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Pituitary Tumor Transforming Gene Causes Aneuploidy and p53-dependent and p53-independent Apoptosis*

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The pituitary tumor transforming gene, PTTG, is abundantly expressed in several neoplasms. We recently showed that PTTG overexpression is associated with apoptosis and therefore have now studied the role of p53 in this process. In MCF-7 breast cancer cells that express wild type p53, PTTG overexpression caused apoptosis. p53 was translocated to the nuclei in cells expressing PTTG. Overexpression of p53, along with PTTG, augmented apoptosis, whereas expression of the human papillomavirus E6 protein inhibited PTTG-induced apoptosis. In MG-63 osteosarcoma cells that are deficient in p53, PTTG caused cell cycle arrest and subsequent apoptosis that was inhibited by caspase inhibitors. A proteasome inhibitor augmented PTTG expression in stable PTTG transfectants, suggesting that down-regulated PTTG expression is required for cell survival. Finally, MG-63 cells expressing PTTG showed signs of aneuploidy including the presence of micronuclei and multiple nuclei. These results indicate that PTTG overexpression causes p53-dependent and p53-independent apoptosis. In the absence of p53, PTTG causes aneuploidy. These results may provide a mechanism for PTTG-induced tumorigenesis whereby PTTG mediates aneuploidy and subsequent cell transformation.

Pituitary Tumor Transforming Gene (PTTG)[1] is highly expressed in pituitary tumors and other neoplasms (1–3). *In vitro*, PTTG transforms 3T3 fibroblasts (1, 4), but the full mechanism of PTTG action has not been clarified. PTTG induces basic fibroblast growth factor secretion (1, 5) and transactivates DNA transcription (6, 7). PTTG is a mammalian securin that maintains binding of sister chromatids during mitosis (8). We recently studied the cellular characteristics of PTTG (9) and showed that PTTG mRNA and protein expressions are cell cycle-dependent and peak at the G_2/M phase. PTTG is localized to both the nucleus and cytoplasm and is degraded at the initiation of anaphase. PTTG overexpression causes apoptosis and inhibits mitosis (9).

Tumor suppressor p53 subjects cells with severe DNA damage or other stress to apoptosis by transactivating apoptosis-inducing genes. Expression of oncogenes such as *myc* (10–12) activates p53 and renders cells apoptotic. The same oncogenes, however, also activate p53-independent apoptosis (13, 14). In this report, we studied p53 involvement in PTTG-induced cell death in cells expressing or lacking wild type p53. Our results show that PTTG overexpression causes p53-dependent and p53-independent apoptosis. Aneuploidy arises as a result of PTTG overexpression in p53-negative cells. These results suggest that aneuploidy may be a mechanism for PTTG-induced tumorigenesis.

EXPERIMENTAL PROCEDURES

Cell Culture, Plasmids, and Transfection—MCF-7 and MG-63 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal calf serum in a 37 °C, humidified incubator with 5% CO_2. Cells were synchronized with double thymidine block (9). Cell cycle was analyzed with a fluorescence-activated cell sorter. Plasmids encoding wild type and an enhanced green fluorescent protein (EGFP)-tagged human PTTG and the parental plasmid pEGFP-N3 were described before (9). Plasmids encoding p53 and R175H p53 were gifts from Dr. C. W. Miller (Cedars-Sinai Medical Center, Los Angeles, CA). Plasmid encoding human papillomavirus (HPV) E6 protein was described before (15). Cells were transfected with FuGene (Hoffmann-La Roche). Stable MG-63 cell lines expressing EGFP or PTTG-EGFP were established by incubating cells with 1 mg/ml G418 after transfection. Caspase inhibitors I and III were from Calbiochem (La Jolla, CA).

Apoptosis Assay—Cells transfected with EGFP or PTTG-EGFP were fixed with 4% paraformaldehyde and permeabilized with 0.6% Tween 20 and stained with Hoechst 33258 (1:10,000; Molecular Probes, Eugene, OR). Slides were observed with a 40× objective, and green cells with apoptotic nuclear characteristics such as nuclear condensation and fragmentation were scored as apoptotic. Apoptosis was confirmed by terminal dUTP nick-end labeling staining. 200–300 green cells were examined on each slide. Nuclear morphology of green cells was also examined for signs of aneuploidy such as micronuclei and macronuclei.

Immunofluorescent staining and fluorescence microscopy were performed as described (9). An antibody to p53 (1:1,000; Calbiochem) or to mdm2 (16) was used and detected with rhodamine-labeled goat anti-mouse IgG (1:500; Molecular Probes). Cells were finally stained with Hoechst 33258 (Molecular Probes). Several hundred cells were observed in each of the 2 to 3 staining experiments, and representative cells are depicted. Western blotting of PTTG-EGFP was performed as described (9).

Metaphase Karyotyping—Growing cells were treated with 100 ng/ml colcemid (Calbiochem) for 90 min, and G_2/M cells were isolated by brief trypsinization and gentle tapping. These cells were incubated with 75 mM KCl for 30 min and fixed and washed with methanol-acetic acid (3:1) and dropped onto glass slides. Metaphase chromosomes were observed.

RESULTS

PTTG Causes Apoptosis in MCF-7 and MG-63 Cells—Human breast cancer MCF-7 cells express wild type p53 (17), and human osteosarcoma MG-63 cells are deficient in p53 (18). Both MCF-7 and MG-63 cells express PTTG mRNA (data not shown). EGFP had no significant effect on apoptosis in either MCF-7 or MG-63 cells (Fig. 1). Expression of PTTG-EGFP caused apoptosis in both MCF-7 and MG-63 cells. In MCF-7

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[1] The abbreviations used are: PTTG, pituitary tumor transforming gene; EGFP, enhanced green fluorescent protein; PTTG-EGFP, EGFP-tagged PTTG; HPV, human papillomavirus.

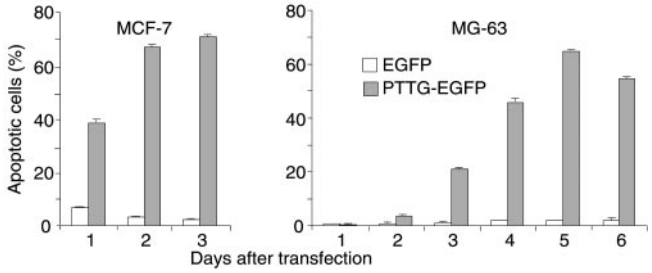

FIG. 1. **PTTG induces apoptosis in MCF-7 and MG-63 cells.** MCF-7 (*left*) and MG-63 (*right*) cells were transfected with EGFP or PTTG-EGFP. On each day after transfection, cells were fixed and stained with Hoechst 33258. Nuclei of green cells were examined with fluorescence microscopy, and the number of cells with apoptotic nuclei was divided by the number of all green cells examined. 200–300 cells were examined for each point.

cells, apoptosis occurred earlier. These results demonstrate that PTTG-induced apoptosis occurs independently of the presence of p53.

PTTG Activates p53-mediated Apoptosis in MCF-7 Cells—p53 mostly distributes in the cytoplasm in normal MCF-7 cells. 64 of 215 cells (30%) expressing PTTG-EGFP had bright nuclear p53 staining (Fig. 2*a*), *versus* 20 of 217 cells (9%) expressing EGFP ($p < 0.05$), suggesting that p53 is translocated to the nuclei as a result of PTTG expression. Very few cells (<1%) expressing PTTG-EGFP revealed nuclear staining of mdm2, a negative regulator of p53, suggesting that mdm2-induced inhibition of p53 export from the nucleus is not responsible for p53 up-regulation. Overexpression of wild type p53 increased apoptosis of MCF-7 cells expressing either EGFP or PTTG-EGFP; however, a preferential increase of apoptosis in cells expressing PTTG-EGFP was observed (Fig. 2*b*). Overexpression of mutant (R175H) p53 did not have a significant impact on MCF-7 cell death. Overexpression of E6, a protein that targets p53 to degradation, eliminated p53 immunoreactivity in most cells expressing PTTG-EGFP (Fig. 2*b*, *inset*). E6 promoted death of cells expressing EGFP, possibly because of lowered checkpoint function, but it protected MCF-7 cells from death induced by PTTG-EGFP. Similar experiments on MG-63 cells showed that p53 did not affect apoptosis induced by PTTG (Fig. 2*c*).

PTTG Arrests MG-63 Cell Cycle—To investigate the relationship between apoptosis and cell cycle changes induced by PTTG, we first observed the same live cells expressing either EGFP or PTTG-EGFP after they were released from a double thymidine block, using fluorescence microscopy (Fig. 3*a*). In the 18 h after release, 16 of 39 cells (41%) expressing EGFP divided, 1 cell (3%) died, and 22 cells (56%) did not change appreciably. EGFP fluorescence was stable among all cells expressing EGFP alone. During the same period, none of the cells expressing PTTG-EGFP divided, 6 of 30 cells (20%) died, EGFP fluorescence disappeared in 3 cells (10%), and 21 cells (70%) exhibited no obvious change. Consistent with our previous observation (9), these results demonstrated that PTTG blocked mitosis, and cells were able to degrade PTTG in interphase.

To determine where the cell cycle was blocked by PTTG, we analyzed the cell cycle of unsynchronized MG-63 cells expressing EGFP or PTTG-EGFP 48 h after transfection (Fig. 3*b*). EGFP itself did not affect the cell cycle (Fig. 3*b*, *left*). PTTG-EGFP-expressing cells had a similar number of cells in S phase (Fig. 3*b*, *right*), but a prominent G_2/M phase (14%) and a corresponding smaller G_1 phase were observed, suggesting a blockade on exit from mitosis.

Caspases have been implicated in apoptosis induced by taxol and human immunodeficiency virus viral protein R (19, 20), both blocking cell cycle at G_2/M phase. We used the total number of green cells and the number of live green cells on day 5 after transfection with PTTG-EGFP as indicators of cell survival. Both caspase inhibitor I (Z-VAD-FMK) and III (Boc-D-FMK) promoted survival of PTTG-EGFP-expressing cells (Fig. 3*c*).

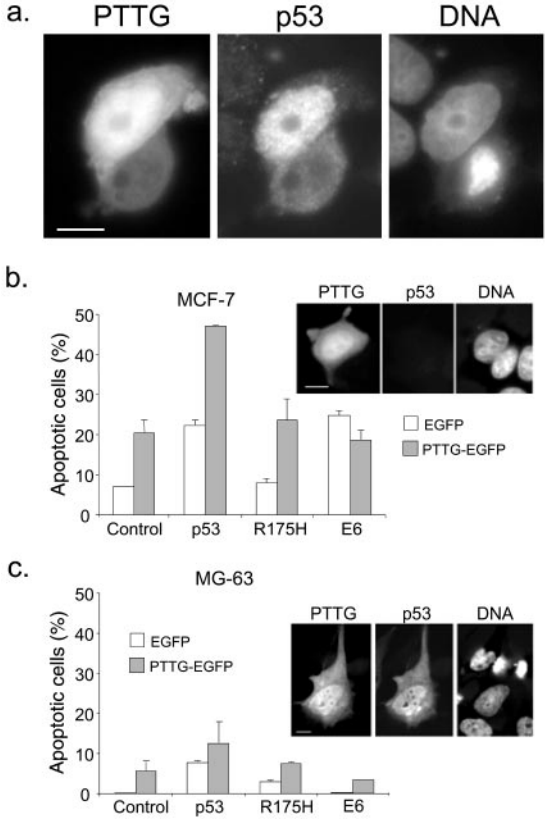

FIG. 2. **PTTG activates p53, and p53 facilitates apoptosis in MCF-7 cells.** *a*, MCF-7 cells were transfected with PTTG-EGFP (2 μg of plasmid/well) and fixed 24 h later. Cells were stained with an antibody to p53 and Hoechst 33258. Images were acquired under appropriate filters. *b*, MCF-7 cells were cotransfected with PTTG-EGFP (0.4 μg of plasmid/well) and p53, R175H p53, or E6 (1.6 μg/well). Cells were fixed 24 h later, and apoptotic cells were counted as described in the legend to Fig. 1. *Inset*, images of a cell co-transfected with PTTG-EGFP and E6. The cells were stained similarly as in *a*. p53 was negative in this cell. Note that in this experiment, MCF-7 cell death rate was lower (compare Fig. 1 and Fig. 2*b*), probably because much less PTTG-EGFP plasmid was used to accommodate the other plasmids. *c*, MG-63 cells were co-transfected with PTTG-EGFP (0.4 μg of plasmid/well) and p53, R175H p53, or E6 (1.6 μg/well). Cells were fixed 48 h later, and apoptotic cells were counted as described in the legend to Fig. 1. *Inset*, images of a cell co-transfected with PTTG-EGFP and p53. The cells were stained similarly to those in *a*. *Bar*, 10 μm.

PTTG Protein Is Continuously Degraded— 7 h after treatment with a proteasome inhibitor, LLnL, there was no significant change in EGFP fluorescence (Fig. 4*a*) or in EGFP protein level, revealed by Western blotting of MG-63 cells stably expressing EGFP (Fig. 4*b*). In cells stably expressing PTTG-EGFP, PTTG-EGFP fluorescence and PTTG-EGFP protein level were both enhanced after LLnL treatment. These results show that PTTG-EGFP was continuously degraded through the ubiquitin pathway and suggested that cells stably expressing PTTG escape apoptosis by down-regulating PTTG.

PTTG Overexpression Causes Aneuploidy—Because PTTG is a mammalian securin that helps keep sister chromatids together, its overexpression may cause abnormal chromosomal separation. In both the parental MG-63 cells and in cells expressing EGFP, signs of aneuploidy were uncommon. In MG-63 cells transiently or stably expressing PTTG-EGFP, the severity and frequency of aneuploidy signs such as micronuclei, macronuclei, or chromosomal bridges were both enhanced (Fig. 5). In

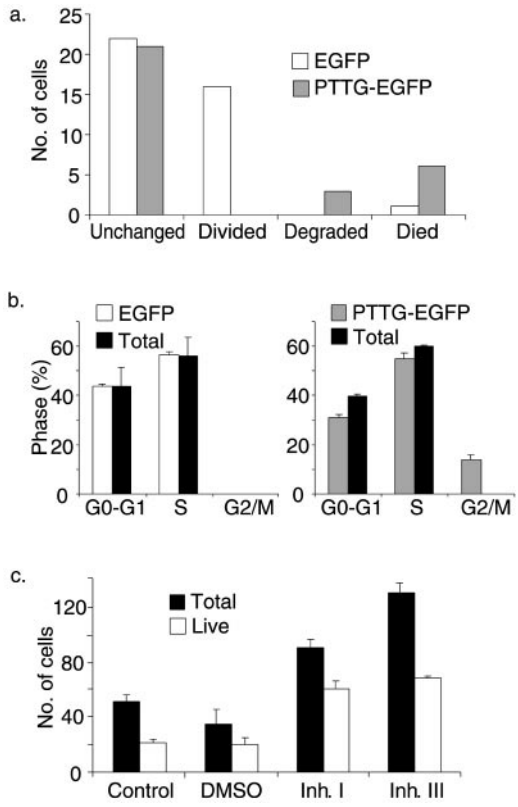

FIG. 3. **PTTG arrests MG-63 cell growth.** *a*, MG-63 cells transiently transfected with EGFP or PTTG-EGFP were synchronized with double thymidine block and released. The same live cells were continuously observed for 18 h after double thymidine block. Shown are the numbers of cells that remained unchanged, divided, degraded EGFP, or died. 39 EGFP-expressing and 30 PTTG-EGFP-expressing cells were observed. *b*, MG-63 cells were transfected with EGFP (*left*) or PTTG-EGFP (*right*). 2 days later, cells were fixed and cell-cycle analyzed for cells that express EGFP or PTTG-EGFP only and all cells (*Total*) including green and non-green cells. *c*, 2,000 MG-63 cells were plated in each well of a 48-well plate. Cells were transfected the next day with PTTG-EGFP and treated with 1% Me_2SO or 100 μM caspase inhibitor I (*Inh. I*) or III (*Inh. III*). Medium was changed every day with fresh drugs added. Shown are total numbers of green cells (*Total*) including live and apoptotic cells and the number of live cells on day 5 after transfection.

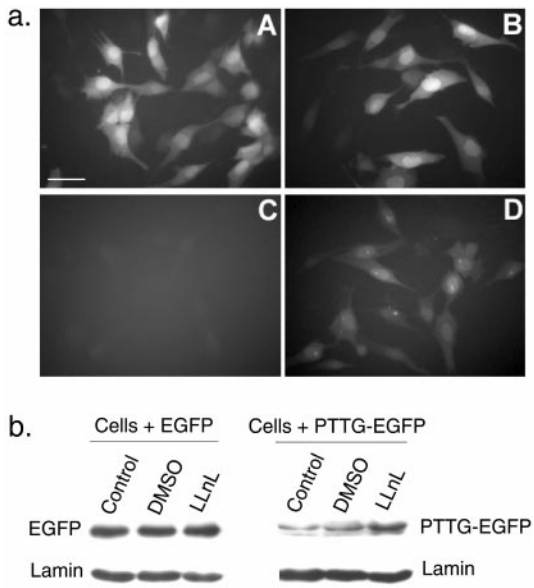

FIG. 4. **Proteasome inhibitor increases PTTG protein expression.** MG-63 cells stably expressing EGFP or PTTG-EGFP were established. Cells were treated with LLnL (50 μM) for 7 h. *a*, fluorescence images were acquired under identical conditions from cells expressing EGFP (*A* and *B*) or PTTG-EGFP (*C* and *D*) before (*A* and *C*) and after (*B* and *D*) LLnL treatment. *Bar*, 50 μm. *b*, in parallel experiments, cells were lysed and EGFP, and PTTG-EGFP proteins were assayed with Western blotting using an anti-EGFP antibody. The same blot was stained with an antibody to nuclear lamin to ensure equal loading.

a representative experiment, only 3 of 253 cells (1%) transiently expressing EGFP were aneuploid, whereas 45 of 133 cells (34%) transiently expressing PTTG-EGFP were aneuploid. In MG-63 cells stably expressing EGFP or PTTG-EGFP, the observed differences in aneuploidy were less dramatic. In a representative counting, 12 of 244 cells (5%) stably expressing EGFP were aneuploid, whereas 21 of 203 cells (10%) stably expressing PTTG-EGFP were aneuploid. The modal number of chromosomes was counted on metaphase chromosome spreads derived from cells stably expressing EGFP or PTTG-EGFP. Both cells had a modal chromosome number of 56, and occasionally (<5%) tetraploid chromosomes were observed with a modal number of about 100. There was no significant difference in the frequency of tetraploid chromosomes between cells expressing EGFP or PTTG-EGFP. The lack of differing in chromosome numbers may be because most aneuploid cells did not divide or because of the low frequency of aneuploid cells.

PTTG-EGFP did not induce aneuploidy in MCF-7 and human choriocarcinoma JEG-3 cells, which also express wild type p53 (data not shown). Aneuploidy was observed in some MCF-7 and JEG-3 cells transfected with PTTG-EGFP and HPV E6 protein. Because the transfection efficiency in MCF-7 cells was low, we counted the rate of aneuploidy only in JEG-3 cells where transfection efficiency was higher. In one representative experiment, 24 of 650 cells (3.7%) expressing PTTG-EGFP and HPV E6 protein were aneuploid, whereas only 5 of 647 cells (0.8%) expressing EGFP and HPV E6 protein were aneuploid. These results suggested that p53 prevents aneuploidy induced by PTTG.

DISCUSSION

In this study, we have elucidated mechanisms for PTTG-induced apoptosis. Although all results shown were derived from the comparison of PTTG-EGFP and EGFP, PTTG-EGFP appears to faithfully represent PTTG, because they exhibit similar cellular characteristics (9) and caused similar cell death (data not shown). Several lines of evidence from our study indicate that p53 mediates PTTG-induced apoptosis in MCF-7 cells. PTTG up-regulated and translocated p53 to the nucleus, overexpression of p53 augmented PTTG-induced apoptosis, and the HPV E6 protein, a p53 inactivator, prevented PTTG-induced apoptosis. It is not clear, however, how p53 is activated by PTTG. *myc* and *ras* activate p53 by activating ARF, a tumor suppressor protein (12, 21) that in turn causes nuclear accumulation of mdm2, preventing p53 nuclear export and subsequent degradation (22, 23). We did not observe a simultaneous nuclear accumulation of p53 and mdm2, suggesting that the ARF mechanism may not apply to PTTG-induced apoptosis, yet PTTG may induce p53 nuclear accumulation by inhibiting mdm2 expression through other mechanisms (23). The dominant negative p53 mutant did not inhibit PTTG-induced apoptosis in MCF-7 cells, possibly indicating that the mutant p53 may not completely inhibit endogenous p53 activity as effectively as the HPV E6 protein. It is also likely that mechanisms other than p53 are also involved in PTTG-induced apoptosis.

PTTG caused apoptosis in p53-negative MG-63 cells, demonstrating that PTTG-induced apoptosis can be p53-independent. It appeared that PTTG causes cell cycle arrest prior to apoptosis. This was evident inasmuch as cells expressing PTTG did not divide after release from double thymidine block, and cycling PTTG-expressing cells were also partially blocked at G_2/M. The PTTG effect on the cell cycle may therefore be both

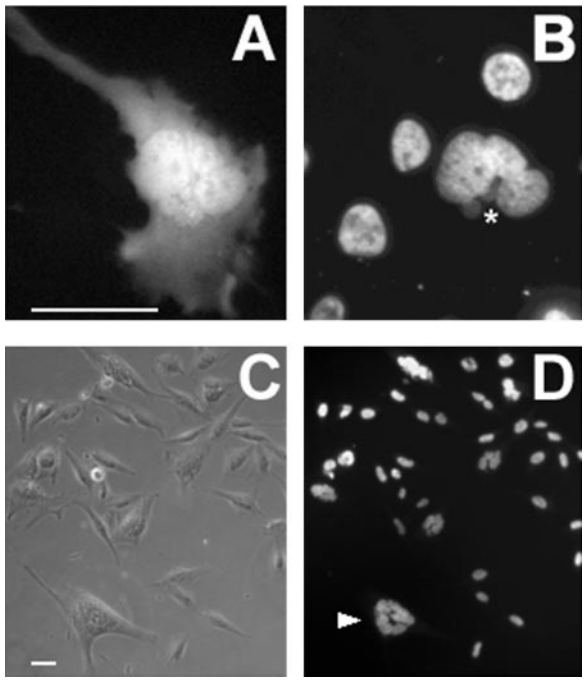

FIG. 5. **PTTG-induced aneuploidy.** MG-63 cells were transiently (*A* and *B*) or stably (*C* and *D*) transfected with PTTG-EGFP. An MG-63 cell transiently expressing PTTG-EGFP and a group of MG-63 cells stably expressing PTTG-EGFP are shown in phase contrast (*A* and *C*) and after staining with Hoechst 33258 to depict the nucleus (*B* and *D*). The *asterisk* indicates two micronuclei, and the *arrowhead* indicates a cell with eight apparent nuclei. *Bar*, 50 μ m.

direct and indirect. The securin function of PTTG (8, 9) would predict that exit from mitosis is inhibited in cells overexpressing PTTG, and overexpression of a nondegradable PTTG disrupts sister chromatid separation (8). The direct impact of PTTG overexpression on mitosis does not explain why only a small percentage of cells were blocked at G_2/M after one to two doubling times, therefore suggesting an additional indirect mechanism, *i.e.* that PTTG activates a surveillance mechanism other than p53, which in turn causes cell cycle arrest. The nature of this surveillance is not clear, but the checkpoint kinases chk1 and chk2 are possible candidates, because they have been involved in p53-independent apoptosis induced by DNA damage (24, 25). Apoptosis may thus result from PTTG-induced cell cycle arrest.

We show evidence that PTTG causes aneuploidy in MG-63 cells. Transient and stable PTTG expression is associated with a higher frequency of aneuploidy. PTTG degradation is required to start the anaphase (8, 9). When a cell overexpresses PTTG, sister chromatids may not separate completely, resulting in a cell with one or more extra chromosomes manifesting as micronuclei. Sister chromatids may not separate at all, forming cells with multiple nuclei or macronuclei. It appears that p53 plays a role in preventing PTTG-induced aneuploidy, because aneuploidy was evident in p53-deficient MG-63 cells and in MCF-7 and JEG-3 cells after endogenous p53 was inhibited. Similar observations have been made on the *myc* (26) and *ras* (27) oncogenes that induce chromosome instability only after p53 is inactivated.

The dual effects of PTTG on apoptosis and aneuploidy are distinct but closely related in tumorigenesis. Apoptosis does not occur as a result of aneuploidy, because the majority of cells were not aneuploid before they entered apoptosis. Rather, by clearing cells that can become aneuploid, apoptosis appears to be a means of protecting against aneuploidy and the resultant tumorigenesis. The dual effects of PTTG on apoptosis and aneuploidy suggest a mechanism for PTTG-induced tumorigenesis. PTTG overexpression activates p53 and a p53-independent apoptosis pathway. Where both apoptotic systems fail, PTTG-induced aneuploid cells survive and divide, prompting daughter cells to be tumorous. This model implies that PTTG-induced tumorigenesis is a slow process, and tumors do not form until apoptosis surveillance systems fail. On the other hand, if PTTG expression is down-regulated by the ubiquitin pathway as we show in this paper, the resultant slightly increased PTTG expression may still escape surveillance and cause aneuploidy. Thus PTTG potentially can contribute to tumorigenesis even when the apoptosis surveillance systems are intact. Because aneuploidy is assumed to be less severe when PTTG is down-regulated, tumorigenesis should also be slow.

In summary, we show that PTTG induced both apoptosis and aneuploidy, and the results suggest that apoptosis may play an important role in preventing tumorigenesis. We have now established mice deficient in PTTG[2] and will more directly address the mechanism of PTTG-induced tumorigenesis in these animals.

REFERENCES

1. Zhang, X., Horwitz, G. A., Prezant, T. R., Valentini, A., Nakashima, M., Bronstein, M. D., and Melmed, S. (1999) *Mol. Endocrinol.* **13,** 156–166
2. Zhang, X., Horwitz, G. A., Heaney, A. P., Nakashima, M., Prezant, T. R., Bronstein, M. D., and Melmed, S. (1999) *J. Clin. Endocrinol. Metab.* **84,** 761–767
3. Heaney, A. P., Singson, R., McCabe, C. J., Nelson, V., Nakashima, M., and Melmed, S. (2000) *Lancet* **355,** 712–715
4. Kakar, S. S., and Jennes, L. (1999) *Cytogenet. Cell Genet.* **84,** 211–216
5. Heaney, A. P., Horwitz, G. A., Wang, Z., Singson, R., and Melmed, S. (1999) *Nat. Med.* **5,** 1317–1321
6. Dominguez, A., Ramos-Morales, F., Romero, F., Rios, R. M., Dreyfus, F., Tortolero, M., and Pintor-Toro, J. A. (1998) *Oncogene* **17,** 2187–2193
7. Wang, Z., and Melmed, S. (2000) *J. Biol. Chem.* **275,** 7459–7461
8. Zou, H., McGarry, T. J., Bernal, T., and Kirschner, M. W. (1999) *Science* **285,** 418–422
9. Yu, R., Ren, S., Horwitz, G. A., Wang, Z., and Melmed, S. (2000) *Mol. Endocrinol.* **14,** 1137–1146
10. Hermaking, H., and Eick, D. (1994) *Science* **265,** 2091–2093
11. Wagner, A. J., Kokontis, J. M., and Hay, N. (1994) *Genes Dev.* **8,** 2817–2830
12. Zindy, F., Eischen, C. M., Randle, D. H., Kamijo, T., Cleveland, J, L., Sherr, C. J., and Roussel, M. F. (1998) *Genes Dev.* **12,** 2424–2433
13. Sakamuro, D., Eviner, V., Elliott, K. J., Showe, L., White, E., and Prendergast, G. C. (1995) *Oncogene* **11,** 2411–2418
14. Trudel, M., Lanoix, J., Barisoni, L., Blouin, M. J., Desforges, M., L'Italien, C., and D'Agati, V. (1997) *J. Exp. Med.* **186,** 1873–1884
15. Scheffner, M., Werness, B. A., Huibregtse, J. M., Levine, A. J., and Howley, P. M. (1990) *Cell* **63,** 1129–1136
16. Chen, J., Marecahl, V., and Levine, A. J. (1993) *Mol. Cell. Biol.* **13,** 4107–4114
17. Elstner, E., Linker-Israeli, M., Said, J., Umiel, T., de Vos, S., Shintaku, I. P., Heber, D., Binderup, L., Uskokovic, M., and Koeffler, H. P. (1995) *Cancer Res.* **55,** 2822–2830
18. Chandar, N., Billig, B., McMaster, J., and Novak, J. (1992) *Br. J. Cancer* **65,** 208–214
19. Panvichian, R., Orth, K., Day, M. L., Day, K. C., Pilat, M. J., and Pienta, K. J. (1998) *Cancer Res.* **58,** 4667–4672
20. Shostak, L. D., Ludlow, J., Fisk, J., Pursell, S., Rimel, B. J., Nguyen, D., Rosenblatt, J. D., and Planelles, V. (1999) *Exp. Cell Res.* **251,** 156–165
21. Palmero, I., Pantoja, C., and Serrano, M. (1998) *Nature* **395,** 125–126
22. Tao, W., and Levine, A. J. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 6937–6941
23. Lu, W., Pochampally, R., Chen, L., Traidej, M., Wang, Y., and Chen, J. (2000) *Oncogene* **19,** 232–240
24. Mailand, N., Falck, J., Lukas, C., Syljuasen, R. G., Welcker, M., Bartek, J., and Lukas, J. (2000) *Science* **288,** 1425–1429
25. Suganuma, M., Kawabe, T., Hori, H., Funabiki, T., and Okamoto, T. (1999) *Cancer Res.* **59,** 5887–5891
26. Yin, X. Y., Grove, L., Datta, N. S., Long, M. W., and Prochownik, E. V. (1999) *Oncogene* **18,** 1177–1184
27. Hundley, J. E., Koester, S. K., Troyer, D. A., Hilsenbeck, S. G., Subler, M. A., and Windle, J. J. (1997) *Mol. Cell. Biol.* **17,** 723–731

[2] Z. Wang, R. Yu, and S. Melmed, manuscript in preparation.