DNA Topoisomerase II Poison TAS-103 Transactivates GC-Box-dependent Transcription via Acetylation of Sp1*

Received for publication, September 13, 2004, and in revised form, October 26, 2004
Published, JBC Papers in Press, November 8, 2004, DOI 10.1074/jbc.M410499200

Takayuki Torigoë‡‡, Hirotu Izumi‡, Tetsuro Wakasugi‡, Ichiro Niina‡, Tomonori Igarashi‡, Takeshi Yoshida‡, Izumi Shibuya‡, Kazuo Chijiiwa‡, Ken-ichi Matsuo**, Hideaki Itoh‡, and Kimitoshi Kohno‡ ‡‡

From the ‡Department of Molecular Biology, the ‡Department of Surgery I and the ‡Department of Physiology I, University of Occupational and Environmental Health, School of Medicine, 1-1 Iseigaoka, Yahatanishi-ku, Kitakyushu, Fukuoka 807-8555, Japan, the Department of Surgery I, University of Miyazaki, Miyazaki-gun, Miyazaki 889-1692, Japan, and **Hanno Research Center, Taiho Pharmaceutical Co., Ltd., Hanno, Saitama 357-8527, Japan

Drug-induced modifications of transcription factors play important roles in both apoptosis and survival signaling. The data presented here show that the DNA topoisomerase II poison TAS-103 transactivated the SV40 promoter in a GC-box-dependent manner and induced Sp1 acetylation in cells expressing p300. This activity was not observed in cells lacking p300. TAS-103 treatment also enhanced the p300 content of the nucleus and the interaction of p300 with Sp1. Cellular susceptibility to TAS-103 was correlated with p300 expression but not with topoisomerase II expression. Furthermore, the presence of p300 significantly sensitized cancer cells to TAS-103 but not to cisplatin. Taken together, these findings demonstrate novel genomic responses to anticancer agents that modulate Sp1 acetylation and Sp1-dependent transcription in an apoptotic pathway.

Sp1 was one of the first transcription factors to be identified in mammalian cells. It is a member of the zinc-finger family, which functions by binding the GC-box within the DNA sequences of promoters (1, 2). Sp1 is expressed ubiquitously in various mammalian cells and is implicated in the transcription of many genes, particularly housekeeping genes and those that are involved in cell growth and development (3, 4). Changes in the cellular content of Sp1, the Sp1/Sp3 ratio, and its GC-box DNA binding activity influence the regulation of Sp1 transcriptional activity (5).

Transcriptional co-activators have an inherently low transcriptional activity. Their only active role in the transcriptional process occurs through their interaction with transcription factors (6). p300 is a transcriptional co-activator that can acetylate a variety of transcription factors and histones (7, 8). The histones are localized within transcriptionally active euchromatin, and their interaction with p300 plays a critical role in transcription regulation. A wide range of biological processes such as the cell cycle, differentiation, and tumor growth are regulated by p300 (9). This protein has the potential to activate p53 target genes and might thus act as a suppressor of tumor cell growth (10–12). Recently, p300 has been shown to function together with Sp1 in GC-box-dependent transcription (13, 14).

The DNA topoisomerase (topo) II poison and anticancer agent TAS-103 induces cellular acidosis through changes in the mitochondrial membrane potential (15). We demonstrated previously that the Sp1 DNA binding activity and interaction of Sp1 with TATA-binding protein are enhanced under conditions of low pH. Therefore, cellular pH might also be crucial for the expression of Sp1 target genes (16), including TAS-103-induced cellular acidosis.

In this study, we show that the treatment of cells with TAS-103 leads to the acetylation of Sp1, resulting in a dramatic induction of Sp1-dependent promoters. We also demonstrate that p300 expression sensitizes cancer cells to TAS-103. We propose that p300-dependent acetylation of Sp1 triggered through the DNA-damage signaling pathway and cellular acidosis leads to the enhanced expression of Sp1 target genes in TAS-103-induced apoptosis. This discovery has important implications for current views of the molecular mechanisms by which anticancer agents trigger genome-wide responses. These important responses might occur through the posttranscriptional modification of transcription factors.

EXPERIMENTAL PROCEDURES

Reagents, Cell Culture, and Antibodies—TAS-103 was kindly provided by the Taiho Pharmaceutical Co., Ltd. (Tokyo, Japan). Cisplatin and etoposide were purchased from Sigma, and camptothecin was purchased from Daichi Seiyaku (Tokyo, Japan).

Human epidermoid cancer KB cells were cultured in Eagle’s minimal essential medium, human breast cancer MCF7 cells and human glioblastoma T98G cells were cultured in Dulbecco’s modified Eagle’s medium, and human colon cancer cell lines (HTC15, CaCo2, LoVo, and SW620) were cultured in RPMI 1640 medium (all from Nissui Seiyaku Co., Tokyo, Japan) containing 10% fetal bovine serum and 0.292 mg/ml L-glutamine. All of the cells were cultured at 37 °C in a humidified chamber containing 5% CO2.

Anti-pan-acetylation (sc-6849) and anti-topo-IIα (sc-5346) antibodies were purchased from Santa Cruz Biotecnology (Santa Cruz, CA). Anti-FLAG (anti-M2) and anti-hemagglutinin (anti-HA)-peroxidase antibodies were purchased from Sigma and Roche Applied Science, respectively.

Preparation of Luciferase-Reporter and Expression Plasmids—Simian virus 40 (SV40) promoter luciferase-reporter plasmids were constructed using the pGL3-promoter vector containing the SV40 promoter (Promega, Madison, WI) designated SV40 Luc1, which contained six copies of the SV40 promoter luciferase-reporter plasmids were constructed using the pGL3-promoter vector containing the SV40 promoter (Promega, Madison, WI) designated SV40 Luc1, which contained six copies of the SV40 promoter.

An expression plasmid for FLAG-Sp1 was obtained using FLAG-tagged Sp1 cDNA fragments (a gift from R. Tjian, University of Cali-
TAS-103 Induces Acetylation of Sp1

Fig. 1. TAS-103 increases Sp1-dependent promoter activity. A, transcriptional activity of the SV40 promoter in response to TAS-103 treatment. KB cells were transiently transfected with the pGL3-promoter vector containing the SV40 promoter, SV40 Luc1. After transfection for 12 h, the cells were incubated for a further 12 h in fresh medium with or without TAS-103 (0, 1, 2, and 4 μM). Luciferase activity was measured as described under “Experimental Procedures.” B2 indicates the pGL3-basic vector. Results are the mean ± S.D. of at least three independent experiments. Error bars indicate the mean ± S.D. B, schematic representation and relative promoter activity of the deletion construct. The deletion construct of SV40 Luc1 subcloned into the pGL3-basic vector upstream of the luciferase reporter gene was named SV40 Luc2 and did not contain a GC-box. KB cells were transiently transfected and treated with TAS-103 (4 μM) before the luciferase assay was performed as described above. The sequences that serve as the recognition site for Sp1 (GC-box) are shown as black boxes. B2 indicates the pGL3-basic vector. Results are the mean ± S.D. of at least three independent experiments. Error bars indicate the mean ± S.D.

A

B

Transcript Transfection and Luciferase Assay—Transient transfections were performed as described previously (17, 18) with minor modifications. Cells were seeded into 12-well tissue culture plates at a concentration of 4 × 10⁴ cells/well. The next day, the cells were transfected with a luciferase-reporter plasmid using Superfect reagent according to the manufacturer’s instructions. The β-galactosidase expression plasmid pCH110 (Amersham Biosciences) was co-transfected as an internal control. The cells were then washed and incubated at 37 °C for 12 h in fresh medium with or without TAS-103. Subsequently, the cells were lysed with reporter lysis buffer (Promega).

Luciferase activity in the lysed cells was detected using a Picogene kit (Toyoinki, Tokyo, Japan) according to the manufacturer’s instructions. The light intensity was measured for 2 s with a luminometer (Luminescencer JNRII AB-2300, ATTO, Tokyo, Japan). The results shown represent at least three independent experiments and were normalized to β-galactosidase activity measured using an enzyme assay kit (Promega).

Preparation of Cellular Extracts—Nuclear extracts were prepared using buffer C as described previously (17, 18). Extracts of whole cells were prepared by lysing cells in buffer X comprising 50 mM Tris-HCl (pH 8.0), 120 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, and 10% glycerol. The cellular debris was removed from the extract by centrifugation and the supernatant was stored at −70 °C. The protein concentrations of nuclear and whole-cell extracts were determined using the Bradford method.

Western Blot Analysis—Nuclear and whole-cell extracts were separated on 7.5% SDS-PAGE gels. Proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA) using a semidry blotter. Western blot analysis was performed with an appropriate dilution of each antibody followed by visualization using enhanced chemiluminescence.

In Vivo Binding Assay—An in vitro binding assay (transient transfection and co-immunoprecipitation assay) was performed as described previously (18) with minor modifications. MCF7 cells growing in 60-mm plates were co-transfected with HA- and FLAG fusion plasmids along with Superfect reagent according to the manufacturer’s instructions. Three hours after transfection, the cells were washed with phosphate-buffered saline and the medium was renewed. After 36 h, the medium was discarded and fresh medium was added with or without anticancer agents (TAS-103 and cisplatin) as indicated. Whole-cell or nuclear extracts were prepared as described above. These were incubated for 2 h at 4 °C with 2 μg of anti-M2 antibody and 10 μl of protein A/G-agarose (Qiagen). The beads were then washed three times with buffer X. The immuno precipitated samples were analyzed using 7.5% SDS-PAGE followed by Western blot analysis.

Cytotoxicity Assay—Cells were seeded in 96-well tissue culture plates at a concentration of 5 × 10³ cells/well. On the following day, drugs were added to the medium at increasing concentrations. After 48 h, the surviving cells were assayed with TetraColar ONE (Seikagaku Corporation, Tokyo, Japan) for 2 h at 37 °C according to the protocol provided and absorbance was measured at 450 nm. The IC₅₀ value was defined as the drug concentration needed to reduce cell growth by 50% relative to the control.

RESULTS

TAS-103 Treatment Markedly Induced SV40 Promoter Activity in a GC-box-dependent Manner—We reported previously that the cellular level of Sp1 increased severalfold in human epidermoid cancer KB cells after exposure to 4 μM TAS-103 (17). We also demonstrated that the SV40 promoter, which contains six GC-boxes, was activated markedly by TAS-103 treatment in a dose-dependent manner (Fig. 1A). The activity
of SV40 Luc1 was maximal following 4 μM TAS-103 treatment for 12 h. To confirm that this effect was GC-box-dependent, we constructed the reporter plasmid SV40 Luc2, which lacks a GC-box. TAS-103 did not induce SV40 promoter activity from this construct (Fig. 1B).

Co-activator p300 Has Critical Roles in TAS-103-induced SV40 Promoter Activity—We next examined TAS-103-induced SV40 promoter activity in various human cancer cell lines. Both KB cells and human glioblastoma T98G cells induced transactivation of the SV40 promoter ~80- and 30-fold following TAS-103 treatment, respectively, whereas human colon cancer HCT15 cells did not (Fig. 2A). However, the Sp1 content of the nucleus induced by TAS-103 treatment was increased only severalfold in these three cell lines (data not shown).
Mutations of p300 have frequently been detected in colon cancer cell lines including HCT15 cells (12). p300 expression was induced by treatment with TAS-103 in KB and T98G cells but not in HCT15 cells (Fig. 2B). Furthermore, transient transfection of a p300 expression plasmid was able to enhance TAS-103-induced SV40 promoter activity by ~20-fold in HCT15 cells (Fig. 2C). This finding suggests that the induction of Sp1 acetylation might be involved in the molecular mechanisms underlying the TAS-103-induced transactivation of Sp1-dependent promoters.

**Treatment of Cells with TAS-103 Led to the Acetylation of Sp1 through Interaction with p300**

p300 Expression Was Correlated with Sensitivity to TAS-103 Treatment in Human Colon Cancer Cell Lines—Bearing in mind the strong link between p300 expression and Sp1 activation after treatment with TAS-103, we next examined whether p300 was involved in cellular sensitivity to TAS-103. Four human colon cancer cell lines, either with or without p300 expression, were used in a cytotoxicity assay. HCT15 and CaCo2 cells lacked p300 expression, whereas LoVo and SW620 cells showed significant p300 expression (Fig. 4A, upper panel). The levels of topo-II expression varied among these cell lines (Fig. 4A, middle panel). Cellular sensitivity to TAS-103 was dependent upon p300 expression. Cells expressing p300 showed 10–30-fold greater sensitivity to TAS-103 compared with cells lacking p300 (Fig. 4B). Cellular expression of p300 also sensitized cells to the well-characterized topo-II poison, etoposide (Fig. 4C). However, cells expressing p300 showed only a 2–9-fold greater sensitivity to etoposide compared with cells lacking p300. Furthermore, among cells lacking p300, CaCo2 cells showed higher expression of topo-IIa and higher sensitivity to etoposide than did HCT15 cells. In addition, among the cells expressing p300, LoVo cells showed higher expression of topo-IIa and higher sensitivity to etoposide than did SW620 cells. These results indicate that topo-IIa expression might be involved in cellular sensitivity to etoposide when cells are categorized into two groups based on p300 status. On the other hand, the cellular sensitivity to cisplatin was not associated with p300 expression (Fig. 4D). The levels of topo-IIa expression have been shown to be involved in the sensitivity of cancer cells to topo II poisons (20); however, no such correlation was observed in the sensitivity of these human colon cancer cell lines to TAS-103 (Fig. 4, A and B). These data indicate that the acetylation of Sp1 via an interaction with p300 is involved in TAS-103-induced apoptosis in cancer cells.

**DISCUSSION**

We have previously identified several transcription factors involved in genomic responses to anticancer agents including Y-box-binding protein-1 (YB-1), activating transcription factor 4 (ATF4), zinc-finger factor 143 (ZNF143), and mitochondrial transcription factor A (mtTFA) (21–24). This study provides evidence to support the novel finding that Sp1 contributes to the stress response of cancer cells following treatment with anticancer agents. We used the SV40 promoter-luciferase reporter to examine whether TAS-103 could activate Sp1-dependent transcription. This was based on the knowledge that Sp1 was originally identified as a transcription factor from HeLa cells that binds to GC-boxes within the 21-bp repeat elements of the SV40 promoter (1, 2). We found that TAS-103 dramatically activated SV40 promoter activity in KB cells (Fig. 1A) by inducing the acetylation of Sp1
through physical interaction with p300 (Fig. 3). Acetylation of Sp1 might be important in bringing about high-level transactivation of the Sp1-dependent promoter. These findings could explain the discrepancy observed in cells expressing p300 such as KB and T98G cells in which TAS-103 induced extensive transactivation of the SV40 promoter (Fig. 2A), whereas the Sp1 protein level was increased only severalfold by TAS-103 treatment (data not shown). To our knowledge,
were clustered within the zinc-finger domain. The acetylated transcription. Lysine residues that could be acetylated by p300 transferase that functions in chromatin remodeling during camptothecin did not transactivate the SV40 promoter (data not shown). Taken together, these findings indicate that the mode of action of TAS-103 might differ from that of etoposide. Martens et al. (32) reported that p300 is a major constituent of the nuclear matrix, similar to topo-IIα (33). p300 does not interact directly with topo-IIα. Rather they co-localize with each other. One possible explanation for why p300 expression sensitized cells to etoposide is that both proteins were localized in the nuclear matrix attachment region, which might be critical for inducing a cleavable complex with etoposide.

Our demonstrated results suggest that treatment with TAS-103 might induce apoptosis by activating both p300- and Sp1-dependent gene expressions. Sp1 directly or indirectly regulates several proteins including Bax, Fas ligand, and caspase-8 that induce apoptosis (34–36). Therefore, further studies should examine the relationship between TAS-induced apoptosis and the expression of these genes. Our present data indicate that TAS-103 is potentially unique in its ability to activate Sp1 target genes through both Sp1 acetylation and cellular acidification (Fig. 5). Collectively, these results demonstrate that Sp1 participates directly in the drug-induced signaling pathway through posttranscriptional modification.

**REFERENCES**

1. Dynan, W. S., and Tjian, R. (1983) Cell 32, 669–680
2. Dynan, W. S., and Tjian, R. (1983) Cell 35, 79–87
3. Suske, G. (1999) Gene (Amst.) 238, 291–300
4. Black, A. R., Black, J. D., and Azizkhan-Clifford, J. (2001) J. Cell. Physiol. 188, 143–160
5. Bouwman, P., and Philipsen, S. (2002) Mol. Cell. Endocrinol. 195, 27–38
6. Imhof, A., Yang, X. J., Oryzko, V. N., Nakatani, Y., Wolffe, A. P., and Ge, H. (1997) Curr. Biol. 7, 689–692
7. Chan, H. M., and La Thangue, N. B. (2001) J. Cell Sci. 114, 2363–2373
8. Vo, N., and Goodman, R. H. (2001) J. Biol. Chem. 276, 13505–13508
9. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
10. Klochkod-Yevin, A., and Yaniv, M. (2001) Biochem. Biophys. Acta 1551, M1–M10
11. Grossman, S. R. (2001) Eur. J. Biochem. 268, 2773–2778
12. Ionov, Y., Matsui, S., and Cowell, J. K. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 1273–1278
13. Xiao, H., Hasegawa, T., and Isobe, K. (2000) J. Biol. Chem. 275, 1371–1376
14. Suzuki, T., Kimura, A., Nagai, R., and Horikoshi, M. (2000) Genes Cells 5, 29–41
15. Roeder, J., Laniaux, A., Wattiez, N., Mahieu, C., Osheroff, N., and Bailly, C. (2000) Cancer Res. 60, 4077–4084
16. Torigoe, T., Izumi, H., Yoshida, Y., Ishiguchi, H., Okamoto, T., Itoh, H., and Kohno, K. (2005) Nucleic Acids Res. 33, 4323–4330
17. Torigoe, T., Izumi, H., Ishiguchi, H., Urakami, T., Ise, T., and Kohno, K. (2002) J. Biol. Chem. 277, 36534–36543
18. Izumi, H., Ohia, R., Nagatsui, G., Ise, T., Nakayama, Y., Nomoto, M., and Kohno, K. (2003) Biochem. J. 373, 713–722
19. Izumi, H., Ise, T., Murakami, T., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Yoshida, T., Tanabe, M., and Kohno, K. (2003) Biochem. Biophys. Acta 1629, 97–104
20. Takano, H., Kohno, K., Katsus, M., Matsuda, T., and Kuwano, M. (1992) Anticancer Drugs 3, 323–330
21. Kohno, K., Izumi, H., Uchiumi, T., Ashizuka, M., and Kuwano, M. (2003) BioEssays 25, 691–698
22. Torigoe, T., Izumi, H., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Tanabe, M., Ise, T., and Kohno, K. (2002) J. Biol. Chem. 277, 36534–36543
23. Izumi, H., Ohia, R., Nagatsui, G., Ise, T., Nakayama, Y., Nomoto, M., and Kohno, K. (2003) Biochem. J. 373, 713–722
24. Izumi, H., Ise, T., Murakami, T., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Yoshida, T., Tanabe, M., and Kohno, K. (2003) Biochem. Biophys. Acta 1629, 97–104
25. Takano, H., Kohno, K., Katsus, M., Matsuda, T., and Kuwano, M. (1992) Anticancer Drugs 3, 323–330
26. Torigoe, T., Izumi, H., Urakami, T., Ishiguchi, H., Uramoto, H., and Kohno, K. (2003) Cancer Res. 63, 3729–3734
27. Kohno, K., Izumi, H., Uchiumi, T., Ashizuka, M., and Kuwano, M. (2003) BioEssays 25, 691–698
28. Terabe, M., Izumi, H., Ise, T., Higuchi, S., Yamori, T., Yasumoto, K., and Kohno, K. (2003) Cancer Res. 63, 8592–8595
29. Ishiguchi, H., Izumi, H., Torigoe, T., Yoshida, Y., Kubota, H., Tsuji, S., and Kohno, K. (2004) Int. J. Cancer 111, 900–909
30. Yoshida, Y., Izumi, H., Torigoe, T., Ishiguchi, H., Itoh, H., Kang, D., and Kohno, K. (2003) Cancer Res. 63, 3729–3734
31. Laurencot, C., Andrew, A. P., and Kennedy, K. A. (1995) Oncol. Res., 363–369
32. Gellitch, R. A., Gissing, H. A., Zhub, J. Y., Engler, R. L., and Babior, B. M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5965–5968
33. Gellitch, R. A., Nordberg, J., Skovronski, E., and Babior, B. M. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 654–658
34. Beck, W. T. (1887) Biochem. Pharmacol. 36, 2879–2887
35. Torigoe, T., Izumi, H., Ise, T., Murakami, T., Uramoto, H., Ishiguchi, H., Yoshida, Y., Tanabe, M., Nomoto, M., and Kohno, K. (2002) Anticancer Drugs 13, 237–245

**FIG. 5. Schematic summary of TAS-103-induced Sp1 target gene expression.** TAS-103 treatment leads to the acetylation of Sp1 via an interaction with p300. Furthermore, TAS-103 can induce cellular acidosis (15), and DNA binding by Sp1 and its interaction with TATA-binding protein (TBP) are enhanced under conditions of low pH (16). Acetylated Sp1 plus cellular acidosis might be important for TAS-103-induced transactivation of Sp1 target genes. Ac indicates the acetylated form of Sp1.
30. Izumi, H., Torigoe, T., Ishiguchi, H., Uramoto, H., Yoshida, Y., Tanabe, M., Ise, T., Murakami, T., Yoshida, T., Nomoto, M., and Kohno, K. (2003) *Cancer Treat. Rev.* **29**, 541–549
31. Tsunoda, T., Tanimura, H., Yamaue, H., Ishimoto, K., Kobunai, T., and Yamada, Y. (2001) *Anticancer Res.* **21**, 3897–3902
32. Martens, J. H., Verlaan, M., Kalkhoven, E., Dorosman, J. C., and Zantema, A. (2002) *Mol. Cell. Biol.* **22**, 2598–2606
33. Adachi, Y., Kas, E., and Laemmli, U. K. (1989) *EMBO J.* **8**, 3997–4006
34. Thornborrow, E. C., and Manfredi, J. J. (2001) *J. Biol. Chem.* **276**, 15598–15608
35. McClure, R. F., Heppelmann, C. J., and Paya, C. V. (1999) *J. Biol. Chem.* **274**, 7756–7762
36. Liedtke, C., Groger, N., Manns, M. P., and Trautwein, C. (2003) *J. Biol. Chem.* **278**, 27593–27604