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Simian Virus 40 T Antigen Can Regulate p53-Mediated Transcription Independent of Binding p53

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A simian virus 40 (SV40) T-antigen mutant containing only the N-terminal 136 amino acids, able to bind to Rb and p300 but not p53, partially inhibited p53-mediated transcription without affecting the ability of p53 to bind DNA. These results suggest that SV40 T antigen can regulate p53-mediated transcription either directly through protein-protein association or indirectly through interaction with factors which may function to confer p53-mediated transcription.

The p53 tumor suppressor is a transcriptional regulator which can stimulate the expression of a subset of genes involved in growth control and DNA repair. In particular, p53 stimulates transcription of p21 (5) (a cyclin kinase inhibitor), GADD45 (10) (a protein involved in DNA repair), and Bax (15) (a stimulator of apoptosis) by binding directly to their promoters. The transcriptional activity of p53 has been shown to be important, in certain cell types, for p53-mediated cell cycle inhibition, for apoptosis, and potentially for tumor suppression (18). The p53 protein forms stable complexes with oncoproteins from several DNA viruses, including simian virus 40 (SV40) T antigen, adenovirus E1B-55K, and the human papillomavirus E6 proteins (12, 14, 19, 23). The interaction between the viral oncoproteins T antigen and E1B with p53 either alters or abolishes the function of p53, whereas the enhanced degradation of p53 by E6 results in a reduction in the level of functional p53 protein (13, 20). The ability of these viral oncoproteins to bind p53 correlates with their ability to transform cells (16, 19, 22). Interestingly, it has been reported that it is the amino terminus of SV40 T antigen, not the p53 binding domain, that is involved in alleviating wild-type p53-mediated cell growth arrest (17). We and others have demonstrated previously that the interaction of T antigen with p53 results in a reduction of p53-mediated transcriptional activation (2, 6, 8). In this report, we have examined the ability of SV40 T antigen to regulate p53-mediated transcription independent of direct interaction with p53.

To determine if a mutant T-antigen protein, unable to bind to p53, could affect p53-mediated transcription, a p53-dependent reporter plasmid, G5p53CAT, was cotransfected with a p53 expression vector and a T-antigen mutant defective in p53 binding (1137) into mink lung CCL-64 cells. 1137 expresses the first 121 amino acids of T antigen, which contain at least two elements important for transformation: cr2 that binds pRB and an amino-terminal motif that shows homology with cr1 of the adenovirus E1A protein (see Fig. 2B). As is shown in Fig. 1, 1137 was able to reduce p53-mediated transcription in a dose-responsive manner. However, wild-type T antigen was more efficient at abrogating p53-mediated transcription in this transient assay, as has been previously noted (8). To determine the regions in the 1137 T antigen required for the observed repression, different T-antigen 1137 mutants were tested for their ability to affect p53-mediated transcription. The 1137 T-antigen mutants used (Fig. 2B) were an Rb binding site mutant (1137Rb), a mutant with a deletion of the cr1-like sequences (1135-1137), and a mutant defective for p53 binding and the cr1 and cr2 functions (1135-1137Rb). When the control and the T-antigen mutants were cotransfected with CMV-p53 and G5p53CAT into CCL-64 cells, the 1137 and 1137Rb mutants affected the transcriptional activity of p53 at a slightly higher level than 1135-1137 (Fig. 2A). These results suggest that the cr1 motif, to a greater extent, and the Rb binding domain, to a lesser extent, contribute to the observed inhibition by T antigen. In addition, given that 1135-1137RB partially repressed p53-mediated transcription, an additional activity of T antigen may also be involved.

The level of p53 increases after DNA damage caused by irradiation, resulting in G_1 arrest of the cell cycle (9). In addition, ionizing radiation, UV radiation, and methylmethane sulfonate were found to induce p53 transcriptional activity when a reporter construct which contains functional p53-binding sites was stably introduced into cells (26). To determine if a stably expressed N terminus of T antigen has a significant effect on the transcriptional activity of endogenous p53, an assay was employed in which p53 was induced by UV radiation. A cell line (Tc) was generated by transfection of murine CH310T1/2 cells with the p53-dependent G5p53CAT reporter plasmid (8). Subsequently, the Tc10 cells were infected with a pLJ-derived retroviral vector containing coding sequences for either wild-type T antigen or a similar construct, N136, which encodes the first 136 amino acids of T antigen. The infected cells were G418 selected, and expression of the different T antigens was confirmed by Western blot (data not shown).

In order to examine p53 in these different cell lines, each stable cell line was treated with the same doses of UV radiation and the levels of p53 protein, DNA binding, and transcriptional activity were examined. UV treatment induced the steady-state level of p53 in the control cells and N136-expressing cells in a dose-dependent manner (Fig. 3A). In contrast, the p53 levels were similar in the nontreated and UV-treated T-antigen cells, apparently due to stabilization of p53 by T antigen. Interestingly, the DNA binding activity of p53, measured with and without the addition of 421 antibody, was induced by UV treatment in a dose-dependent manner to approximately the same extent in all three cell lines (Fig. 3B). p53 binding in the presence of full-length T antigen (Tc10T) was only slightly lower compared to the other two cell lines. Exactly

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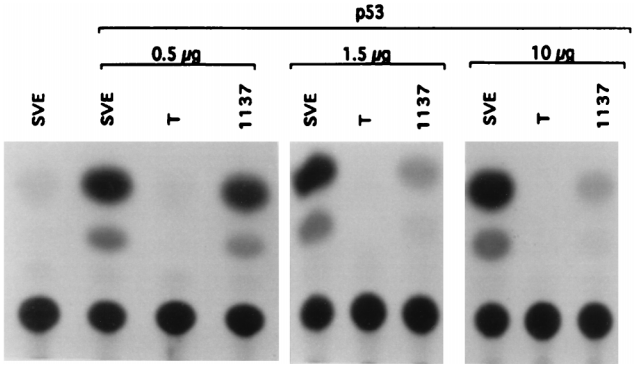

FIG. 1. Effect of SV40 T antigen and 1137 proteins on human p53-mediated transcription. The p53-dependent G5p53CAT reporter (8) with six copies of the consensus p53-binding half sites (5′-CTAGAGGCATGTCTGATC-3′) was cotransfected into CCL-64 cells with the human wild-type p53 expression plasmid CMV-p53 and increasing doses of either SV40 wild-type T antigen (lanes T) or 1137 expression plasmid (lanes 1137) or the SV40 early promoter containing control plasmid SVE (lanes SVE) (8). A Rous sarcoma virus-luciferase expression plasmid was included as an internal control for normalizing transfection efficiency, which did not vary more than twofold.

why T antigen was unable to block p53 DNA binding activity is unclear, but this inability may be due to an excess of p53, to modification of p53 following UV treatment, or to an alternative mechanism. When p53-dependent chloramphenicol acetyltransferase (CAT) activity was measured in the UV-treated cells, 10- and 20-fold induction was observed in the Tc10B control cells at 3 and 8 J/m^2, respectively (Fig. 3C). However, similar to the cell line containing wild-type T antigen, the cell line containing the N136 T-antigen mutant also had reduced p53 transcriptional activity after UV treatment. Taken together, these results demonstrate that an SV40 T antigen deficient in p53 binding also can affect p53-mediated transcription. Moreover, the ability of the N136 T-antigen mutant to repress p53-mediated transcription correlates with its ability to overcome the p53-mediated block to the cell cycle (17).

We have observed that the N terminus of T antigen, which does not have a functional p53 binding domain, can reduce p53-transcriptional activity. Since 1137 does not affect the DNA binding activity of p53, it most likely affects p53 transactivation once it is bound to the promoter. The SV40 T-antigen N terminus can associate with other cellular proteins

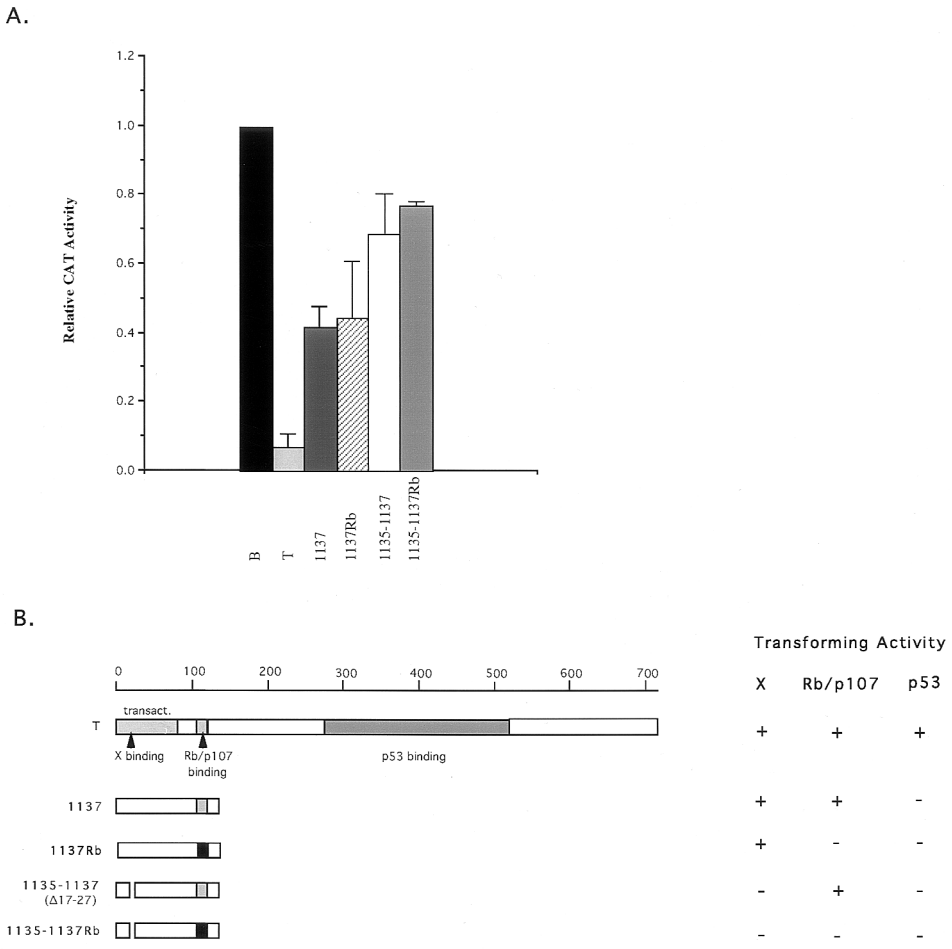

FIG. 2. (A) Effects of different SV40 T-antigen mutants on p53-mediated transcription in CCL-64 cells. The reporter plasmid G5p53CAT was cotransfected into CCL-64 with the human p53 expression vector CMV-p53 and 3 μg of the different SV40 T-antigen mutants in the RSV-B expression plasmid. The vector plasmid (RSV-B) was used as a negative control, and the pSV2-luciferase plasmid was included to normalize the transfection efficiency. Error bars, standard errors of the means. (B) Diagram of the different T-antigen constructs used and their abilities (+) to bind to p53, Rb/p107, and the third putative transforming factor, X. 1137 contains the N-terminal 121 amino acids of T antigen, 1137Rb has a point mutation at the Rb/p107 binding site of 1137, 1135-1137 contains a deletion mutation of amino acids 17 to 27, and 1135-1137Rb has both the point mutation of 1137Rb and the deletion mutation of 1135-1137.

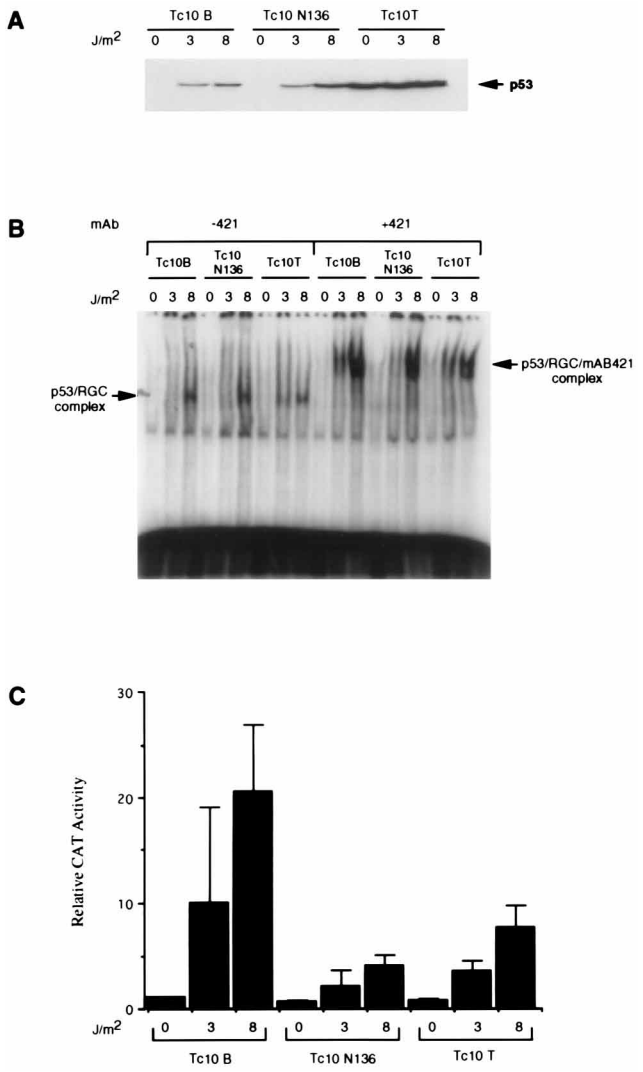

FIG. 3. (A) Following UV stimulation of the Tc10 cell lines, p53 levels were examined by Western blotting. The first three lanes contain Tc10 cells infected with an empty retrovirus vector (Tc10 B), the middle three lanes contain Tc10 cells stably infected with a retroviral vector carrying the N136 SV40 T antigen construct (Tc10 N136), the last three lanes contain Tc10 cells stably infected with a retroviral vector carrying the SV40 large T antigen (Tc10T). The lanes received UV radiation at 0, 3, and 8 J/m^2 as indicated. (B) Gel shift binding assays were performed to evaluate the ability of p53 to bind the ribosome group complex binding site (2). The lanes were run in the presence (+) or absence (−) of p53 monoclonal antibody (mAb) 421. (C) CAT assays were performed with the Tc10 cell lines treated with the various doses of UV radiation indicated. The cell lines were subjected to 0, 3, or 8 J/m^2 of UV radiation, and 16 h later the CAT activity was measured. The results are from four separate experiments. Error bars, standard errors of the means.

such as pRb, p107, and p130, and members of the CREB-binding protein (CBP) family such as p300 (24). The amino terminal of T antigen also is able to bind directly to TFIID in vivo (3). The association of T antigen with one or more of these factors may play a role in the observed regulation of p53-mediated transcription. Rb, p107, and p130 have been shown to associate with $TAF_{II}250$, one component of TFIID, to mediate transcriptional regulation (21; unpublished observations). p53 is able to stimulate transcription in vitro through an interaction with $dTAF_{II}60$ and $dTAF_{II}40$, which in turn interact with $TAF_{II}250$ and TBP (25). Interestingly, the amino terminus of T antigen can bind weakly to $dTAF_{II}60$ and $dTAF_{II}110$, potentially affecting p53-mediated transcription. In addition, p300 and other CBP family members have been implicated as transcriptional adapters for certain complex transcriptional regulatory elements, interacting directly with a number of transcription factors (1, 4, 7, 11, 25).

Our results suggest that there are at least two ways for SV40 T antigen to affect the transcriptional activity of p53. Firstly, T antigen can bind directly to the p53 central region and block specific DNA binding (6, 8). Secondly, T antigen may affect p53-mediated transcription indirectly through interaction with a protein(s), such as a p300 family member or Rb. Alternatively, T antigen could be binding directly to TFIID (3). Clearly, a better understanding of the factors involved in conferring p53-mediated transcription is necessary to understand the complex role of p53 in cell cycle control, apoptosis, and tumor suppression.

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