LETTER TO THE EDITOR Jpn. J. Cancer Res. 84, 818–820, July 1993

Unusual Interactions of Adenovirus 5 and 12 E1B Proteins with p53

The p53 protein was originally discovered as a polypeptide of 53,000 daltons that can form a complex with the large T-antigen of the DNA tumor virus SV40. It has lately attracted a great deal of attention when researchers came to realize that mutations in its gene are the most frequently observed genetic lesion in human cancers. Subsequent work showed that the p53 gene, like the *myc* oncogene, can cooperate with an activated *ras* oncogene in the transformation of primary rodent cell cultures. This indicated that the gene is a *myc*-like dominant oncogene. Later work demonstrated that the p53 gene used in these initial studies was not wild-type but contained a mutation, which resulted in the substitution of a single amino acid in the p53 protein. Non-mutated p53 genes, as opposed to the mutated ones, were unable to cooperate with *ras* and, in fact, acted in the opposite way, i.e. as a transformation-suppressor or tumor-suppressor gene. It is now clear that wild-type p53 indeed is a negative growth regulator, which fulfils an important, but still incompletely understood role in the regulation of cell division. The mutant p53 genes found in human tumors have lost the tumor- or growth-suppressor function, but some have acquired a dominant oncogene activity, which would explain the cooperation with *ras*. (For further details see references.[1,2])

Recently, it was found that p53 has the properties of a transcription factor, which stimulates transcription of certain genes by binding to a specific sequence in the promoter. Certain promoters that lack the specific sequence are suppressed, rather than stimulated by p53; this would imply that p53 is not an ordinary transcription factor, but a factor that can either stimulate or repress, depending on the nature of the promoter. An interesting finding was that p53 not only interacts with the SV40 large T-antigen, but also with the transforming 55 kD E1B protein of human adenovirus 5 (Ad5), and with one of the transforming proteins, E6, of the human papilloma virus 16 (HPV16).

Van den Heuvel has investigated the consequences of the interaction of p53 with the Ad5 E1B protein. Human adenoviruses are classified according to their oncogenicity in certain rodents into oncogenic and non-oncogenic types. Cells transformed by oncogenic viruses (e.g. Ad12) are oncogenic in syngeneic immunocompetent hosts, whereas cells transformed by non-oncogenic viruses (e.g. Ad5) are not. However, both types of transformed cells are oncogenic in immunodeficient nude mice, though Ad12-transformed cells induce tumors more efficiently than Ad5-transformed cells. The transforming region of the Ad5 or Ad12 genome, region E1, consists of two subregions, E1A and E1B, which are both required for complete oncogenic transformation and which each code for 2 proteins. In cells transformed by the non-oncogenic Ad5, one of the viral E1B proteins, with a molecular weight of 55 kD, forms a tight complex with p53. Due to the formation of this complex, most, if not all of the p53 is sequestered into a cytoplasmic (i.e., extranuclear) body (Fig. 1). As a consequence, Ad5-transformed cells lack free nuclear p53. In contrast, no such association has been detected in Ad12-transformed cells, in which p53 occurs in the nucleus, where it belongs.[3,4] In both Ad5- and Ad12-transformed cells p53 is metabolically highly stable, and in that respect it resembles mutant forms of p53, which are also stable.

Previous work had shown that the difference in oncogenicity between Ad5- and Ad12-transformed cells in nude mice is dependent on the origin of the E1B region. Cells containing E1B of Ad12 are highly oncogenic, those with E1B of Ad5 are weakly oncogenic. Van den Heuvel investigated whether the difference in oncogenicity in nude mice between cells transformed by Ad5 or Ad12 is caused by the different interaction of their 55 kD E1B proteins with p53. He introduced the Ad5-55 kD E1B gene into highly oncogenic Ad12-transformed cells in which p53 occurs free in the nucleus, and found that in these cells all detectable p53 became sequestered into a cytoplasmic body due to the association with the Ad5-E1B protein. The cells showed a greatly reduced oncogenicity in nude mice, possibly as a result of the disappearance of free p53. The tumors that developed in the end after a long latency period lacked Ad5-E1B expression, so that p53 could return to the nucleus[5]. This suggested that in Ad-transformed cells free nuclear p53 enhances oncogenicity, a property known to be associated with mutant p53.

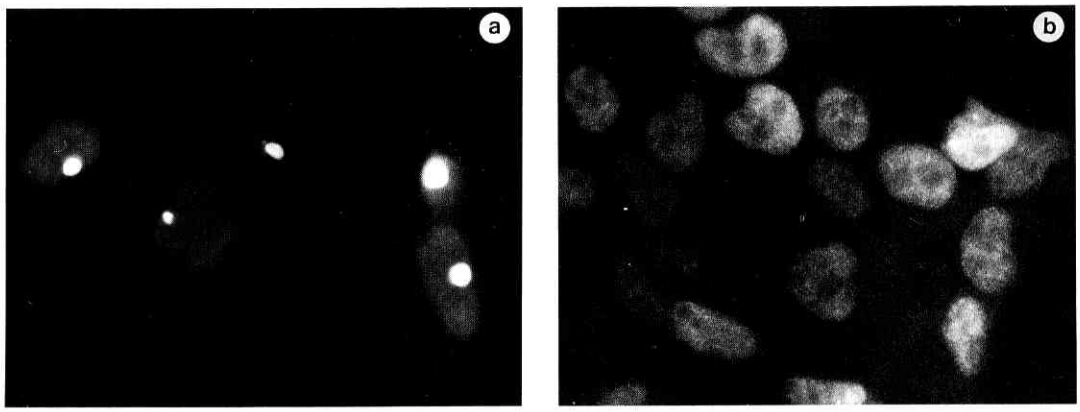

Fig. 1. Immunofluorescence of baby rat kidney cells transformed with the E1 region of non-oncogenic Ad5 (a), using an antibody against p53. All detectable p53 is concentrated in a cytoplasmic body, together with part of the 55 kD viral E1B protein. Nuclear DNA was counterstained with propidium iodide to make the nuclei visible. For comparison, the immunofluorescence pattern of p53 is shown for cells transformed by oncogenic Ad12 (b), in which p53 has the usual nuclear distribution.

The metabolic stability of p53 in Ad-transformed cells indicated that it might indeed be mutated. Surprisingly, however, Van den Heuvel demonstrated that p53 in Ad-transformed cells is wild-type rather than mutated. Thus, wild-type p53 can acquire dominant oncogene activity, possibly due to modifications induced by the Ad12-55 kD E1B protein. Further work showed that Ad5-E1B, but not Ad12-E1B, also interferes with *in vitro* cell transformation as induced by various combinations of oncogenes, e.g. *myc*+*ras*. When the Ad5-55 kD E1B gene was transfected together with *myc*+*ras*, transformation was strongly suppressed, whereas the corresponding Ad12-E1B protein not only failed to suppress, but even enhanced the transformation frequency.[6] These data suggested that inactivation of p53 by Ad5-55 kD E1B prevents outgrowth of transformed cells, or prevents cell transformation. Surprisingly, in the presence of Ad-E1A, the Ad5-E1B protein does not suppress transformation. Together, these data suggest that wild-type p53 plays a positive role in cell transformation or in cell proliferation, but that the presence of Ad-E1A bypasses the need for p53.

In summary, the experiments with the Ad5- and Ad12-55 kD E1B proteins have shown that, (1) *wild-type* p53 can acquire a dominant oncogene activity which contributes to tumorigenicity; (2) in cultured rat cells, wild-type p53 can smehow positively contribute to cell transformation or to cell proliferation; and (3) the Ad5-55 kD E1B protein, but not the corresponding Ad12 protein, acts as a tumor- or growth-suppressor protein. The conclusions on p53 function are surprising, since most available data classify *wild-type* p53 as a protein that negatively regulates cell division. While this may be correct, it is conceivable that this negative function controls an essential step in the cell cycle, which should be passed before the cell can proceed further. The E1A oncogene, and possibly other oncogenes, can bypass the need for this p53-controlled step. The observation that mice lacking both p53 alleles can develop normally, indicates that the function of p53 is more subtle than has been believed, at least *in vivo*.

(Received March 8, 1993/Accepted March 31, 1993)

A. J. van der Eb

Sylvius Laboratory
Wassenaarsewg 72
2333 AL Leiden
The Netherlands

REFERENCES

1) Levine, A. J. and Momand, J. Tumor suppressor genes: the p53 and retinoblastoma sensitivity genes and gene products. *Biochim. Biophys. Acta,* **1032,** 119–136 (1990).
2) Montenarh, M. Biochemical properties of the growth suppressor/oncoprotein p53. *Oncogene,* **7,** 1673–1680 (1992).
3) Sarnow, P., Ho, Y. S., Williams, J. and Levine, A. J. Adenovirus E1B-58kD tumor antigen and SV40 large tumor antigen are physically associated with the same 54kD cellular protein in transformed cells. *Cell,* **28,** 387–394 (1982).
4) Zantema, A., Schrier, P. I., Davis-Olivier, A., Van Laar, T., Vaessen, R. T. J. M. and Van der Eb, A. J. Adenovirus serotype determines association and localization of the large E1B tumor antigen with cellular tumor antigen p53 in transformed cells. *Mol. Cell. Biol.,* **5,** 3084–3091 (1985).
5) Van den Heuvel, S. J. L., Van Laar, T., Kast, W. M., Melief, C.J. M., Zantema, A. and Van der Eb, A. J. Association between the cellular p53 and the adenovirus 5 E1B-55kD proteins reduces the oncogenicity of Ad-transformed cells. *EMBO J.,* **9,** 2621–2629 (1991).
6) Van den Heuvel, S. J. L. Van Laar, T., The, I. and Van der Eb, A. J. The large E1B proteins of adenovirus type 5 and type 12 have different effects on p53 and distinct roles in cell transformation. *J. Virol.,* **67,** (1993), in press.