Induction of Activating Transcription Factor 1 by Nickel and Its Role as a Negative Regulator of Thrombospondin I Gene Expression

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ABSTRACT

Thrombospondin I (TSP I) is an extracellular matrix glycoprotein that influences cell adhesion, motility, and growth. On the basis of its effects on endothelial cell proliferation, TSP I has attracted interest as a potential regulator of solid tumor growth through modulation of tumor blood supply. The regulation of TSP I expression is of critical importance for designing new approaches in tumor therapy. Recently, we have shown that TSP I expression is lost in nickel-transformed cells. In this paper, we identified an activating transcription factor (ATF/cAMP-responsive element-binding protein binding site as a negative regulatory site in the S'-flanking sequence of mouse TSP I promoter. We identified ATF-1 as a major component of the ATF/cAMP-responsive element-binding protein binding complex. This Mr 35,000 nuclear ATF-1 protein was shown to be present in higher amounts in nickel-transformed 3T3 cells that do not express TSP I. Acute treatment of 3T3 cells with NiCl2 resulted in the induction of this transcription factor, and this induction was correlated temporally with the suppression of TSP I expression in the same cells. These results show that nickel exposure causes accumulation of the ATF-1 transcription factor, which is responsible for the down-regulation of transcription of TSP I, and possibly other tumor suppressor genes during nickel-induced cellular transformation.

INTRODUCTION

TSP I is a multifunctional, large extracellular matrix glycoprotein (Mr, 420,000) that is synthesized by a variety of cells, such as neutrophils, monocytes, macrophages, endothelial cells, fibroblasts, keratinocytes, and smooth muscle cells (1–3). Because TSP I protein influences cell-to-cell (4) and cell-to-matrix interactions, it plays an important role in embryogenesis (5, 6), wound repair (2, 7), inflammation (2, 8), and tumor regression (2, 9–14). Loss of TSP I expression has been found in carcinogen- and oncogene-transformed cells (15–17). It was shown further that TSP I inhibits angiogenesis, and there was good correlation between lower levels of TSP I expression in tumor cells and angiogenic activity (9, 10, 12–14). TSP I inhibits proteases, including plasmin and urokinase, which depress metastatic activity of tumor cells (18). Overexpression of TSP I in tumor cells restored the normal cellular phenotype and suppressed their tumorigenesis in vivo by preventing growth of blood vessels (11, 13, 14).

Numerous factors that affect TSP I gene expression were studied over the last decade. It was found that TSP I expression can be stimulated by serum (19, 20), and that serum-inducible elements were present in higher amounts in nickel-transformed 3T3 cells that do not express TSP I. Acute treatment of 3T3 cells with NiCl2 resulted in the induction of this transcription factor, and this induction was correlated temporally with the suppression of TSP I expression in the same cells. These results show that nickel exposure causes accumulation of the ATF-1 transcription factor, which is responsible for the down-regulation of transcription of TSP I, and possibly other tumor suppressor genes during nickel-induced cellular transformation.

MATERIALS AND METHODS

Cell Culture. The BALB/c 3T3 mouse fibroblast cell line from American Type Culture Collection and the derived nickel-resistant and transformed B200 cells were used in this study (28). The transformed phenotype (i.e., ability to grow in soft agar, and so forth) of B200 cells has been described previously (29). The 3T3 and B200 cells were cultured in α-MEM supplemented with 10% FCS, 2 mM glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained at 37°C in 5% CO2. B200 cells were resistant to the growth suppression of 200 μM NiCl2. Because these cells inherently maintain their nickel resistance and transformed phenotype, all of the experiments reported here were carried out without culturing B200 cells in the presence of NiCl2.

3T3 cell viability after treatment with 1 mM of NiCl2 for 24 h was monitored by trypan blue staining and was found to be 80–85%.

Analysis of Methylation Status of TSP I Promoter Region by Southern Blot. Genomic DNA from 3T3 and B200 was digested with a 4-fold excess of restriction enzymes (MspI, HpaII, and CfoI). Ten μg of digested DNA were fractionated in a 1% agarose gel and transferred to nylon Nitran membranes.
concentration of the extracts was measured using a protein determination kit to ensure the reproducibility of the data. All CAT activities were normalized to β-galactosidase activities. Four cycles of freeze-thawing in PBS buffer. CAT and β-gal expression were measured using a specific TSP I/β-gal probe and [3H]-labeled probe (5 x 10^4 cpm) added last to a 20-μL reaction mixture containing nuclear extract (4 μg of nuclear proteins), 7.5% glycerol, 0.5 μg of poly(deoxyinosinic-deoxycytidylic acid), 5 mM MgCl₂, 60 mM NaCl, and 10 mM KCl, in 10 mM HEPES buffer (pH 7.9). The double-stranded unlabeled ATF/CREB oligonucleotides was used as a specific competitor.

For supershift experiments, 8 μg of nuclear proteins were incubated for 1 h with antibodies at 4°C prior to the oligonucleotide addition. Anti-ATF-1, anti-ATF-2, anti-ATF-3, anti-ATF-4, anti-CREB-1, and anti-CREB-2 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Samples were electrophoresed in a 6% nondenaturing polyacrylamide gel in 0.5× Tris-borate-EDTA buffer (10× TBE, 0.89 M Tris-borate, 0.025 mM EDTA) for 3 h at 12 V/cm. The gels were vacuum dried and exposed to X-ray film (Eastman Kodak Co., Rochester, NY).

Western Blot Analysis. Fifteen μg of the same nuclear extracts that were used for a mobility shift assay were separated on 10% SDS-PAGE, and the proteins were transferred to nitrocellulose membranes (Schleicher & Schuell). Nonspecific binding was blocked by soaking the membrane in 5% nonfat dry milk in TBS buffer (20 mM Tris-HCl (pH 8.0), and 150 mM NaCl with 0.1% Tween 20) overnight at 4°C. The membrane was washed (four times for 15 min each) with TBS buffer and incubated for 1 h with anti-ATF-1 antibodies at room temperature. After washing (four times for 15 min each) with TBS buffer, the membrane was incubated with a second antismouse peroxidase-conjugated antibody (Cappel, Durham, NC) and used for washing again with TBS buffer (four times for 15 min each). Chemiluminescent substrate Luminol (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) was added, and the membrane was exposed to X-Omat AR 2 film (Eastman Kodak Co.). After the exposure, the membrane was rinsed with water and silver stained to evaluate protein levels. The molecular weight of ATF-1 was determined using prestained molecular weight markers (Bio-Rad Laboratories).

Northern Blot Analysis. Total RNA was isolated using an RNA preparation kit (RNAzol; Cinna/Biotecx Laboratories, Inc., Houston, TX). The RNA (15 μg) was fractionated in a 1.0% formaldehyde agarose gel and then transferred to nitrocellulose membranes (BA-85; Schleicher & Schuell). The membranes were hybridized with a 32P-labeled TSP I cDNA probe (16). Ethidium bromide staining was used to evaluate the nucleic acid loading of each lane.

RESULTS

Low Levels of TSP I Transcription in Nickel-transformed B200 Cells. The TSP I transcription levels in 3T3 and B200 cells were examined by Northern blot analysis using hamster TSP I cDNA as a probe. The analysis showed that the TSP I transcription in B200 cells was almost negligible compared to that of 3T3 cells (Fig. 1). The inactivation of TSP I expression could be accounted for either by hypermethylation of TSP I promoter or by inactivation of transcriptional regulation in B200 cells.

Analysis of Methyltransferase Status of the 5′ End of TSP I in 3T3 and B200 Cells. To analyze the methylation status of the TSP I promoter region in 3T3 and B200 cells, genomic DNA was digested with the methylation-sensitive restriction endonucleases HpaII and CfoI and with the methylation-insensitive restriction endonuclease MspI. Southern blot analysis did not reveal differences in methylation pattern between 3T3 and B200 cells. The analysis was repeated three times with identical results. A representative autoradiograph is shown in Fig. 2a.

Electrophoretic Mobility Shift Assays. Nuclear protein extracts from 3T3 and B200 cells were prepared by the method of Dignam et al. (31). The protein concentration of the extracts was measured using a protein determination kit (Bio-Rad Laboratories, Hercules, CA) with BSA used as a standard. The normal NF-1, SRE, and ATF (AGCTTCGCGGCGCCGAGGGG-3′) and mutated ATF (−210) (AGCTTCGCGCGCCGAGGGG-3′) fragments of TSP I promoter containing potential binding sites were purchased from National Biosciences, Inc. (Plymouth, MN). These oligonucleotides were end labeled with [γ-32P]ATP (Amersham Life Science, Arlington Heights, IL) and T4 polynucleotide kinase (New England BioLabs, Inc., Beverly, MA). The electrophoretic mobility shift assay was performed with labeled probe (5 × 10⁴ cpm) added last to a 20-μL reaction mixture containing nuclear extract (4 μg of nuclear proteins), 7.5% glycerol, 0.5 μg of poly(dexoxyinosinic-dexoxyycytidylic acid), 5 mM MgCl₂, 60 mM NaCl, and 10 mM KCl, in 10 mM HEPES buffer (pH 7.9). The double-stranded unlabeled ATF/CREB oligonucleotides was used as a specific competitor.

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whereby cytosine is converted to uracil, but methylated cytosine remains nonreactive. The target DNA was amplified by PCR and sequenced. The results obtained with this method again did not show differences in the methylation status between cell lines (data not shown). The map of methylated and nonmethylated CpG sites on TSP I promoter as shown in Fig. 2 demonstrates the obtained results.

Fifty-three CpG dinucleotides are located within the 1.4-kb fragment of the mouse TSP I promoter region, but most of these sites are not associated with recognition sites of methylation sensitive restriction endonucleases. CpG dinucleotides are also located within the coding region of the TSP I gene and in the introns. To overcome tedious analysis of all CpG dinucleotides in TSP I genomic DNA, we decided to use 5-azacytidine, a known demethylating agent, in an attempt to reactivate TSP I expression. Cells were treated with 5 μM 5-azacytidine for 48 h, and the expression was analyzed by Northern blot. No reactivation of TSP I gene was found, suggesting further that inactivation of the gene was due to DNA methylation (not shown).

Potential Binding Sites for Transcription Factors on the Mouse TSP I Promoter. The location of potential binding sites for transcription factors in the mouse TSP I promoter has been published by Lawler et al. (26). The region from −1400 to +52 contains almost all known important regulatory sequences, including six SP1 (CCGGCCG) sites at −1210, −644, −180, −80, −55, and −40; two AP-1 sites (TGAG/CTCA) at positions −527 and −430; five AP-2-binding sites (CCCT/AG/CCG) at positions −1220, −673, −447, −354, and −24; two NF-1-binding sites (TGGC/A(N)5GCCA) at positions −1265 and −1158; one NFκB-binding site (GGG-GACTCT) at position −260; one SRE (TCCTTATITGG) at position −1188; and one ATF/CREB (TGACGTCC) responsive element at position −1098.

Using a MacVector 4.5 computer program, we have analyzed the promoter region of mouse TSP I and have identified two additional recognition sites for NF-IL6 transcription factor at positions −865 and −827. The consensus sequence for this transcription factor, TTNNGNAAT, was also found in the promoters of MDRI genes from hamster to human (32) and has been suggested to be activated via a ras-dependent signal transduction pathway (33). There is also a Rb control element (CCACCC; Ref. 34) within this fragment of TSP I promoter. This Rb element overlapped with the AP-2-binding site at position −447. Rb regulatory elements have also been identified in the promoter region of the c-fos and c-myc proto-oncogenes and transforming growth factor β1, and insulin-like growth factor II, as well as other genes (35). We also found that mouse TSP I promoter contained three potential p53-binding sites (T/CTGCCT) at positions −590, −10, and +34. p53 has been shown to be very important in the regulation of human TSP I expression (24). The structure of the 1.4-kb portion of mouse TSP I promoter region, as well as all deletion fragments used in this study, is depicted in Fig. 3.

Identification of Binding Sites for Negative Regulators of TSP I Expression. A 1.4-kb fragment corresponding to the 5' end of mouse TSP I was obtained by PCR from the genomic DNA of 3T3 cells. This fragment was cloned into promoterless CAT plasmid (pTSP-CAT1) as described in “Materials and Methods.” Four additional constructs with smaller fragments of the TSP I promoter were also prepared. Potential binding sites and the activity of each fragment in driving CAT expression are shown in Fig. 3. Transient transfection of these constructs into 3T3 cells revealed an approximately 4-fold stimulation of CAT activity when the first 400 bp of the 5' end of the 1.4-kb fragment was deleted, suggesting the presence of a negative regulatory element within the region deleted (Fig. 3). Additional deletions of the promoter, up to −200 bp, resulted in a decrease of CAT activity; however, all plasmids (pTSP-CAT2–pTSP-CAT5) showed higher activity compared to the original 1.4-kb fragment (pTSP-CAT1) (Fig. 3).

Six potential binding sites for transcription factors were located in
ATF-1 Transcription Factor Is a Negative Regulator of TSP I.

Because any one of the large family of ATF/CREB transcription factors can potentially bind to the ATF/CREB-binding element (36–38), we used antibodies raised against each of the six members of the family to identify those involved in DNA binding. Only antibodies against transcription factor ATF-1 showed a supershift, indicating that this transcription factor was involved in DNA binding to the ATF/CREB site, and most likely, this ATF-1 transcription factor was involved in the down-regulation of TSP expression by nickel (Fig. 6A). Similar results were obtained with nuclear extracts from B200 cells, confirming that ATF-1 was the factor involved in binding to ATF/CREB site in both cell lines (data not shown).

To substantiate further that ATF-1 is a negative regulator of TSP I expression, we analyzed nuclear extracts from 3T3 and B200 cells by Western blot. The molecular weight range of ATF-1 was shown to be between Mₙ 35,000 and 40,000 (39). Using antibodies against ATF-1, we found that a nuclear protein with the molecular weight of approximately Mₙ 35,000 was present at higher levels in nuclear extracts of B200 cells (Fig. 6B).

Acute Treatment of 3T3 Cells with Nickel Induces ATF-1 Transcription Factor and Suppresses TSP I Expression. Acute treatment of 3T3 cells with 1.0 mM NiCl₂ for 24 h resulted in suppression of TSP I gene expression (Fig. 1). It is interesting to note here that in acutely treated cells, we did not observe complete inactivation of TSP I gene expression (Fig. 1). This suggested that another negative regulator(s) is also involved in inactivation of TSP I expression in B200 cells. We tested whether NiCl₂ treatment would result in the

Oligonucleotides representing sequences of these three responsive elements were tested in gel mobility shift assays for binding with nuclear extracts isolated from 3T3 and B200 cells. Clone B200 represented a nickel-transformed and resistant clone from 3T3 cells that did not express TSP I (see Ref. 16 and Fig. 1). We expected to observe more binding of a negative transcription factor in extracts from B200 cells if this factor was involved directly in the down-regulation of TSP I. No differences in binding of nuclear extracts were found for NF-1 and SRE oligonucleotides (data not shown); however, when an oligonucleotide probe for the ATF/CREB-binding site was tested, more binding was found in nuclear extracts from nickel-transformed B200 cells compared with parental TSP I expressing 3T3 cells (Fig. 4). One specific DNA-protein complex was demonstrated in gel shift assays for both nuclear extracts isolated from 3T3 and B200 cells (Fig. 4). This complex was specific, because it was efficiently competed by a 50-fold molar excess of unlabeled specific nucleotides (Fig. 4).

To obtain additional evidence that the ATF/CREB-binding site was responsible for negative regulation of TSP I, we constructed a 1.4-kb promoter mutant reporter plasmid (pTSP-CAT1M) that contained a single-point mutation at the ATF/CREB-binding site (T → C) in the TSP I promoter (see “Materials and Methods”). The high level of expression of this plasmid when transfected into 3T3 cells was comparable to the expression of the fragment that did not contain an ATF-binding site (pTSP-CAT2) and was much higher than the whole promoter without this point mutation (Fig. 5). Additionally, nickel exposure down-regulated the expression of the intact TSP I promoter (see below) but did not down-regulate the TSP I promoter that had a point mutation in the ATF/CREB-binding element (Fig. 5). To verify that this base substitution indeed resulted in less protein binding to DNA, we tested the mutated oligonucleotide in a gel mobility shift assay. Fig. 4 shows significantly less binding of transcription factor(s) from nuclear extracts of both 3T3 and B200 cells to the mutated ATF/CREB oligonucleotide.

Fig. 4. Mobility shift analysis of nuclear extract binding to normal and mutated ATF/CREB DNA-binding sites. Nuclear extracts were prepared from 3T3 or B200 cells, and gel mobility shift assays were conducted as described in “Materials and Methods.” Protein binding from nuclear extracts of 3T3 cells and B200 cells was compared to the wild-type or mutated probe. Competition with a 50-fold molar excess of the unlabeled ATF/CREB oligonucleotide confirmed specificity of binding.
cells. 3T3 cells were transfected with plasmids pTSP-CAT1 (1), pTSP-CAT1 followed by pTSP-CAT1M (same plasmid as pTSP-CAT1) but with a point-mutated ATF/CREB site (T→C; 3), pTSP-CAT1M followed by exposure to 1.0 mM NiCl₂ for 24 h after transfection (4), and pTSP-CAT2 (5). The CAT activity of each plasmid was determined and expressed relative to the amount of CAT obtained for construct pTSP-CAT1. The data are expressed as the means (bars, SD) from four independent experiments (for details, see "Materials and Methods").

induction of ATF-1 in 3T3-treated cells in a time-related manner. Indeed, this protein was induced in nuclear extracts from 3T3 cells that had been treated with 1.0 mM NiCl₂ for 24 h (Fig. 7A). This finding added additional support to the idea that ATF-1 was a transcription factor that mediated at least in part nickel-induced down-regulation of TSP I expression. Gel mobility shift assays using the ATF/CREB sequence showed more binding activity in extracts of 3T3 cells treated with 1.0 mM NiCl₂, as well as more binding in nickel-resistant and transformed B200 cells, confirming that this induced protein was involved in binding to DNA (Fig. 7B). Other carcinogenic metals, such as cadmium and cobalt, neither affected TSP I expression nor ATF-1 induction, suggesting that this effect of nickel was not shared by other carcinogenic metal ions.

**DISCUSSION**

To analyze the molecular mechanisms of inactivation of TSP I in nickel-resistant and -transformed B200 cells, we have cloned a 1.4-kb fragment of the 5' end of TSP I and carried out Southern blot and PCR analyses using methylation-sensitive restriction endonucleases. Earlier, we provided strong evidence that nickel silenced expression of some genes by enhancing DNA methylation (40), and we expected to find similar effects in the 3T3 cell system.

No differences, however, were found in the restriction patterns of B200 cells compared with parental 3T3 cells. In addition, detailed sodium bisulfite analysis did not reveal any differences in the methylation status of the TSP I promoter regions of 3T3 and B200 cells. Finally, 5-azacytidine treatment did not reactivate TSP I expression in B200 cells, providing additional support for the conclusion that methylation was not involved in silencing of TSP I in B200 cells.

A functional analysis of TSP I promoter allowed us to identify a negative regulatory region of the TSP I promoter in mouse 3T3 cells and to find that ATF-1 transcription factor was a negative regulator of TSP I gene expression in these cells. The promoter region of mouse TSP I was first analyzed by Lawler et al. (26). A computer analysis of the 5' region of the gene revealed the presence of several potential regulatory elements, including AP-1, AP-2, SP-1, SRE, and ATF/CREB, but the significance of each of these elements in the regulation of TSP I expression in different tissues was not studied. All known transcription factors (i.e., serum induced) involved in regulation of TSP I are considered to be positive regulators, and negative regulators have not as yet been described in the literature. There is, however, some indirect evidence suggesting the existence of such negative regulator(s). Superinduction of TSP I expression by inhibition of protein syntheses using cyclohexamide (20) is suggestive of the presence of a negative regulator(s), because one explanation for a superinduction mechanism involves the blockage of protein synthesis for a negative regulator(s).

Earlier, we have shown that TSP I is inactivated in Chinese hamster and mouse nickel-transformed cells (16). Using two different parts of the human TSP I promoter, -1659 to +749 and -234 to +749, linked to CAT, we found that both constructs were down-regulated in nickel-transformed Chinese hamster cells, with the longer construct being suppressed more efficiently. These data showed that the human TSP I promoter was down-regulated at the transcriptional level in nickel-transformed hamster cells. The fact that both constructs were down-regulated in nickel-transformed hamster cells suggested that more than one negative regulator may exist. In this paper, we found one site with negative regulatory activity in the promoter area of mouse TSP I spanning from -1396 to +52. However, we cannot exclude the possibility that there were additional sites with negative regulatory activity upstream of -1396 or in the region of the first exon and intron spanning up to +750. We also cannot exclude the existence of additional negative regulatory sites within the analyzed region of the promoter, and more constructs with smaller deletions will need to be obtained to further characterize this region. The possibility that more than one negative regulator is functional in mouse B200 cells is supported by our demonstration of incomplete inactivation of TSP I in acutely treated 3T3 cells.

Carcinogenic nickel compounds exhibit weak mutagenic activity in all mutation assays tested (41–44); however, they are at the same time potent human and animal carcinogens. For example, nickel chloride has been shown to be the most potent chemical agent that immortalized Syrian hamster fibroblasts in vitro, surpassing such well-known...
chemical carcinogens as N-methyl-N-nitrosoarene, dimethyalsulfate, and benzo(a)pyrene diol epoxide (45). A single dose of 0.25 mM NiCl₂ for 24 h was sufficient to produce 60 and 9 of 10 immortalized cultures in two separate experiments, with no spontaneous immortalization occurring in these experiments (45). In experiments carried out in our laboratory, a single dose of 1 mM NiCl₂ for 24 h was sufficient to produce morphological transformation and even anchorage-independent growth of Chinese hamster embryo fibroblasts (46). This implied that nickel treatments described in this paper (1 mM of NiCl₂ for 24 h) could potentially result in transformation of 3T3 cells. Thus, activation of ATF-1 expression may be an early event in nickel-induced transformation.

The mechanism by which the ATF-1 transcription factor is induced by nickel is not known. It is unlikely to be due to a direct effect of nickel, because there are no known nickel-responsive elements that regulate gene expression in any form of life. Because nothing is known about the effects of nickel on mRNA stability, it is difficult to speculate that this may be how nickel enhances the levels of ATF-1 transcripts. Stabilization of mRNAs could be one of the epigenetic mechanisms by which nickel exerts its carcinogenic activity, but additional work is required to understand how nickel may have positive effects on gene expression. Interesting features of the up-regulation of ATF-1 in mouse cells are that it can be caused by direct exposure of cells to nickel, and this change is persistent even in the absence of nickel in transformed cells.

Little is known about the regulation of gene expression of ATF-1, the stability of its mRNA, or the half-life of the protein in different cells. ATF-1 was found to be a major protein purified from HeLa cells grown in suspension (39), and ATF-1 transcripts were found to be more abundant in these cells compared to ATFa, ATF-2, and CREB transcripts (47). However, because no comparison with normal human epithelial cells has been done, it is not clear whether the high level of ATF-1 expression was characteristic of epithelial tissues or if this high level of expression was related to the transformed state of HeLa cells. ATF-1 was found to be overexpressed in human lymphomas and in activated lymphocytes, suggesting an important role of this transcription factor in cell growth and malignant transformation (48). In lymphomas, overexpression of ATF-1 was not due to enhanced transcription but rather resulted from increased stability of ATF-1 mRNA in these cells (48).

Previously, we have shown that Rb is an important positive regulator of TSP I expression (16), and we found low levels of Rb expression in human and mouse nickel-transformed cells. It is interesting to note that an ATF-binding site was also identified in the promoter of Rb and was shown to be important for its expression (49). Thus, it is conceivable that ATF-1 transcription factor can also be a negative regulator of Rb expression. p53 has been shown to be another positive regulator of TSP I gene expression (24). Because p53 was found to be mutated in nickel-immortalized human epithelial cells (50), it is also possible that p53 is inactivated or mutated in nickel-transformed B200 cells, resulting in down-regulation of TSP I expression. In summary, we have found that the ATF/CREB site functions as a negative regulatory site in the mouse TSP I promoter. We also have shown that ATF-1 transcription factor is a negative regulator that binds to ATF/CREB-binding site in mouse 3T3 cells. This transcription factor was also involved in down-regulation of TSP I in nickel-transformed cells. Additionally, acute exposure of 3T3 cells to nickel resulted in induction of ATF-1, and this was correlated temporally with the down-regulation of TSP I.

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REFERENCES


5 A.K. Salnikow and M. Costa, manuscript in preparation.


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