The large T antigen of simian virus 40 binds and inactivates p53 but not p73

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The p73 proteins α and β were identified based on their similarity to the tumour suppressor gene product p53. p53 and the p73 proteins activate transcription from p53-responsive promoters. The large T antigen of simian virus 40 (SV40) forms a specific complex with p53 and inhibits p53-mediated transcription. Here we show that the large T antigens from SV40 and JC virus strongly reduce the transcriptional activity of p53 but do not detectably affect the ability of the p73 proteins to transactivate. p53 but not the p73 proteins associate with SV40 T antigen in vitro. Finally, p53 colocalizes with a cytoplasmic mutant of SV40 T antigen, whereas both variants of p73 fail to colocalize with cytoplasmic T antigen. These results indicate that T antigen selectively binds and inactivates p53 but does not detectably affect the p73 proteins.

The p53 tumour suppressor protein is a key regulator of cell proliferation and apoptosis, and its integrity and/or activity are impaired in most malignant tumours (Levine, 1997). Some of the regulatory properties of p53 are based on its ability to modulate transcription. Certain promoters contain specific p53 binding sites that allow transcriptional activation by p53. Many of the genes regulated by p53 can influence cell cycle progression and induction of apoptosis, thus partially explaining the tumour suppressing activity of p53.

The large T antigen of simian virus 40 (SV40) binds and inactivates p53 (Jiang et al., 1993; McCormick et al., 1981; Mietz et al., 1992; Sarnow et al., 1982), and this may contribute to the induction of cell cycle progression by T antigen (Dobbelstein et al., 1992). This mechanism may also lead to induction of the mesotheliomas that were recently reported to be associated with SV40 DNA persistence in patients (Carbone et al., 1997; De Luca et al., 1997). Gene products from other tumour viruses also form a complex with p53 and inhibit p53-mediated transcriptional activation. Examples are the E6 proteins from oncogenic human papillomaviruses (Mietz et al., 1992; Werness et al., 1990) and the E1B-55 kDa protein from adenovirus type 5 (Sarnow et al., 1982; Yew & Berk, 1992).

More recently, Kaghad et al. (1997) identified a novel gene product, termed p73, that closely resembles p53. At least two different proteins are expressed from the corresponding gene through a mechanism of alternative splicing. The two forms of p73 were named p73α and p73β. They differ only within their carboxy-terminal portions; the amino-terminal and central domains are identical. Both proteins can activate transcription from a p53-responsive promoter (Jost et al., 1997; Kaghad et al., 1997), and they have the potential to induce apoptosis (Jost et al., 1997). For these reasons, it has been speculated that oncogene products antagonizing p53 may also target p73 (Kaghad et al., 1997).

Given the reported structural and functional similarities between p53 and p73 proteins, we tried to determine whether or not viral oncoproteins might bind and inactivate p73 proteins in addition to p53. Surprisingly, three different assays revealed that SV40 T antigen selectively antagonizes p53 but does not seem to affect the p73 proteins.

To analyse the effect of SV40 T antigen on the transcriptional activity of p53 and the p73 proteins, expression plasmids were transfected using a lipid preparation (FuGene 6, Boehringer Mannheim) into Saos-2 cells, a human osteosarcoma-derived cell line that lacks endogenous p53. A cotransfected reporter construct containing a p53-responsive promoter that activates the expression of firefly luciferase was employed to monitor p53-like transcriptional activity. p53 as well as both p73 proteins activated reporter expression when transiently expressed in this assay (Fig. 1; compare lane 1 with lanes 2, 5 and 8), confirming previous results (Jost et al., 1997). It was noted, however, that p73α was considerably less active than p53, whereas p73β displayed stronger transcriptional activity. This ratio of activities was consistently observed.

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Fig. 1. Transcriptional activity of p53 and the p73 proteins in the presence of SV40- and JCV T antigen. Saos-2 cells were transfected with expression plasmids for p53 (50 ng), p73α (50 ng), p73β (10 ng), SV40 T antigen (1 µg), and JCV T antigen (1 µg) as indicated below the lanes. When T antigen expression plasmids were not transfected, the same amount of an expression plasmid for β-galactosidase (pCMVsportβgal, Life Technologies) was added instead. In each case, 500 ng of a reporter plasmid, pBP100luc (Roth et al., 1998), which contains the first mdm2 intron activating the expression of luciferase, was cotransfected. After overnight incubation, the cells were harvested and luciferase activity was determined. The value obtained with p53 alone was arbitrarily set to 100% and the other values were normalized accordingly. The column heights reflect the average of at least three independent experiments.

When using several different p53-responsive promoters (our unpublished observations). The difference in the potential to activate transcription may be due to the reported difference in the ability of these proteins to form oligomers. While p73β is known to oligomerize with itself efficiently, p73α forms oligomers with poor efficiency, at least when assayed in a yeast two-hybrid system (Kaghad et al., 1997). When SV40 T antigen was coexpressed, it drastically reduced the transcriptional activation by p53 (Fig. 1, lane 3), but it did not significantly alter the activity measured with the p73 proteins (Fig. 1, lanes 6 and 9). Since p73 loss was suggested to play a role in the formation of neuroblastomas (Kaghad et al., 1997), we also tested whether a neurotropic virus might affect the function of p73. JC virus (JCV) is known to cause a disease (progressive multifocal leukencephalopathy) primarily affecting the central nervous system, and its gene products and replication mechanisms have strong similarities to SV40. Therefore, the large T antigen from JCV was tested for its ability to inhibit p73. However, while JCV T antigen inhibited p53 (Fig. 1, lane 4), as reported previously (Bollag et al., 1989; Staib et al., 1996), it did not detectably interfere with p73 activity (Fig. 1, lanes 7 and 10). We conclude that both SV40 and JCV express large T antigens that selectively inhibit p53 activity but do not affect the potential of p73 proteins to activate transcription.

Next, we asked if the observed difference in transcriptional inactivation might reflect different abilities of p53 and p73 to physically associate with SV40 T antigen. To test this, p53 and the two p73 proteins were synthesized by in vitro transcription using T7 RNA polymerase and translation in a rabbit reticulocyte lysate (Promega). Radiolabelled proteins of the expected size were obtained (Fig. 2, lanes 1–3). These were incubated at 30°C for 30 min with a lysate of COS7 cells, a cell line that constitutively expresses SV40 T antigen (10⁶ COS7 cells were lysed in 1 ml of lysis buffer containing 50 mM Tris–HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 0.01% Nonidet P-40). SV40 T antigen was then immunoprecipitated by a murine monoclonal antibody (Pab419) by a previously described procedure (Yew & Berk, 1992). The precipitated material was analysed by SDS–PAGE and autoradiography. While p53 was efficiently coprecipitated with T antigen (Fig. 2, lane 4), little or no p73 protein of either species was found to associate with T antigen. Thus, p53 efficiently forms a complex with SV40 T antigen in vitro, while both p73 proteins fail to do so.

Finally, we addressed the question whether the differential binding efficiencies of p53 and p73 to SV40 T antigen also result in differential abilities to colocalize in transfected cells. To allow direct comparison, epitope-tagged versions of p53 and p73 were transiently expressed in Saos-2 cells. The proteins were detected by immunofluorescent staining of the

Fig. 2. Association of SV40 T antigen with p53 but not p73. p53 and the two p73 proteins were synthesized and radioactively labelled with [35S]methionine by in vitro translation using a reticulocyte lysate. The proteins were then incubated with a lysate of COS7 cells that contain SV40 T antigen. This was followed by immunoprecipitation with an antibody (Pab 419) against SV40 T antigen, SDS–PAGE and autoradiography on a BioImager (Fuji). The proteins obtained by in vitro translation are shown in lanes 1–3, and the material coprecipitated with SV40 T antigen is shown in lanes 4–6.
Fig. 3. Intracellular colocalization of cytoplasmic T antigen with p53 but not p73. Saos-2 cells were transfected with expression plasmids for N-terminally HA-tagged p53, p73α and p73β (100 ng each) along with a mutant SV40 T antigen (K128A; 400 ng of the expression plasmid) that fails to enter the nucleus (Kalderon et al., 1984a, b). As a control, wild-type SV40 T antigen was transfected (m, n). After 24 h incubation, the cells were fixed, permeabilized and stained as described (Dobbelstein et al., 1992) with a rabbit antibody to the HA tag (Santa Cruz) and a murine monoclonal antibody to SV40 T antigen (Pab 419), followed by secondary antibodies (Jackson) coupled to Texas Red and fluorescein isothiocyanate, respectively.

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be a more central player in the prevention of tumorigenesis. This hypothesis is further supported by recent studies of p73 expression in malignant tumours. In contrast to p53, p73 was frequently found to be expressed and not mutated in tumours derived from several tissues. Further, the p73 gene did not display the characteristics of a tumour suppressor gene in these studies (Mai et al., 1998; Nomoto et al., 1998; Sunahara et al., 1998; Takahashi et al., 1998). Activities unique to p53 and absent from p73 still have to be identified, but candidates are the growth-suppressing activity of the proline-rich region near the amino terminus of p53 (Walker & Levine, 1996) or the ability of p53 to bind components of the TFIIH complex (Wang et al., 1995, 1996).

Other p53-related proteins have been identified recently (Bian & Sun, 1997; Osada et al., 1998; Schmaler & Bamberger, 1997; Trink et al., 1998; Zeng et al., 1998). It will be interesting to determine (a) if these proteins can also activate transcription from p53-responsive promoters and (b) whether or not they can be inhibited and bound by p53-antagonists from tumour viruses. These experiments might provide a starting point to probe the functional similarity between these different p53 homologues and p53 itself.

What is the role of the p73 proteins if they are not simply indistinguishable ‘p53-Doppelgänger’ (Kaelin, 1998)? Unlike p53, p73 expression is not detectably induced by UV irradiation or other genotoxic effects (Kaghad et al., 1997). This argues that p73 proteins are not primarily ‘guardians of the genome’ (Lane, 1992). One possible role of p73 and other p53 homologues might be the induction of differentiation of certain tissues during development. This would be in accordance with the tissue-specific expression that has been reported at least for the p51/KET proteins (Osada et al., 1998; Schmaler & Bamberger, 1997).

Possibly, the new p53 homologues are not primarily involved in tumour suppression. However, their differences from p53 may act as a guideline to identify the unique functions of p53 that ultimately prevent the formation of malignant tumours.

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