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Transglutaminase-dependent Modulation of Transcription Factor Sp1 Activity

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Modification of transcription factors would result in significant changes in the expression of related genes. Recently, the presence of transglutaminase (TGase) has been reported in nuclei, the biological significances of which have attracted a great concern. In this study, we tested the possibility that nuclear TGase would crosslink and regulate the activity of a glutamine-rich transcription factor Sp1. The addition of purified guinea pig liver TGase increased the binding activity of Sp1 to the target DNA sequence by gel electrophoretic mobility shift assay. The activity of the human $p21^{WAF1}$ promoter containing six Sp1 binding sites was increased by the co-transfection of the TGase 2 gene, and two Sp1 sites at –82 and –69, relative to the transcription start site, were essential for the increased activity in human renal embryonic 293T cells. The activity of a minimal promoter containing three consensus Sp1 binding sites was increased by co-transfection of human TGase 2 gene. The amount of Sp1 protein was increased dramatically in TGase 2-transfected 293T cells and the Sp1 protein itself from HeLa cell nuclear extracts was crosslinked readily by purified TGase at 37°C in the presence of Ca^{2+}. These results suggest that the nuclear TGase might modulate the activity of the Sp1 transcription factor probably via the posttranslational or transcriptional modification of the factor by TGase.

Keywords: $p21^{WAF1}$ Promoter; Sp1; Transglutaminase 2.

Introduction

Transglutaminase 2 (TGase 2, TGase C, tissue TGase, or guinea pig liver TGase) is a Ca^{2+}-dependent enzyme that catalyzes the acyl-transfer reaction between the γ-carboxamide group of a peptide-bound glutamine residue and either the ε-amino group of a peptide-bound lysine residue yielding an isopeptide bond or the primary amino group of a polyamine resulting in ε (γ-glutamyl)-polyamine bond formation (Davies *et al.*, 1988; Folk, 1980; Folk and Chung, 1985). It also has GTP-binding and hydrolyzing activity which might be involved in the receptor-mediated intracellular signaling via phospholipase C δ1 (Feng *et al.*, 1996; Lee *et al.*, 1989; Nakaoka *et al.*, 1994).

TGase shows three distinct localizations, cytosol (73%), plasma membrane (20%) and nuclei (7%) (Bruce and Peter, 1983). In the case of nuclear TGase, there are a few candidate substrate proteins enriched in glutamine residues in the nuclei (Folk, 1980). For example, core histones or abnormal CAG repeat proteins such as huntingtin may be good substrates for nuclear TGase (Ballestar *et al.*, 1996; Karpuj *et al.*, 1999). In addition, other nuclear proteins, such as transcription factors, could be probable substrates for nuclear TGase. In this respect, we have tested the possibility that nuclear TGase might crosslink nuclear transcription factors, especially the ubiquitous glutamine-rich transcription factor, Sp1.

Transcription factor Sp1 is a Zn^{2+}-dependent protein that binds to GC-rich DNA sequences and regulates the constitutive and induced expression of a variety of mammalian genes (Kadonaga *et al.*, 1987). It has been shown to interact physically or functionally with other transcription factors such as TFIID, E2F1, YY1, pRb, or NF-kappaB (Lee *et al.*, 1993; Lin *et al.*, 1996; Saluja *et al.*, 1998; Udvadia *et al.*, 1993). It has two glutamine-rich domains outside the DNA binding domain (Courey and Tjian, 1988), which are important for the Sp1-mediated transcriptional activation: (a) one of the domains was responsible for mediating transcriptional activation of SV40 early promoter in Drosophila tissue culture cells (Courey and Tjian, 1988); (b) one of

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the domains was required for interaction with a glutamine-rich region of a human TFIID component, hTAFII130 (Saluja *et al*., 1998; Tanese *et al*., 1996); (c) physical interaction between c-Jun and at least one of the glutamine-rich domains of Sp1 was important to mediate TGF-β-induced activation of $p21^{WAF1/Cip1}$ promoter (Kardassis *et al*., 1999).

Therefore, we assumed if TGase 2 could crosslink the Sp1 protein as a glutamine donor substrate, it would affect the Sp1-mediated transcriptional activation of genes. Moreover, since Sp1 is a ubiquitous transcription factor (Kardassis *et al*., 1999), the modulation of Sp1 activity by TGase would affect the expression of a spectrum of genes, resulting in significant cellular responses.

Materials and Methods

Materials Purified Sp1, HeLa cell nuclear extracts and Sp1 binding consensus oligonucleotide were obtained from Promega (USA). Purified guinea pig liver transglutaminase was purchased from Sigma (USA). The γ-^{32}P-ATP was obtained from Amersham (UK). Dulbecco's modified Eagle's medium, fetal bovine serum, and penicillin G sodium/streptomycin sulfate were purchased from GIBCO BRL (USA).

DNA constructs The 3,257-bp human TGase 2 cDNA was cloned into the pcDNA3.1 vector (Invitrogen, USA). A minimal luciferase reporter plasmid, Sp1-luc (Sowa *et al*., 1997), and luciferase reporter plasmids containing various deletions of the human p21 promoter sequence (Nakano *et al*., 1997; Sowa *et al*., 1997) were kindly provided by Dr. Sakai and Dr. Sowa (Kyoto Prefectural University of Medicine, Kyoto, Japan). Briefly, Sp1-luc was generated by subcloning the fragment which contains three consensus Sp1 binding sites (GGGCGG) without the TATA box from the SV40 promoter into a luciferase reporter vector, pGL3-Basic (Promega, USA). pWWP was generated by subcloning the 2,400-bp human wild-type $p21^{WAF1/Cip1}$ promoter into the pGL3-Basic vector. The following reporter plasmids were then constructed: pWPdel-BstX I, containing all six Sp1 binding sites; pWP101, lacking the first (−119) and second (-109) binding site; pWP101-mtSp1-3, containing the functional fourth (−69) to sixth (−55) binding site; pWP101-mt-Sp1-5,6, containing the functional third (−82) and fourth (−69) binding site; and pWPdel-Sma I, containing the fifth (−60) and sixth (−55) binding site.

Gel electrophoretic mobility shift assay Purified Sp1 protein (1 or 0.2 fpμ) was incubated in a reaction mixture (30 μl) consisting of 4.2 mM HEPES, pH 7.9, 42 mM KCl, 1 mM $MgCl_2$, 11% glycerol, 20 mM dithiothreitol, 0.07 μg/ml poly(dI-dC), and γ-^{32}P-labeled oligonucleotides (40,000 cpm) at room temperature for 1 h. The sequence of oligonucleotide used in this assay was 5′-ATTCGATCGGGGCGGGGC-GAG-3′ (Promega, USA). Each protein–DNA complex was analyzed using 4% nondenaturing polyacrylamide gels at 4°C for 2 h. The gel was then dried and exposed to a film with an intensifying screen at −70°C. The autoradiogram was scanned with a Hewlett-Packard Scanjet 6200C scanner.

Cell culture and transient transfection Human renal embryonic 293T cells were maintained in Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 100 units/ml penicillin G sodium sulfate, and 100 μg/ml streptomycin sulfate. The cells were transfected by Lipofectamine-Plus reagent (GIBCO BRL, USA) for 6 h, cell lysates were collected for luciferase activity assay 48 h later.

Luciferase activity assay The luciferase activities of the cell lysates were determined according to the manufacturer's instructions (Promega, USA) and normalized to the cell lysate protein level.

Western blot analysis Protein samples were subjected to 10% SDS-polyacrylamide gel electrophoresis. The gel was blotted onto a nitrocellulose membrane, which was incubated with a specific antibody for Sp1 (Santa Cruz, USA), β-actin (Sigma, USA), or TGase 2 and then with a secondary antibody conjugated with horseradish peroxidase. Chemiluminescence was performed with an ECL kit (Amersham, UK) in accordance with the manufacturer's instructions.

Results

Increased Sp1 activity by TGase 2 To test whether TGase 2 could modulate the activity of Sp1, we checked the Sp1 binding force to the consensus-sequenced oligonucleotide using gel electrophoretic mobility shift assay. The binding activity of purified human Sp1 protein to the target DNA molecule was increased in the presence of purified guinea pig liver TGase dose-dependently, from 0.0005 unit/ml (0.02 μg/ml) to 0.5 unit/ml (20 μg/ml) concentration (Fig. 1A). A cold probe eliminated the band (Fig. 1A), which showed the band was a result of specific Sp1-target DNA binding. On the other hand, 20 μg/ml of boiled guinea pig liver TGase, γ-glutamyl transpeptidase (GGTP), or bovine serum albumin (BSA) did not change the Sp1 activity (Fig. 1B), which illustrated that the change in Sp1 activity by TGase 2 was not a nonspecific protein effect. The Sp1 protein from HeLa cell nuclear extracts as well as purified Sp1 protein showed the same results (data not shown). These results suggest that TGase 2 can regulate Sp1 activity *in vitro*.

Increased human $p21^{WAF1}$ promoter activity by TGase 2 expression To check whether the effect of TGase 2 on the modulation of Sp1 activity operates at the cellular level, we analyzed the luciferase activity of the human $p21^{WAF1}$ promoter, containing six consensus Sp1 binding sites in human embryonic renal 293T cells. The luciferase activity of pWWP, containing the wild-type promoter, was increased 2.1-fold by a coexpression of the human TGase 2 gene (Figs. 2A and 2B). To

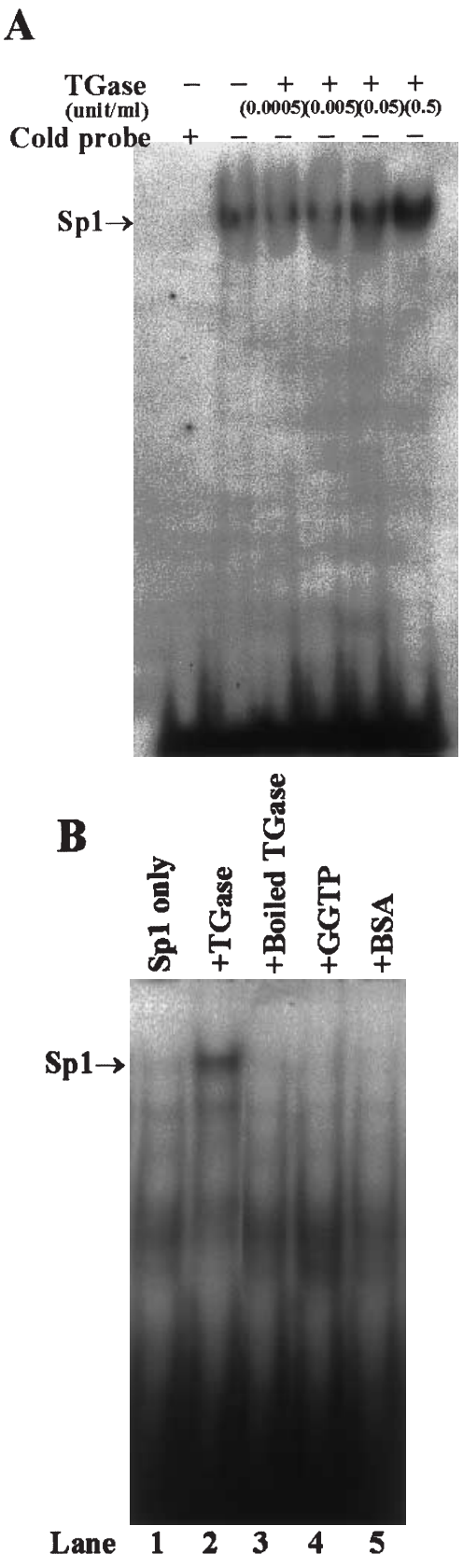

Fig. 1. Gel electrophoretic mobility shift assay for Sp1 activity. (**A**) The binding activity of purified Sp1 protein (one foot print unit) was enhanced in the presence of purified guinea pig liver TGase dose-dependently in the 0.0005–0.5 unit/ml concentration range. (**B**) 0.2 foot print unit of Sp1 protein alone did not cause any detectable band (lane 1). Enhanced binding activity of Sp1 by 20 μg/ml (0.5 unit/ml) of TGase (lane 2) was not observed in the presence of the same concentrations of boiled TGase (lane 3), γ-glutamyl transpeptidase (GGTP, lane 4) or bovine serum albumin (BSA, lane 5). The gel electrophoretic mobility shift assay was performed according to the method described in **Materials and Methods**.

determine whether the Sp1 binding sites were involved in the increased promoter activity, we compared the luciferase activity in various Sp1 binding site deletion constructs (Fig. 2A). The luciferase activity of pWPdel-*BstX* I containing only six Sp1 binding sites and the TATA box was still increased about 1.5-fold by TGase 2 expression, and the activity recovered to 1.9-fold in the first and second Sp1 binding site deleted construct, pWP101 (Fig. 2B). The pWP101-mtSp1-3 containing the mutated third binding site showed reduced fold induction (1.4-fold) and pWPdel-*Sma* I containing the fifth and sixth Sp1 binding site and the TATA box showed no fold induction (Fig. 2B). The pWP101-mtSp1-5,6 lacking the functional fifth and sixth Sp1 binding site did not show any promoter activity (Fig. 2B). All these data strongly suggest that the third (located at −82) and fourth (located at −69) Sp1 binding sites are the primary responsive sites for TGase 2 expression and the fifth and sixth binding sites are essential for the basal promoter activity. Moreover, this result demonstrates the possibility that TGase 2 can modulate Sp1 activity *in vivo*.

Increased activity of a minimal promoter by TGase 2 expression To confirm that TGase 2 can modulate the promoter activity through the Sp1 binding site, we analyzed the luciferase activity of the Sp1-luc plasmid (Fig. 2A), containing SV40 promoter-derived three consensus Sp1 binding sites but no TATA box, in the 293T cells. A transient coexpression of the human TGase 2 gene increased the luciferase reporter activity of Sp1-luc plasmid about 1.5-fold compared with the control expression of the empty pcDNA3.1 vector, whereas the expression of the TGase 2 gene did not alter the luciferase activity of the control pGL3-Basic vector (Fig. 2A and Fig. 3). This strongly suggests that TGase 2 can modulate promoter activity through the Sp1 binding site.

Sp1 crosslinking by TGase 2 To investigate the mechanism for the increase in Sp1 activity by TGase 2 *in vitro*, and the increase in promoter activity by TGase 2 in 293T cells, we checked the possibility that Sp1 protein might be crosslinked by the enzyme. The HeLa cell nuclear extracts were subjected to incubation with purified

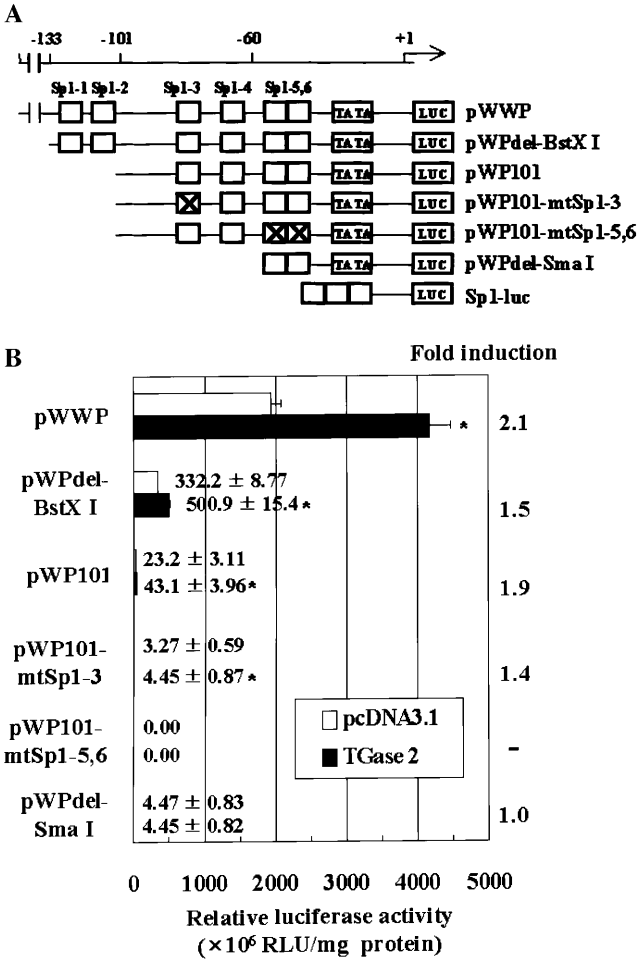

Fig. 2. (**A**) Schematic representation of full size (pWWP), deletion (pWPdel-*Bst*X I, pWP101, pWPdel-*Sma* I), and mutant (pWP101-mtSp1-3, pWP101-mtSp1-5,6) human $p21^{WAF1/Cip1}$ promoter–luciferase plasmids, and three tandem repeats of consensus Sp1 sites driving luciferase plasmid (Sp1-luc) and control vector (pGL3-Basic) used in transfection assays. The open boxes represent the Sp1 elements of the promoter and the crossed boxes represent the mutated Sp1 elements of the promoter. The 5′ end of the promoter is indicated by vertical bars and numbered relative to the transcription start site. (**B**) Transactivation of $p21^{WAF1/Cip1}$ promoter by TGase 2. The indicated p21 promoter-luciferase constructs (1 μg) were cotransfected into 293T cells separately with 0.2 μg empty pcDNA3.1 vector or human TGase 2 cDNA in 6 well plates. Each construct was described in **Materials and Methods**. The results are reported as mean values ± standard deviations from three different experiments. ∗ represents $p < 0.05$ by the Mann Whitney U test.

guinea pig liver TGase at 37°C in the presence of a cofactor, Ca^{2+}, and were analyzed by the Western blot method with a Sp1 specific antibody after SDS–polyacrylamide gel electrophoresis. As shown in Fig. 4A, the enzyme incubation caused the formation of high-molecular-weight-complex forms of Sp1 proteins (lane 2), which was shown clearly in the presence of the

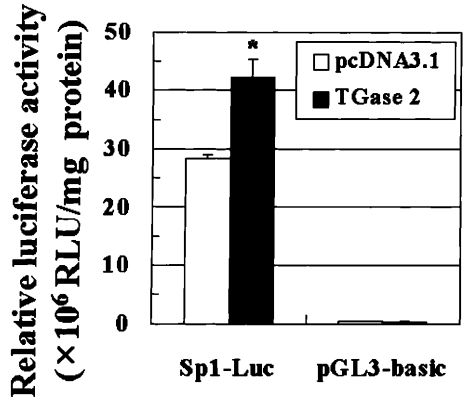

Fig. 3. Activation of the Sp1-luc promoter by TGase 2. One microgram of Sp1-luc or pGL3-Basic plasmid was cotransfected into 293T cells with 0.2 μg of empty pcDNA3.1 vector or human TGase 2 cDNA in 6-well plates. Each construct was described in **Materials and Methods**. The results are reported as mean values ± standard deviations from three different experiments. ∗ represents $p < 0.05$ by the Mann Whitney U test.

cofactor, Ca^{2+} (lane 3). This demonstrates that the Sp1 protein can be oligomerized or polymerized by TGase 2 through covalent crosslinks *in vitro*, by which Sp1 activity may also be modulated *in vivo*.

Although the original 293T cell nuclear extracts showed a weak expression of Sp1 protein, the amount of Sp1 protein was dramatically increased by TGase 2 gene transfection (Fig. 4B). This suggests the possibility that TGase 2 can modulate Sp1 activity by altering the amount of Sp1 protein, which may be due to delaying Sp1 protein degradation or the transcriptional activation of the Sp1 gene.

Discussion

In the present study, we tested the possibility of activity modulation of transcription factor Sp1 by TGase 2. By gel electrophoretic mobility shift assay, the addition of a purified guinea pig liver TGase increased the activity of human Sp1 protein dose-dependently (Fig. 1A). The fact that boiled TGase did not increase the Sp1 activity demonstrated the importance of the tertiary structure and the intact activity of the enzyme for its effect on the Sp1 activity (Fig. 1B). The specific effect of TGase 2 on Sp1 activity could be illustrated by excluding the nonspecific effects of other proteins (Fig. 1B).

Many genes are related with Sp1 transcription factor for their expression, but in this experiment, we selected the human $p21^{WAF1}$ promoter in order to focus on the regulation mechanism of the cell cycle, aging and cancer. The wild-type human $p21^{WAF1}$ promoter contains six Sp1 binding motifs, which are essential to the transactivation processes of the promoter by butyrate, histone deacetylase, nerve growth

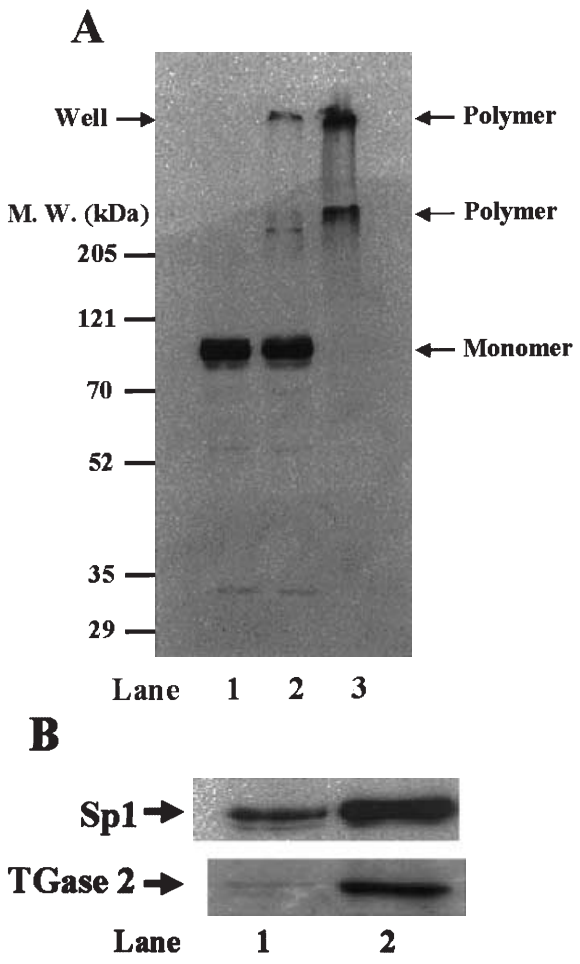

Fig. 4. (**A**) Crosslinking of Sp1 proteins by TGase 2. Forty micrograms of HeLa cell nuclear extracts (lane 1) and 20 μg of purified guinea pig liver TGase were coincubated in a buffer (10 mM HEPES, 100 mM KCl, 50 μM EDTA, 2.5 mM $MgCl_2$ and 6% glycerol) at 37°C for 1 h in the absence (lane 2) or presence (lane 3) of 10 mM $CaCl_2$. Sp1 protein was detected by Western blot analysis as described in **Materials and Methods**. (**B**) Sp1 protein level in 293T cell nuclear extracts. Original 293T cell nuclear extracts showed weak expression of Sp1 and TGase 2 (lane 1), whereas the amount of Sp1 protein was dramatically enhanced by TGase 2 gene transfection (lane 2). Each 2 μg of empty pcDNA3.1 vector or human TGase 2 cDNA was transfected into 293T cells in a 100 pi dish. Nuclear extracts (80 μg) were subjected to Western blot analysis as described in **Materials and Methods**.

factor, progesterone or c-Jun (Kardassis *et al*., 1999; Nakano *et al*., 1997; Owen *et al*., 1998; Sowa *et al*., 1997; Yan and Ziff, 1997). Of the six Sp1 binding motifs, it has been reported that the third and the fourth binding motif are the major responsive sites for promoter activation and that the fifth and the sixth binding site are essential for basal promoter activity (Nakano *et al*., 1997; Owen *et al*., 1998; Sowa *et al*., 1997). Consistent with these reports, it was observed that TGase 2 could also transactivate the promoter activity through the Sp1 binding sites, especially the third (located at −82) and the fourth (located at −69) binding site (Fig. 2B).

The various deletion mutants of $p21^{WAF1}$ promoter have the TATA box (Fig. 2A). It was confirmed that TGase 2 could regulate promoter activity through the Sp1 binding motifs by an experiment using Sp1-luc plasmid lacking the TATA box (Fig. 3).

Since TGase 2 has GTPase activity in addition to transglutamination activity (Feng *et al*., 1996; Lee *et al*., 1989; Nakaoka *et al*., 1994), it is not simple to speculate upon the nature of the mechanism by which TGase 2 enhances Sp1 activity by gel electrophoretic mobility shift assay (Fig. 1A) and the promoter activity via Sp1 binding sites (Figs. 2B and 3); however, Sp1 has two glutamine-rich domains, which are functionally important and might be good candidate substrate sites for TGase 2 (Courey and Tjian, 1988; Kardassis *et al*., 1999; Saluja *et al*., 1998; Tanese *et al*., 1996). Therefore, we tested the crosslinking of the factor by TGase 2 and found that Sp1 protein from HeLa cell nuclear extracts was readily oligomerized covalently by the enzyme *in vitro* (Fig. 4A). As a consequence, it can be suggested that the enhancement of Sp1 activity by TGase 2 *in vitro* might be associated with either a conformational change of the factor or its covalent crosslinking.

In addition, it was observed that the amount of Sp1 protein was increased in the TGase 2 overexpressed 293T cells (Fig. 4B), which suggests that TGase 2 might regulate Sp1 activity by the control of the transcription process or protein degradation process of Sp1 proteins in 293T cells. It is also possible that TGase 2 might regulate Sp1 activity by the oligomerization of the factor. Although we could not observe any significant changes in the activities of other transcription factors such as AP-1, TFIID, NF-kappaB from HeLa cell nuclear extracts (data not shown), the possibility should be considered that TGase 2 might affect promoter activity through the modulation of other transcription factors in 293T cells.

TGase 2 has been reported to be implicated in diverse cellular functions, including differentiation (Birckbichler *et al*., 1976; Scott *et al*., 1982), inhibition of cell growth (Cai *et al*., 1991; Katoh *et al*., 1996; Mian *et al*., 1995), cellular senescence (Park *et al*., 1999), apoptosis (Fesus *et al*., 1987; Oliverio *et al*., 1999; Piacentini *et al*., 1991) and drug resistancy (Han and Park, 1999a; 1999b). It is well known that $p21^{WAF1/Cip1}$ plays important roles in the control of cellular senescence (Brown *et al*., 1997), apoptosis (Kang *et al*., 1999) and differentiation (Topley *et al*., 1999). Sp1 has been reported to be associated with the transactivation processes in many gene promoters including cell cycle-regulating genes such as $p21^{WAF1/Cip1}$ promoter (Kardassis *et al*., 1999; Owen *et al*., 1998; Sowa *et al*., 1997). Therefore, it might be possible that TGase 2 could be

involved in processes related with p21 $^{WAF1/Cip1}$ gene activation through the modulation of Sp1 activity. The possibility of the modulation of Sp1 activity by TGase 2 in other genes should be evaluated further.

It is not yet known whether TGase 2 can modify other transcription factors, but the possibility that nuclear TGase 2 may modify and regulate many other transcription factors in addition to Sp1 would provide a novel mode of transcriptional control, which has the potential to affect a variety of biological systems significantly.

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References

Ballestar, E., Abad, C., and Franco, L. (1996) Core histones are glutaminyl substrates for tissue transglutaminase. *J. Biol. Chem.* **271**, 18817–18824.

Birckbichler, P. J., Orr, G. R., and Patterson, M. K. Jr. (1976) Differential transglutaminase distribution in normal rat liver and rat hepatoma. *Cancer Res.* **36**, 2911–2914.

Brown, J. P., Wei, W., and Sedvy, J. M. (1997) Bypass of senescence after disruption of p21CIP1/WAF1 gene in normal diploid human fibroblasts. *Science* **277**, 831–834.

Bruce, S. E. and Peter, T. J. (1983) The subcellular localization of transglutaminase in normal liver and in glucagon-treated and partial hepatectomized rats. *Biosci. Rep.* **3**, 1085–1090.

Cai, D., Ben, T., and De Luca, L. M. (1991) Retinoids induce tissue transglutaminase in NIH-3T3 cells. *Biochem. Biophys. Res. Commun.* **175**, 1119–1124.

Courey, A. J. and Tjian, R. (1988) Analysis of Sp1 *in vivo* reveals multiple transcriptional domains including a novel glutamine-rich activation motif. *Cell* **55**, 887–898.

Davies, P. J. A., Chiocca, E. A., Basilion, J. P., Podder, S., and Stein, J. P. (1988) Transglutaminase and their regulation: implications for polyamine metabolism. *Adv. Exp. Med. Biol.* **250**, 391–401.

Feng, J. F., Rhee, S. G., and Im, M. J. (1996) Evidence that phospholipase δ1 is the effector in the G_h (transglutaminase II)-mediated signaling. *J. Biol. Chem.* **271**, 16451–16454.

Fesus, L., Thomazy, V., and Falus, A. (1987) Induction and activation of tissue transglutaminase during programmed cell death. *FEBS Lett.* **224**, 13771–13776.

Folk, J. E. (1980) Transglutaminases. *Annu. Rev. Biochem.* **49**, 517–531.

Folk, J. E. and Chung, S. I. (1985) Transglutaminases. *Methods Enzymol.* **113**, 358–375.

Han, J. A. and Park, S. C. (1999a) Reduction of transglutaminase 2 expression is associated with an induction of drug sensitivity in PC14 human lung cancer cell line. *J. Cancer Res. Clin. Oncol.* **125**, 89–95.

Han, J. A. and Park, S. C. (1999b) Hydrogen peroxide mediates doxorubicin-induced transglutaminase 2 expression in PC14 human lung cancer. *Exp. Mol. Med.* **31**, 88–98.

Hirano, F., Tanaka, H., Hirano, Y., Hiramoto, M., Handa, H., Makino, I., and Scheidereit, C. (1998) Functional inferference of Sp1 and NF-kappaB through the same DNA binding site. *Mol. Cell Biol.* **18**, 1266–1274.

Kadonaga, J. T., Carner, K. R., Masiarz, F. R., and Tjian, R. (1987) Isolation of cDNA encoding transcription factor Sp1 and fuctional analysis of the DNA binding domain. *Cell* **51**, 1079–1090.

Kang, K. H., Kim, W. H., and Choi, K. H. (1999) p21 promotes ceramide-induced apoptosis and antagonizes the antideath effect of bcl-2 in human hepatocarcinoma cells. *Exp. Cell Res.* **253**, 403–412.

Kardassis, D., Papakosta, P., Pardali, K., and Moustakas, A. (1999) c-Jun transactivates the promoter of the human p21$^{WAF1/Cip1}$ gene by acting as a superactivator of the ubiquitous transcription factor Sp1. *J. Biol. Chem.* **274**, 29572–29581.

Karpuj, M. V., Garren, H., Slunt, H., Price, D. L., Gusella, J., Becher, M. W., and Steinman, L. (1999) Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc. Natl. Acad. Sci. USA* **96**, 7388–7393.

Katoh, S., Nakagawa, N., Yano, Y., Satoh, K., Konoh, H., and Ohkubo, Y. (1996) Transglutaminase induced by epidermal growth factor negatively regulates the growth signal in primary cultured hepatocytes. *Biochem. J.* **313**, 305–309.

Lee, J. S., Galvin, K. M., and Shi, Y. (1993) Evidence for physical interaction between the zinc-finger transcription factors YY1 and Sp1. *Proc. Natl. Acad. Sci. USA* **90**, 6145–6149.

Lee, K. N., Birckbichler, P. J., and Patterson, M. K. Jr. (1989) GTP hydrolysis by guinea pig liver transglutaminase. *Biochem. Biophys. Res. Commun.* **162**, 1370–1375.

Lin, S. Y., Black, A. R., Kostic, D., Pajovic, S., Hoover, C. N., and Azizkhan, J. C. (1996) Cell cycle-regulated association of E2F1 and Sp1 is related to their functional interaction. *Mol. Cell Biol.* **16**, 1668–1675.

Mian, S., Alaoui, E., Lawry, J., Gentile, V., Davies, P. J. A., and Griffin, M. (1995) The importance of GTP-binding protein tissue transglutaminase in the regulation of cell cycle progression. *FEBS Lett.* **370**, 27–31.

Nakano, K., Mizuno, T., Sowa, Y., Orita, T., Yoshino, T., Okuyama, Y., Fujita, T., Ohtani-Fujita, N., Matsugawa, Y., Tokino, T., Yamagishi, H., Oka, T., Nomura, H., and Sakai, T. (1997) Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. *J. Biol. Chem.* **272**, 22199–22206.

Nakaoka, H., Perez, D. M., Baek, K. J., Das, T., Husain, A., Misono, K., Im, M. J., and Graham, R. M. (1994) Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. *Science* **264**, 1593–1596.

Oliverio, S., Amendola, A., Rodolfo, C., Spinedi, A., and Piacentini, M. (1999) Inhibition of tissue transglutaminase increases cell survival by preventing apoptosis. *J. Biol. Chem.* **274**, 34123–34128.

Owen, G. I., Richer, J. K., Tung, L., Takimoto, G., and Horwitz, K. B. (1998) Progesteron regulates transcription of the p21^{WAF1} cyclin-dependent kinase inhibitor gene through Sp1 and CBP/p300. *J. Biol. Chem.* **273**, 10696–10701.

Park, S. C., Yeo, E. J., Han, J. A., Hwang, Y. C., Choi, J. Y., Park, J. S., Park, Y. H., Kim, K. O., Kim, I. G., Seong, S. C., and Kwak, S. J. (1999) Aging process is accompanied by increase of transglutaminase C. *J. Gerontol. A. Biol. Sci. Med. Sci.* **54**, B78–83.

Piacentini, M., Autuori, F., Dini, L., Farrace, M. G., Ghibelli, L., Piredda, L., and Fesus, L. (1991) Tissue transgluta-

minase is specifically expressed in neonatal rat liver cells undergoing apoptosis upon epidermal growth factor stimulation. *Cell Tissue Res.* **263**, 227–235.

Saluja, D., Vassallo, M. F., and Tanese, N. (1998) Distinct subdomains of human TAFII130 are required for interactions with glutamine-rich transcruptional activators. *Mol. Cell Biol.* **18**, 5734–5743.

Scott, K. F. F., Meyskens, F. L. Jr., and Russell, D. H. (1982) Retinoids increase transglutaminase activity and inhibit ornithine decarboxylase activity in chinese hamster ovary cells and in melanoma cells stimulated to differentiate. *Proc. Natl. Acad. Sci. USA* **79**, 4093–4097.

Sowa, Y., Orita, T., Minamikawa, S., Nakano, K., Mizuno, T., Nomura, H., and Sakai, T. (1997) Histone deacetylase inhibitor activates the WAF1/Cip1 gene promoter through the Sp1 sites. *Biochem. Biophys. Res. Commun.* **241**, 142–150.

Tanese, N., Saluja, D., Vassallo, M. F., Chen, J. L., and Admon, A. (1996) Molecular cloning and analysis of two subunits of the human TFIID comlex: hTAFII130 and hTAFII100. *Proc. Natl. Acad. Sci. USA* **93**, 13611–13616.

Topley, G. I., Okuyama, R., Gonzales, J. G., Conti, C., and Dotto, G. P. (1999) p21(WAF1/Cip1) functions as a suppressor of malignant skin tumor formation and a determinant of keratinocyte stem-cell potential. *Proc. Natl. Acad. Sci. USA* **96**, 9089–9094.

Udvadia, A. J., Rogers, K. T., Higgins, P. D., Murata, Y., Martin, K. H., Humphrey, P. A., and Horowitz, J. M. (1993) Sp-1 binds promoter elements regulated by the RB protein and Sp-1-mediated transcription is stimulated by RB coexpression. *Proc. Natl. Acad. Sci. USA* **90**, 3265–4369.

Yan, G. and Ziff, E. B. (1997) Nerve growth factor induces transcription of the p21 WAF1/CIP1 and cyclin D1 genes in PC12 cells by activating the Sp1 transcription factor. *J. Neurosci.* **17**, 6122–6132.