Cell, Vol. S116, S67–S69, January 23, 2004 Copyright ©2004 by Cell Press

P53 is a Tumor Suppressor Gene

Commentary

Arnold J. Levine,[1]* Cathy A. Finlay,[2] and Philip W. Hinds[3]
[1]Institute for Advanced Study
Einstein Drive
Princeton, New Jersey 08540
and
Cancer Institute of New Jersey
195 Little Albany St.
New Brunswick, New Jersey 08903
[2]Department of High Throughput Biology
GlaxoSmithKline
5 Moore Drive
Research Triangle Park, North Carolina 27709
[3]Harvard Medical School
Department of Pathology
Armenise 433
200 Longwood Avenue
Boston, Massachusetts 02115

During the decade of the 1970s a great deal of progress was made in elucidating how viruses cause cancer. The RNA tumor viruses were shown to harbor cellular genes, often containing mutations, that overexpressed a protein causing the cancer. Long-latency RNA tumor viruses integrated next to or near a cellular gene that was then overexpressed and contributed to the formation of the tumor. By contrast, the DNA tumor viruses encoded their own genes that had no cellular ortholog, but were essential for the initiation and maintenance of the transformed state and tumorigenesis. These viral genes encoded proteins that were expressed in tumor cells and were seen as foreign by animals harboring these tumors. The animals produced antibodies directed against these viral antigens, and for this reason, these viral proteins were called tumor antigens. Accordingly, the transforming genes contained in the SV40 tumor virus were called the large and small tumor antigens, or T and t antigens.

In 1979, David Lane and Lionel Crawford (Lane and Crawford, 1979) and Daniel Linzer and Arnold Levine (Linzer and Levine, 1979) reported that the SV40 T-antigen was bound to a cellular protein that came to be called p53 and that the concentration of the p53 protein was much higher in SV40 or other transformed cells than in normal cells. In fact, temperature-sensitive mutants of T-antigen were shown to control the levels of the p53 protein in a temperature-sensitive fashion (Linzer et al., 1979), and serum from animals bearing SV40-induced tumors contained antibodies to the p53 protein (Linzer and Levine, 1979). At this same time, Lloyd Old and his colleagues showed that animals immunized with spontaneous transformed and tumorigenic cells also made antibodies to the p53 protein (DeLeo et al., 1979). Based upon these studies, p53 came to be classified as a tumor antigen. These observations were the first clue to the mechanism of how the SV40 tumor antigen might transform cells and the next step in this investigation was to clone the gene for p53.

Several groups raced to obtain the p53 cDNA, and Moshe Oren took on this challenge in the Levine laboratory. In 1983 (Oren and Levine, 1983), he succeeded in using immunopurified polysomes to isolate a cDNA containing a portion of the 3′ untranslated region of the p53 mRNA. This fragment was used to hybrid select the P53 mRNA that was then translated in vitro and immunoprecipitated using p53 antibodies (this assay was the state of the art at the time). As Moshe Oren went back to the Weizmann Institute to start his own laboratory, his group and the Levine laboratory rushed to create or find a cDNA library that might have a full-length clone of the p53 cDNA (not at all the state of the art at that time). The Levine laboratory approached Art Levinson at Genentech (previously a graduate student in the Levine lab), and he passed the project onto David Goeddell's lab, where one of their top cDNA cloners, Dianne Pennica, used mRNA from mouse F9 teratocarcinoma cells, isolated by Nancy Reich, to make a cDNA library that indeed had a full-length clone of p53 (Pennica et al., 1984). At the time, nobody thought that p53 was going to be important enough to patent the cDNA clone, since SV40 transformation was not considered to be as good a model for human cancer as were oncogenes from the RNA tumor viruses. Back at the Weizmann Institute, Moshe Oren employed a mouse liver genomic library (which, in hindsight, was apparently contaminated by some clones from a transformed cell library) to find a full-length p53 gene (Bienz et al., 1984). A comparison of the amino acid sequences predicted by the Oren genomic clone and the Pennica cDNA clone showed that they were identical except at amino acid position 135 (out of 390 amino acids), where Oren had a valine and Pennica had an alanine. Not much was made of this difference at the time.

The next step in this adventure was to determine if p53 was an oncogene that could transform cells. At the time there were two kinds of oncogenes: those that could immortalize cells in culture but had no impact upon the other properties of the transformed phenotype (myc, E1A), and those (ras, E1B) that could fully transform permanent cell lines, but could only transform primary cells in culture when added to these cells along with myc or E1A (Land et al., 1983). At this time, Cathy Finlay, a postdoctoral fellow who obtained her degree at the Wistar Institute working on cellular senescence, and Phil Hinds, a new graduate student, joined the Levine laboratory and began working on the transforming properties of the p53 cDNA clone we had isolated. When our cDNA clone had no transforming activity that we could observe, we were a bit surprised, but we were even more troubled by our negative result when it was shown by Moshe that his p53 genomic clone plus ras cDNA clones could transform primary rat embryo fibroblasts (Eliyahu et al., 1984). Further, two additional labs had similar results with different p53 clones (Jenkins et al., 1984; Parada et al., 1984). At this point, we exchanged clones with Moshe and could easily reproduce

Correspondence: alevine@ias.edu

his results with his clone, and so the 135 alanine-to-valine difference began to loom larger. The trouble was, we didn't know whether this difference was a harmless polymorphism, whether a mutation at codon 135 activated or inactivated p53's transforming ability (nor indeed which sequence was wild-type), nor even whether the presence of introns in the transforming Oren genomic clone might produce a more active splice variant. What complicated our thinking at first was that our cDNA clone came from a teratocarcinoma library (and turned out to be wild-type) and Moshe's clone came from normal mouse liver (and turned out to be a mutant).

Careful mapping and marker rescue experiments (this was before the advent of PCR) by Phil Hinds solidly established the nontransforming F9 sequence as wild-type and identified the val135 mutation as required for transformation by the Oren genomic construct (Hinds et al., 1989). Further, we began to appreciate the fact that most transformed cell lines in culture contain a diverse set of mutant p53 proteins and all teratocarcinoma cell lines or tumors contain a wild-type p53 protein (see Lutzker and Levine, 1996). Our first real clue to the significance of p53 mutation in cancer was obtained when Cathy Finlay carried out transformation studies mixing the two different p53 sequences (mutant and wild-type) with E1A plus ras or myc plus ras in transformation assays. Phil Hinds then analyzed each of the transformed clones that came from the experiment, examining the p53 proteins expressed in these cells to see if they were mutant or wild-type. These experiments gave rather surprising and clear-cut answers. Our wild-type p53 cDNA clone and a genomic clone bearing the wild-type sequence blocked the ability of myc plus ras or E1A plus ras to transform cells in culture. The few transformed clones that came from these experiments expressed myc and ras or E1A and ras but had a mutant p53 protein even though we had supplied sequences encoding wild-type p53. The only transformed cells that survived this experiment had selected for a mutant p53. Further, p53 cDNA clones with several different artificial or natural mutations could cooperate with ras and transform cells (Finlay et al., 1988) or even aided E1A plus ras transformation (Finlay et al., 1989). Thus, the P53 wild-type gene was acting like an anti-oncogene or tumor suppressor gene in these assays. When coupled with observations of a similar mutational spectrum of p53 in murine erythroleukemias (Ben David et al., 1988; Munroe et al., 1988), these data indicated to us that the p53 mutant protein had lost tumor suppressor activity and at the same time could act upon overexpression as a dominant-negative p53 mutant to inactivate endogenous wild-type p53 in primary cells and cooperate with ras in transformation. As we were writing the manuscript describing these studies (Finlay et al., 1989), we appreciated the rather complete reversal of interpretations in the field that this brought about; p53 began its life as a tumor antigen. It then was classified as an oncogene that could immortalize cells and cooperate with ras. Now the wild-type gene was going to be shown to be a tumor suppressor, leading to the consequent realization that SV40 transforms cells by inactivating the p53 protein function (Finlay et al., 1989).

After the paper about all this was submitted to *Cell* and in review, A. Levine went to a Cold Spring Harbor meeting held at the Banbury Center to talk about this work. It was at that meeting that B. Vogelstein presented results demonstrating that two colon cancers from humans contained point mutations in one copy of the P53 gene and that the other allele was lost (Baker et al., 1989). This was a completely different path to the same conclusion that P53 was a tumor suppressor gene. But more than that, Vogelstein had shown that the p53 gene plays a role in human cancers. Research spanning the 1990s and into the 21st century has shown this to be quite correct. P53 mutations are common in human cancers (55% of human cancers), and families have now been identified that inherit p53 mutations and develop cancers with 100% penetrance (Vogelstein et al., 2000). The p53 protein has been shown to be a transcription factor regulating a set of genes that can mediate cell cycle arrest and apoptosis, facilitate DNA repair, or alter other cellular processes. The function of the p53 protein is to act as a checkpoint responding to a wide variety of stress signals that can originate from external or internal events. DNA damage, hypoxia, heat shock (denatured proteins), mitotic spindle damage, nucleoside triphosphate pool sizes, nitric oxide signaling, and even oncogene mutational activation in a cell, will all signal to p53 and elicit a specific response by the cell (Vogelstein et al., 2000). The p53 protein functions as an integrator of upstream signals and then acts as a central node in a signal transduction network that responds to minimize mutations and other errors that can lead to cancers or other pathologies (Vogelstein et al., 2000). In recent years, the regulation of the p53 protein (the central node in this network) has been a large part of the focus of this field, and this network will serve as a model for integrative biology and the evolution of cellular networks. Many additional oncogenes and tumor suppressor genes reside in this network, and it clearly plays a central role in our present day understanding of cancer in humans.

This review of the history of these experiments is a good example of how science moves ahead in a path that is often torturous, with many false leads and dead ends. It is clear, however, that progress is made because there are many scientists and many laboratories that add key observations to a field and that these are used to build a structure upon which the next advance is made. The p53 field, which began in 1979, has benefited from hundreds of laboratories, sharing reagents and ideas, producing thousands of papers – some subset of which will make an important impact upon patients and their diseases. This all began with the conviction that the study of simple model systems, the DNA and RNA tumor viruses, could well lead to the understanding of the origins of cancer in humans. These virus groups lead us to the tumor suppressor genes and the oncogenes. While we have more to learn, we have made a good start.

References

Baker, S.J., Fearon, E.R., Nigro, J.M., Hamilton, S.R., Preisinger, A.C., Jessup, J.M., vanTuinen, P., Ledbetter, D.H., Barker, D.F., Nakamura, Y., et al. (1989). Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. Science *244*, 217–221.

Ben David, Y., Prideaux, V.R., Chow, V., Benchimol, S., and Bern-

stein, A. (1988). Inactivation of the p53 oncogene by internal deletion or retroviral integration in erythroleukemic cell lines induced by Friend leukemia virus. Oncogene *3*, 179–185.

Bienz, B., Zakut-Houri, R., Givol, D., and Oren, M. (1984). Analysis of the gene coding for the murine cellular tumour antigen p53. EMBO J. *3*, 2179–2183.

DeLeo, A.B., Jay, G., Appella, E., Dubois, G.C., Law, L.W., and Old, L.J. (1979). Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. Proc. Natl. Acad. Sci. USA *76*, 2420–2424.

Eliyahu, D., Raz, A., Gruss, P., Givol, D., and Oren, M. (1984). Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. Nature *312*, 646–649.

Finlay, C.A., Hinds, P.W., Tan, T.H., Eliyahu, D., Oren, M., and Levine, A.J. (1988). Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. Mol. Cell. Biol. *8*, 531–539.

Finlay, C.A., Hinds, P.W., and Levine, A.J. (1989). The p53 proto-oncogene can act as a suppressor of transformation. Cell *57*, 1083–1093.

Hinds, P., Finlay, C., and Levine, A.J. (1989). Mutation is required to activate the p53 gene for cooperation with the ras oncogene and transformation. J. Virol. *63*, 739–746.

Jenkins, J.R., Rudge, K., and Currie, G.A. (1984). Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. Nature *312*, 651–654.

Land, H., Parada, L.F., and Weinberg, R.A. (1983). Cellular oncogenes and multistep carcinogenesis. Science *222*, 771–778.

Lane, D.P., and Crawford, L.V. (1979). T antigen is bound to a host protein in SV40-transformed cells. Nature *278*, 261–263.

Linzer, D.I., and Levine, A.J. (1979). Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. Cell *17*, 43–52.

Linzer, D.I., Maltzman, W., and Levine, A.J. (1979). The SV40 A gene product is required for the production of a 54,000 MW cellular tumor antigen. Virology *98*, 308–318.

Lutzker, S., and Levine, A.J. (1996). A functionally inactive p53 protein in embryonal carcinoma cells is activated by DNA damage or cellular differentiation. Nat. Med. *2*, 804–810.

Munroe, D.G., Rovinski, B., Bernstein, A., and Benchimol, S. (1988). Loss of a highly conserved domain on p53 as a result of gene deletion during Friend virus-induced erythroleukemia. Oncogene *2*, 621–624.

Oren, M., and Levine, A.J. (1983). Molecular cloning of a cDNA specific for the murine p53 cellular tumor antigen. Proc. Natl. Acad. Sci. USA *80*, 56–59.

Parada, L.F., Land, H., Weinberg, R.A., Wolf, D., and Rotter, V. (1984). Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. Nature *312*, 649–651.

Pennica, D., Goeddel, D.V., Hayflick, J.S., Reich, N.C., Anderson, C.W., and Levine, A.J. (1984). The amino acid sequence of murine p53 determined from a c-DNA clone. Virology *134*, 477–482.

Vogelstsein, B., Lane, D., and Levine, A.J. (2000). Surfing the p53 network. Nature *408*, 307–310.

Cell, Vol. 57, 1083–1093, June 30, 1989, Copyright ©1989 by Cell Press

The p53 Proto-Oncogene Can Act as a Suppressor of Transformation

Cathy A. Finlay, Philip W. Hinds, and Arnold J. Levine
Princeton University
Department of Biology
Princeton, New Jersey 08540-1014

Summary

DNA clones of the wild-type p53 proto-oncogene inhibit the ability of E1A plus *ras* or mutant p53 plus *ras*-activated oncogenes to transform primary rat embryo fibroblasts. The rare clones of transformed foci that result from E1A plus *ras* plus wild-type p53 triple transfections all contain the p53 DNA in their genome, but the great majority fail to express the p53 protein. The three cell lines derived from such foci that express p53 all produce mutant p53 proteins with properties similar or identical to transformation-activated p53 proteins. The p53 mutants selected in this fashion (transformation in vitro) resemble the p53 mutants selected in tumors (in vivo). These results suggest that the p53 proto-oncogene can act negatively to block transformation.

Introduction

The proto-oncogene product, p53, is a cellular protein expressed at low levels in nontransformed cells (Dippold et al., 1981; Benchimol et al., 1982; Thomas et al., 1983; Rogel et al., 1985). In contrast, in tumor-derived and transformed cell lines, the levels of p53 are often elevated (Crawford et al., 1981; Dippold et al., 1981; Benchimol et al., 1982; Rotter, 1983; Thomas et al., 1983; Koeffler et al., 1986). In particular, in cells transformed by SV40 or adenovirus type 5, p53 is found in stable complexes with the transforming proteins from these viruses, the SV40 large T antigen (Lane and Crawford, 1979; Linzer and Levine, 1979), and the E1B-55kd protein, respectively (Sarnow et al., 1982). The levels of p53 in cells containing these complexes are approximately 100-fold higher than in the nontransformed cell, and the half-life of p53 is correspondingly extended (from 20 min to 24 hr) (Oren et al., 1981; Reich et al., 1983). Therefore, the elevation of p53 levels has often been correlated with cellular transformation. The results of several studies have supported the hypothesis that increased levels of p53 effect changes in the phenotype of the normal cell; overexpression of p53 resulted in the immortalization of rodent cells (Jenkins et al., 1984; Rovinski and Benchimol, 1988) and, when assayed in conjunction with an activated *ras* gene, in the full transformation of primary rodent cells to a tumorigenic phenotype (Eliyahu et al., 1984; Parada et al., 1984). Recent studies, however, have demonstrated that the wild-type sequence of the proto-oncogene p53 (p53-wt) does not encode a transforming protein; i.e., no transformants are observed when primary rat embryo fibroblasts are cotransfected with a p53-wt plus an activated *ras* gene (Finlay et al., 1988; Eliyahu et al., 1988; Hinds et al., 1989). The confusion surrounding the transforming activities of p53 resulted from the existence of several independently derived p53 cDNAs and of at least one p53 genomic clone, all of which possess mutations that activate the p53 protein for cooperation with *ras* (Finlay et al., 1988; Eliyahu et al., 1988; Hinds et al., 1989). The mutations (the result of one or two single nucleotide changes) are localized between amino acids 130 and 234 (out of a total of 390 amino acids). In addition, linker insertion mutations in this same region also activate the p53 protein for transformation with the *ras* oncogene (Finlay et al., 1988). In general, the activated p53 proteins have several properties in common: they bind poorly or not at all to the SV40 large T antigen; they form complexes with the constitutively expressed member of the 70 kd heat shock family, hsc70; and they do not express an epitope localized between amino acid residues 88 and 109 that is recognized by the murine-specific, conformation-dependent, monoclonal antibody PAb246. These transformation-activating mutations span a region comprising approximately 25% of the p53 protein. These observations suggest that the transformation-activating mutations could result from a loss-of-function mutation of the p53 protein. These mutants, when overexpressed, could then act in a *trans*-dominant fashion to inactivate the endogenous wild-type p53, perhaps by the formation of nonfunctional multimeric complexes of proteins (Herskowitz, 1987). It has previously been clearly demonstrated that high levels of mutant p53 proteins are required for the efficient cloning and establishment of p53 plus *ras*-transformed cell lines (Hinds et al., 1989). The association between mutant p53 proteins and the endogenous wild-type p53 protein in a complex has also been previously observed (Rovinski and Benchimol, 1988; Eliyahu et al., 1988). Thus, inactivation of the p53-wt protein by an inactive mutant p53 protein in the same complex may actually be involved in the process of p53-mediated transformation.

The inactivation of p53 appears to play an important role in the development of Friend virus-induced erythroleukemias in vivo. Rearrangement of the murine p53 gene occurs in a high proportion of malignant cell lines derived from the spleens of Friend virus-infected mice (Mowat et al., 1985; Chow et al., 1987), resulting in the inactivation of p53 (Ben-David et al., 1988) or the synthesis of elevated levels of antigenically related truncated polypeptides (Rovinski et al., 1987; Munroe et al., 1988). These investigators have suggested that the inactivation of the p53 gene confers a selective advantage for the development of the tumorigenic phenotype. The rearrangement of the p53 gene has also been observed in 3/6 human osteogenic sarcomas (Masuda et al., 1987).

The inactivation of p53 functions could also be involved in the process of viral transformation. Recent studies have demonstrated that both the SV40 large T antigen and the E1A products from Ad5 form specific complexes with the