate, and 200 μg/ml salmon sperm DNA) for at least 6 at 42°C. *Eco*RI restriction fragments from pcDNA3.1-MnSOD and pcDNA3.1-CuZnSOD containing full-length human SOD2 and SOD1 cDNAs, respectively, or AP-2α cDNA were gel-isolated and labeled with ^{32}P using a random primed DNA labeling kit (Boehringer-Mannheim) following the instructions provided by the supplier. The cDNA probes were added to the prehybridization solution and incubated for an additional 12–18 h at 42°C. The membrane was then washed in the 1× saline–sodium citrate buffer (SSC) with 1% sodium dodecyl sulfate (SDS) at 65°C for 30 min, followed by 0.1× SSC at room temperature for 30 min. The membrane was wrapped in plastic wrap and exposed to x-ray film at −80°C for 2–24 h.

Immunoblot analysis

For SOD western blots, total cell proteins were prepared by first scrape harvesting cells

in 0.25 *M* Tris buffer, pH 7.5, and then pelleting the cells by centrifugation at 500 *g* for 2 min at 4°C. The resulting cell pellets were sonicated in 200 μl of Tris buffer, followed by centrifugation at 2,000 *g* for 5 min at 4°C. The protein concentrations in the supernatants were quantified spectrophotometrically using bicinchoninic acid (BCA) reagent (Amersham), and the proteins were used in western analysis. Fifteen micrograms of nuclear or total cell protein isolated from MRC5-VA and MRC5 was boiled for 5 min in the presence of reducing sample buffer (0.5 *M* Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.1% bromphenol blue). Proteins were resolved by electrophoresis in 12.5% polyacrylamide gels at 35 mA for 40 min. The proteins were electroblotted to nitrocellulose membrane in cold transfer buffer (39 m*M* glycine, 48 m*M* Tris base, 0.037% SDS, 20% methanol) at 100 V for 2–4 h. The membranes were blocked in TBST (10 m*M* Tris, 150 m*M* NaCl, and 0.05% Tween 20) containing 3% powdered milk. The membranes were then immunoblotted with antibodies specific for either MnSOD or copper- and zinc-containing superoxide dismutase (CuZnSOD) at final dilutions of 1:1,000. The membranes were washed three times with TBST and probed with horseradish peroxidase-conjugated goat anti-rabbit IgG. The membranes were then washed with TBST again, and the immunoreactive proteins were detected with enhanced chemiluminescence (ECL) reagent (Amersham). For AP-2 western blots, 25 μg of nuclear extract (described below) was loaded per well and subsequently separated on 12.5% SDS-polyacrylamide gels and transferred to nitrocellulose. The membranes were then immunoblotted with antibodies specific for AP-2α (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) at a final dilution of 1:1,000, followed by horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody at 1:10,000 (Amersham Pharmacia Biotech). Detection was performed with ECL reagent (Amersham Pharmacia Biotech).

SOD activity gels

Total cellular proteins were obtained by sonication and measured by BCA reagent (Pierce Biochemical). Protein samples were loaded on 12.5% native polyacrylamide gels with 5% stacking gels after preelectrophoresis for 1 h. After electrophoresis at 4°C, the native gels were stained for SOD activity as previously described (1).

SOD enzyme activity assay

SOD activity was quantified using an indirect competition assay between SOD and an indicator molecule, nitro blue tetrazolium. This assay was performed with crude cellular homogenates as previously described (30). Sodium cyanide (5 m*M*) inhibits CuZnSOD. Therefore, activity in the crude homogenate in the presence of NaCN indicates only MnSOD activity. The difference between total SOD activity and cyanide-insensitive activity represents CuZnSOD activity.

Gel mobility shift assays

A double-stranded oligonucleotide (upper strand, 5′-AGCTCAA**GCCCGCGGGC**TC-3′; lower strand, 5′-TCGAAGA**GCCCGCGGGC**-TTG-3′) was end-labeled with [^{32}P]dCTP by a Klenow fill-in reaction and was used as a probe in gel mobility shift assays. This probe con-

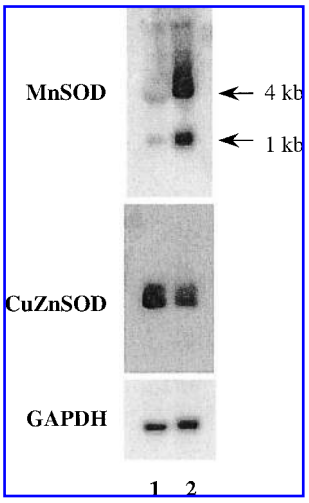

FIG. 1. SV40 transformed human lung fibroblasts displayed decreased steady-state levels of MnSOD mRNA compared with their normal counterpart MRC5 cell strain (upper panel). Steady state levels of CuZnSOD mRNA were slightly higher in MRC5-VA cells than in MRC5 parental cells (**middle panel**). GAPDH mRNA served as the control for RNA loading and transfer (**lower panel**). The experiment was repeated twice, and typical results are shown. Lane 1, MRC5-VA; lane 2, MRC5.

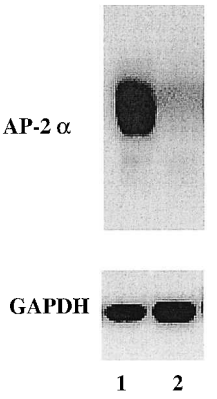

FIG. 2. AP-2 mRNA is abundantly expressed in transformed but not normal human lung fibroblasts. Northern blots containing 10 μg of total RNA were probed for AP-2α (**upper panel**) and GAPDH (**lower panel**) mRNA expression. Typical results from two replicate northern blots are shown. Lane 1, MRC5-VA; lane 2, MRC5.

tained a consensus AP-2 binding site in the context of the human SOD2 promoter from nucleotides −26 to −14 relative to the major transcription start site. Nuclear proteins were extracted from the cells as follows. Cells were scraped into 0.5 ml of cold buffer A (10 m*M* HEPES, pH 7.9, 1.5 m*M* $MgCl_2$, 10 m*M* KCl; and 0.5 m*M* dithiothreitol), lysed with 20 strokes of a Dounce homogenizer (Kontes Scientific Glassware, Vineland, NJ, U.S.A.), and centrifuged for 30 s. After centrifugation, the supernatants were removed, and the pellets were resuspended in buffer C (20 m*M* HEPES, pH 7.9, 25% glycerol, 0.42 *M* NaCl, 1.5 m*M* $MgCl_2$, 0.2 m*M* EDTA, 0.5 m*M* phenylmethylsulfonyl fluoride, and 0.5 m*M* dithiothreitol). These mixtures were placed on ice for 15 min and microcentrifuged for 5 min at 4°C. Then the supernatants were harvested and diluted 1:6 with buffer D (20 m*M* HEPES, pH 7.9, 20% glycerol, 0.1 *M* KCl, 0.2 m*M* EDTA, 0.5 m*M* phenylmethylsulfonyl fluoride, and 0.5 m*M* dithiothreitol). The protein concentrations of the extracts were determined with BCA protein assay reagents (Pierce Biochemical). Gel mobility shift assays were performed by incubating 5 μg of nuclear protein or 10 μg of total cellular protein together with the ^{32}P-radiolabeled oligonucleotide probe in the presence of 1 μg of poly(dIdC) (Pharmacia Inc., Piscataway, NJ, U.S.A.) and 1 × gel shift buffer (10 m*M* Tris, pH 7.5, 50 m*M* NaCl, 1 m*M* $MgCl_2$, 0.5 m*M* EDTA, 0.5 m*M* dithiothreitol, and 4% glycerol) at room temperature for 15 min. The binding reactions were loaded onto a 5% polyacrylamide gel and run at 35 mA for ~40 min in 1 × Tris–borate–EDTA. The gels were wrapped in plastic wrap and exposed to x-ray film (Kodak) overnight at −80°C. To assess the specificity of the binding reaction, antibodies specific to AP-2 were used in gel supershift assays to verify that the DNA binding activity measured was due specifically to AP-2 (Santa Cruz Biotechnology). For gel supershift, 1 μl of anti-AP-2α antibody was incubated with each binding reaction for 30 min before loading onto the gel.

RESULTS

MRC5-VA cells express decreased steady-state levels of MnSOD mRNA, but increased steady-state levels of AP-2α mRNA

To assess comparative levels of antioxidant enzyme expression in MRC5 and MRC5-VA

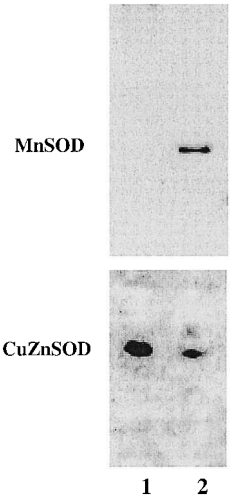

FIG. 3. SV40 transformed human lung fibroblasts displayed markedly decreased MnSOD protein expression compared with their normal counterpart MRC5 cells (upper panel). Levels of CuZnSOD protein expression were slightly higher in the MRC5-VA cells than their normal cell counterpart, MRC5 (**lower panel**). Typical results from two replicate western blots are shown. Lane 1, MRC5-VA; lane 2, MRC5.

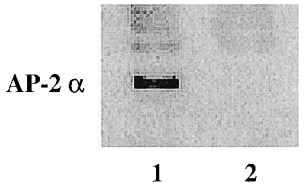

FIG. 4. AP-2α protein is expressed in transformed but not normal human lung fibroblasts. Total cellular proteins (25 μg) were electrophoresed and transferred to nitrocellulose. Western blots were blocked and probed with anti-human AP-2α antibody. Typical results from two replicate western blots are shown. Lane 1, MRC5-VA; lane 2, MRC5.

cells, we initially used northern blot analysis of total cellular RNA. The results, shown in Fig. 1, indicated that steady-state levels of SOD2 mRNA were considerably greater in MRC5 cells than in their SV40 transformed counterpart, MRC5-VA. In contrast, steady-state levels of SOD1 mRNA were slightly higher in the MRC5-VA cells as compared with the MRC5 cells. Expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a constitutively expressed gene, was essentially equivalent in the two cell types and served as the control for RNA loading and transfer. AP-2 is a putative transcriptional regulator of the SOD2 gene. To determine whether AP-2 mRNAs were differentially expressed in these cell lines, we probed the northern blots with an AP-2α cDNA. Results of this experiment are shown in Fig. 2. In contrast to the MnSOD mRNA expression pattern, AP-2α steady-state mRNA levels were abundant in the SV40 transformed MRC5-VA cells, whereas they were barely detectable in the MRC5 normal cells.

AP-2 and MnSOD protein expression are inversely related in MRC5-VA cells

To establish that the differentially expressed SOD2 and AP-2 mRNA transcripts observed in the northern blot analyses were translated into proteins, we performed western blot analyses with rabbit anti-human antibodies specific to MnSOD and AP-2α, respectively. We also determined CuZnSOD protein expression levels by western blot analysis. Our results from the SOD immunoblots, shown in Fig. 3, indicated that MnSOD protein was abundantly expressed in MRC5 cells, but undetectable in MRC5-VA cells. In agreement with the northern blot analysis, CuZnSOD protein levels were also slightly elevated in the MRC5-VA cells compared with to the MRC5 cells. Conversely, AP-2α proteins were only detectable in the SV40 transformed MRC5-VA cells and not in the parent MRC5 fibroblasts (Fig. 4).

MRC5-VA cells express decreased levels of MnSOD enzyme activity

To determine whether the increased MnSOD protein was enzymatically active, we performed two different activity assays. First, we used an in-gel activity assay for SOD activity, the results of which are shown in Fig. 5. The assay measures both CuZnSOD and MnSOD activities that are separable because of their different electrophoretic mobilities. Figure 5 shows that, consistent with the northern and western blots, the activity of CuZnSOD was higher in the SV40 transformed MRC5-VA cell line. In contrast, the levels of MnSOD activity were strikingly different in the two cell lines, with the parent MRC5 cell strain showing considerably greater activity than its SV40 transformed counterpart.

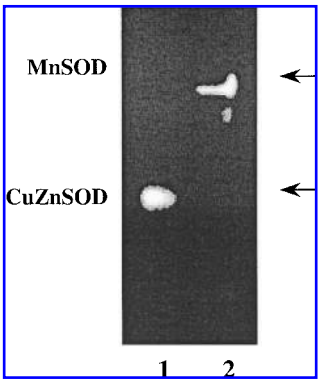

FIG. 5. SV40 transformed human lung fibroblasts exhibited dramatically decreased MnSOD enzyme activity compared with their normal counterpart MRC5 cell strain in an activity gel assay. The CuZnSOD enzyme activities between the two cell types were also different, in accordance with the protein expression data. The lower band was cyanide-sensitive, confirming it as CuZnSOD. Typical results from two replicate activity gels are shown. Lane 1, MRC5-VA; lane 2, MRC5.

Although the SOD activity gels provide a qualitative comparison of the SOD enzyme activities, they are not quantitative assays. To quantify the SOD activities in these two cell types, we performed biochemical enzyme kinetic assays. The results, shown in Table 1, indicate that CuZnSOD activity was elevated in the MRC5-VA cells compared with MRC5 cells. Conversely, MnSOD activity was dramatically higher in MRC5 parent cells than in MRC5-VA cells.

Thus, the SV40 transformed MRC5-VA cells showed decreased MnSOD mRNA, protein, and enzyme activity as compared with its normal counterpart, MRC5. MRC5-VA joins a number of other virally transformed cell lines that display diminished MnSOD expression (22, 28, 32).

MRC5-VA transformed cells but not MRC5 normal cells exhibit constitutive AP-2 DNA binding activity

To determine whether the AP-2α proteins in the MRC5-VA cells were able to interact with a known AP-2 *cis*-element in the SOD2 gene promoter region, we performed gel mobility shift analyses with a radiolabeled probe from a sequence contained within the 5′ flanking region of the human SOD2 gene. Our results, shown in Fig. 6, clearly demonstrate that AP-2α-specific DNA binding activity is constitutively present in MRC5-VA cells, but undetectable in MRC5 parent cells. This DNA binding activity was positively identified as AP-2 by supershift analysis with an anti-AP-2α antibody. These data indicate that AP-2 binding to the α sequence in the human SOD2 promoter is abundant in transformed cells with decreased SOD2 expression and suggest that AP-2 may mediate transcriptional down-regulation of MnSOD expression during transformation.

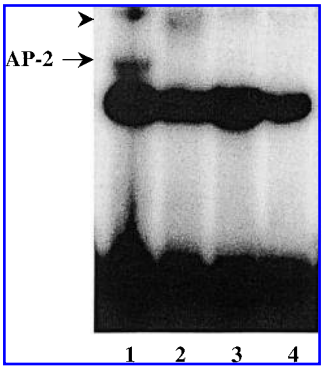

FIG. 6. AP-2 DNA binding activity is constitutively expressed in transformed MRC5-VA cells, but not in normal MRC5 fibroblasts. Nuclear proteins (5 μg) were incubated with a ^{32}P-labeled probe containing an AP-2 binding site from the human SOD2 promoter. The gel shifted band was positively identified as AP-2 with an anti-AP-2α antibody in a supershifted complex. Lane 1, MRC5-VA nuclear extract; lane 2, MRC5-VA nuclear extract + anti-AP-2α antibody; lane 3, MRC5 nuclear extract; lane 4, MRC5 nuclear extract + anti-AP-2α antibody. Typical results from two replicate gel shifts are shown. Arrow, AP-2:DNA complex; arrowhead, supershifted AP-2:DNA:antibody complex.

TABLE 1. SOD ACTIVITIES IN MRC5 AND MRC5-VA CELL LINES

	MnSOD	*CuZnSOD*	*Total SOD*
MRC5	414	22	436
MRC5-VA	65	58	149

SV40 transformed human lung fibroblasts MRC5-VA display decreased MnSOD enzyme activity (units/mg of protein) compared with their normal counterpart MRC5 cell strain. Data presented are the average of two replicates.

DISCUSSION

SOD2 has an unconventional tumor suppressor activity. The MnSOD protein functions to detoxify superoxide radicals formed by one-electron reduction of oxygen by electrons that leak off the mitochondrial electron transport chain during oxidative phosphorylation. In general, tumor cells express lower amounts of MnSOD mRNA, protein, and activity than the normal cells from which they were derived (27). The absence of MnSOD contributes to an altered redox state in the cell, accumulation of reactive oxygen species, and formation of clastogenic free radicals. This heightened cellular prooxidant state reached after diminution of MnSOD activity may then contribute to genomic instability, leading to maintenance and progression of the malignant phenotype.

During our analysis of differential transcription factor activities and availabilities in the MRC5 and MRC5-VA model system, we ob-

served that transcription factor AP-2 is abundant in MRC5-VA, but absent in MRC5 (12). We had previously shown that DNA methylation of an AP-2 binding site in the SOD2 promoter could inhibit not only AP-2 DNA binding, but also SOD2 promoter activity (13). Moreover, we had demonstrated that AP-2 DNA binding was redox-sensitive (11). Considering these previous findings, we reasoned that AP-2 was somehow participating in the transcriptional regulation of SOD2. Thus, the marked difference in AP-2 activities in these two cell types prompted us to examine their expression of SOD2. Our findings indicate that AP-2 activity and SOD2 expression are inversely related and suggest that AP-2 may play an active role in down-regulating MnSOD expression.

In the current study, we determined expression of both AP-2 and SOD2 in these two cell types at the levels of mRNA, protein, and activity. Our results indicated that SOD2 gene expression was strikingly decreased in MRC5-VA cells compared with MRC5 cells at each level of investigation. In contrast, AP-2 expression was up-regulated at each of these levels of investigation. The decrease in antioxidant enzyme expression was specific for SOD2, because SOD1 expression was, in fact, slightly elevated in the SV40 transformed cells. This may be the result of a cellular compensatory mechanism associated with loss of MnSOD expression, or due to transcriptional activation of SOD1 by AP-2 as has been previously suggested (18). The observation that there is diminished expression of SOD2 in the SV40 transformed cells is consistent with previous findings for SOD2 expression in other types of virally transformed cells (22, 28). Interestingly, we have observed a similar inverse relationship between AP-2 and SOD2 in another matched pair of normal and transformed cells, WI38 and WI38-VA13 (12; and unpublished observations). The mechanism underlying MnSOD repression, however, remains unknown. The cell types described here that displayed differential SOD2 and AP-2 expression should provide a valuable model system in which to explore further the mechanisms for differential regulation. Indeed, several insights into mechanism have already emerged from this model system.

We previously identified a possible epigenetic mechanism for transcriptional repression of SOD2 expression involving DNA methylation of intronic regulatory elements in the SOD2 gene (14). Nevertheless, based on our finding that AP-2 activity is abundant in MRC5-VA, but undetectable in MRC5, it is reasonable to hypothesize that AP-2 may act as a transcriptional repressor of SOD2 gene expression. This would not be surprising because AP-2 has been previously shown to act as a repressor of several other genes as discussed above (2, 8, 16, 17, 23). Interestingly, mutations in the SOD2 promoter in certain cancer cell lines with decreased MnSOD expression lead to the acquisition of new AP-2 DNA binding sites. This finding suggests that AP-2 may act as an active repressor of MnSOD expression rather than simply competing with SP1 as has been suggested (31). Clearly more studies are needed to elucidate the mechanism(s) involved, and the description of this model system should help set the stage for definition of these mechanisms.

ACKNOWLEDGMENTS

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ABBREVIATIONS

AP-2, activator protein-2; BCA, bicinchoninic acid; C/EBPα, CAAT enhancer binding protein-α; CuZnSOD, copper- and zinc-containing superoxide dismutase; ECL, enhanced chemiluminescence; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MnSOD, manganese-containing superoxide dismutase; SDS, sodium dodecyl sulfate; SOD, superoxide dismutase; SOD1, superoxide dismutase 1 gene encoding CuZnSOD; SOD2, superoxide dismutase 2 gene encoding MnSOD; SSC, saline–sodium citrate; SV40, simian virus 40; TBST, Tris-buffered saline with Tween 20.

REFERENCES

1. Beauchamp C and Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44: 276–278, 1971.
2. Bisgrove DA and Godbout R. Differential expression of AP-2 alpha and AP-2 beta in the developing chick retina: repression of R-FABP promoter activity by AP-2. *Dev Dyn* 214: 195–206, 1999.
3. Bosher JM, Totty NF, Hsuan JJ, Williams T, and Hurst HC. A family of AP-2 proteins regulates c-erbB-2 expression in mammary carcinoma. *Oncogene* 13: 1701–1707, 1996.
4. Bravard A, Sabatier L, Hoffschir F, Ricoul M, Luccioni C, and Dutrillaux B. SOD2: a new type of tumor-suppressor gene? *Int J Cancer* 51: 476–480, 1992.
5. Chuang LS, Ian HI, Koh TW, Ng HH, Xu G, and Li BF. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* 277: 1996–2000, 1997.
6. Church SL, Grant JW, Meese EU, and Trent JM. Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping. *Genomics* 14: 823–825, 1992.
7. Church SL, Grant JW, Ridnour LA, Oberley LW, Swanson PE, Meltzer PS, and Trent JM. Increased manganese superoxide dismutase expression suppresses the malignant phenotype of human melanoma cells. *Proc Natl Acad Sci U S A* 90: 3113–3117, 1993.
8. Getman DK, Mutero A, Inoue K, and Taylor P. Transcription factor repression and activation of the human acetylcholinesterase gene. *J Biol Chem* 270: 23511–23519, 1995.
9. Green MH, Lowe JE, Petit-Frere C, Karran P, Hall P, and Kataoka H. Properties of *N*-methyl-*N*-nitrosourea-resistant, Mex-derivatives of an SV40-immortalized human fibroblast cell line. *Carcinogenesis* 10: 893–898, 1989.
10. Halliwell B and Gutteridge JMC. *Free Radicals in Biology and Medicine*, 3rd ed. Oxford, U.K.: Clarendon Press, 1999.
11. Huang Y and Domann FE. Redox modulation of AP-2 DNA binding activity *in vitro*. *Biochem Biophys Res Commun* 249: 307–312, 1998.
12. Huang Y and Domann FE. Transcription factor AP-2 mRNA and DNA binding activity are constitutively expressed in SV40 immortalized but not normal human lung fibroblasts. *Arch Biochem Biophys* 364: 241–246, 1999.
13. Huang Y, Peng J, Oberley LW, and Domann FE. Transcriptional inhibition of manganese superoxide dismutase (SOD2) gene expression by DNA methylation of the 5′ CpG island. *Free Radic Biol Med* 23: 314–320, 1997.
14. Huang Y, He T, and Domann FE. Decreased expression of manganese superoxide dismutase in transformed cells is associated with increased cytosine methylation of the SOD2 gene. *DNA Cell Biol* 18: 643–652, 1999.
15. Jacobs JP, Jones CM, and Baille JP. Characteristics of a human diploid cell designated MRC-5. *Nature* 227: 168–170, 1970.
16. Jiang JG, DeFrances MC, Machen J, Johnson C, and Zargenar R. The repressive function of AP-2 transcriptional factor on the hepatocyte growth factor gene promoter. *Biochem Biophys Res Commun* 272: 882–886, 2000.
17. Jiang MS, Tang QQ, McLenithan J, Geiman D, Shillinglaw W, Henzel WJ, and Lane MD. Derepression of the C/EBP alpha gene during adipogenesis: identification of AP-2 alpha as a repressor. *Proc Natl Acad Sci U S A* 95: 3467–3471, 1998.
18. Kim YH, Park KH, and Rho HM. Transcriptional activation of the Cu,Zn-superoxide dismutase gene through the AP-2 site by ginsenoside Rb2 extracted from a medicinal plant, Panax ginseng. *J Biol Chem* 271: 24539–24543, 1996.
19. Li JJ, Oberley LW, St. Clair DK, Ridnour LA, and Oberley TD. Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase. *Oncogene* 10: 1989–2000, 1995.
20. Liang BC, Ross DA, Greenberg HS, Meltzer PS, and Trent JM. Evidence of allelic imbalance of chromosome 6 in human astrocytomas. *Neurology* 44: 533–536, 1994.
21. Liu R, Oberley TD, and Oberley LW. Transfection and expression of MnSOD cDNA decreases tumor malignancy of human oral squamous carcinoma SCC-25 cells. *Hum Gene Ther* 8: 585–595, 1997.
22. Marlhens F, Nicole A, and Sinet PM. Lowered level of translatable RNAs for manganese superoxide dismutase in human fibroblasts transformed by SV40. *Biochem Biophys Res Commun* 129: 300–305, 1985.
23. Miao K, Potter JJ, Anania FA, Rennie-Tankersley L, and Mezey E. Identification of two repressor elements in the mouse alpha2 (I) collagen promoter. *Arch Biochem Biophys* 361: 7–16, 1999.
24. Millikin D, Meese E, Vogelstein B, Witkowski C, and Trent J. Loss of heterozygosity for loci on the long arm of chromosome 6 in human malignant melanoma. *Cancer Res* 51: 5449–5453, 1991.
25. Mitchell PJ, Wang C, and Tjian R. Transcription factor AP-2 is expressed in neural crest cell lineages during mouse embryogenesis. *Cell* 50: 847–861, 1987.
26. Nottoli T, Hagopian-Donaldson S, Zhang J, Perkins A, and Williams T. AP-2 null cells disrupt morphogenesis of the eye, face, and limbs in chimeric mice. *Proc Natl Acad Sci U S A* 95: 13714–13719, 1998.
27. Oberley LW, Oberley TD, and Buettner GR. Cell differentiation, aging and cancer: the possible roles of superoxide and superoxide dismutases. *Med Hypotheses* 6: 249–268, 1980.
28. Oberley LW, McCormick ML, Sierra-Rivera E, and Kasemset-St. Clair D. Manganese superoxide dismutase in normal and transformed human embryonic lung fibroblasts. *Free Radic Biol Med* 6: 379–384, 1989.
29. Shorle H, Meier P, Buchert M, Jaenisch R, and Mitchell PJ. Transcription factor AP-2 essential for cranial clo-

sure and craniofacial development. *Nature* 381: 235–238, 1996.
30. Spitz DR and Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* 179: 8–18, 1989.
31. Xu Y, Krishnan A, Wan XS, Majima H, Yeh CC, Ludewig G, Kasarkis EJ, and St. Clair DK. Mutations in the promoter reveal a cause for the reduced expression of the human manganese superoxide dismutase gene in cancer cells. *Oncogene* 18: 93–102, 1999.
32. Yamanaka N and Deamer D. Superoxide dismutase in WI38 cell cultures: effects of age, trypsinization and SV40 transformation. *Physiol Chem Phys* 6: 95–106, 1974.
33. Yan T, Oberley LW, Zhong W, and St. Clair DK. Manganese-containing superoxide dismutase overexpression causes phenotypic reversion in SV40-transformed human lung fibroblasts. *Cancer Res* 56: 2864–2871, 1996.
34. Zhong W, Oberley LW, Oberley TD, and St. Clair DK. Suppression of the malignant phenotype of human glioma cells by overexpression of manganese superoxide dismutase. *Oncogene* 14: 481–490, 1997.

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1. Sanjit Kumar Dhar, Daret K. St. Clair. 2012. Manganese superoxide dismutase regulation and cancer. *Free Radical Biology and Medicine* **52**:11-12, 2209-2222. [CrossRef]
2. Marie Ennen, Vanessa Minig, Stéphanie Grandemange, Nadège Touche, Jean-Louis Merlin, Vanessa Besancenot, Emilie Brunner, Lionel Domenjoud, Philippe Becuwe. 2011. Regulation of the high basal expression of the manganese superoxide dismutase gene in aggressive breast cancer cells. *Free Radical Biology and Medicine* **50**:12, 1771-1779. [CrossRef]
3. Ae-Kyong Kim. 2010. Modulation of MnSOD in Cancer: Epidemiological and Experimental Evidences. *Toxicological Research* **26**:2, 83-93. [CrossRef]
4. Lu Miao, Daret K. St. Clair. 2009. Regulation of superoxide dismutase genes: Implications in disease. *Free Radical Biology and Medicine* **47**:4, 344-356. [CrossRef]
5. Michael J. Hitchler, Larry W. Oberley, Frederick E. Domann. 2008. Epigenetic silencing of SOD2 by histone modifications in human breast cancer cells. *Free Radical Biology and Medicine* **45**:11, 1573-1580. [CrossRef]
6. O TIROSH, E LEVY, R REIFEN. 2007. High selenium diet protects against TNBS-induced acute inflammation, mitochondrial dysfunction, and secondary necrosis in rat colon. *Nutrition* **23**:11-12, 878-886. [CrossRef]
7. L YU, S VENKATARAMAN, M COLEMAN, D SPITZ, P WERTZ, F DOMANN. 2006. Glutathione peroxidase-1 inhibits UVA-induced AP-2# expression in human keratinocytes. *Biochemical and Biophysical Research Communications* **351**:4, 1066-1071. [CrossRef]
8. Min Li, Yingqun Wang, Mien-Chie Hung, Perry Kannan. 2006. Inefficient proteasomal-degradation pathway stabilizes AP-2# and activates HER-2/neu gene in breast cancer. *International Journal of Cancer* **118**:4, 802-811. [CrossRef]
9. Erika M Becker, David B Lovejoy, Judith M Greer, Ralph Watts, Des R Richardson. 2003. Identification of the di-pyridyl ketone isonicotinoyl hydrazone (PKIH) analogues as potent iron chelators and anti-tumour agents. *British Journal of Pharmacology* **138**:5, 819-830. [CrossRef]