The Tumor Suppressor p53 in the Center of a Strategy Aiming at the Alleviation of Side Effects in Cancer Therapies

Lisa Wiesmüller*

Instabilities in the genetic material of somatic cells (alteration of single nucleotides, loss, exchange, or duplication of chromosome segments, lack of single chromosomes or supernumerary chromosomes) can contribute to the development of hyperproliferative cell populations and tumors, and finally to metastasis, when, as part of a multistep process, oncogenes become activated or tumor suppressors become inactivated.[1] The importance of the maintenance of the genetic stability for tumor suppression becomes especially evident when looking at the anti-oncogenic protein p53; the natural wild-type p53 gene is inactivated in 50–60% of all cancer types by mutation or deletion of one or two alleles.[2, 3] Furthermore, in other tumor types epigenetic mechanisms, such as exclusion from the nucleus, accelerated degradation, or neutralization of the protein by complex formation, indirectly lead to the functional inactivation of p53.[4] During cellular stress situations p53 can halt growth in the cell-cycle stage G1 (before DNA synthesis), more rarely in stage S (during DNA synthesis), or in stage G2/M (after DNA synthesis), or can induce active cell death, called apoptosis (Figure 1).[5]

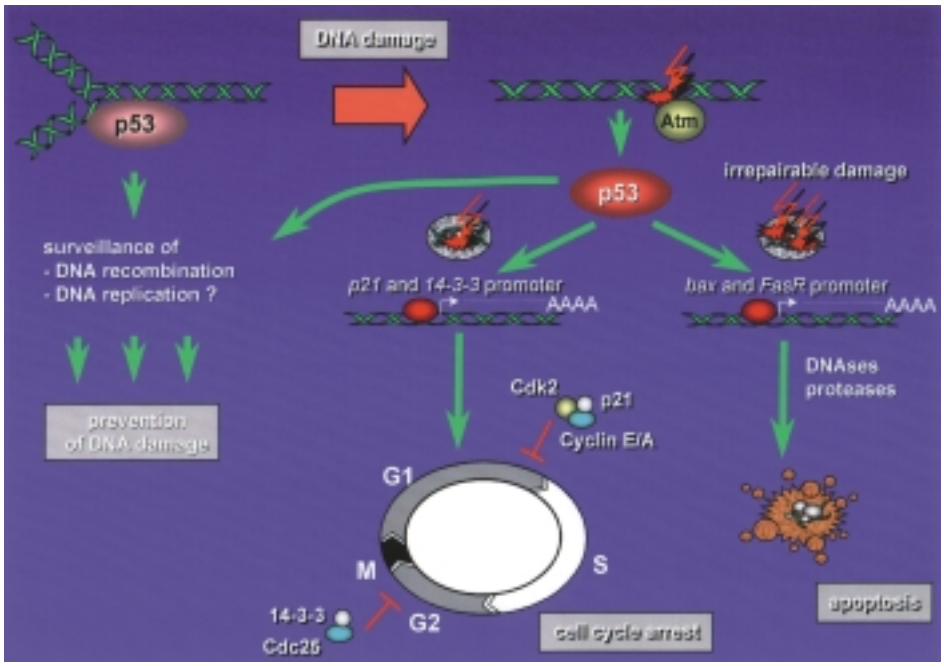

Figure 1. The role of p53 as the "Guardian of the Genome". p53 maintains the genetic integrity during natural cellular proliferation phases by surveillance of recombination and possibly also of replication processes. This figure also comprises the p53-dependent intracellular signal-transduction pathways, which lead to growth arrest or apoptosis during cellular stress situations.

Apoptosis represents a protective mechanism, which eliminates cells after irrepairable damage. This mechanism is critical for the avoidance of malignant tumors and for the success of chemotherapeutic treatments. Apoptosis is a cellular suicide mechanism, during which cellular structures are dissolved and proteins and chromosomes are degraded by proteases and DNAases, respectively. The signals for p53-mediated growth regulation, which were identified at the molecular level, are predominantly DNA strand breaks, which for example, arise after treatment with ionising radiation. Kinases, such as Atm, which recognize a DNA-strand break, transfer phosphate groups to p53.[6] The resulting activation of the p53 molecule allows genes with p53 recognition sequences to be switched on. The product of the target gene $p21^{WAF}$ represents an inhibitor of the G1/S cell-cycle stage specific kinases CyclinE:Cdk2 and CyclinA:Cdk2, and the duplication of damaged DNA is thereby inhibited.[5, 7] The product of the *14-3-3* gene sequesters the phosphatase Cdc25, which is necessary for the activation of the Cdk1 kinase at the G2/M transition. Thus, 14-3-3 inhibits the mitotic division of damaged cells. The target genes which encode death receptors, such as CD95(APO-1/FasR), and a gene which encodes an antagonist of the anti-apoptotic factor Bcl2, Bax, mediate the induction of active cell death.

During the last few years it has been reported from several laboratories that, apart from growth regulatory functions, p53 actively participates in DNA repair processes, particularly recombination.[8] Recombination processes execute DNA exchange between two regions on the genome and represent the final repair mechanism, when DNA double-strand breaks do not allow transfer of the missing information from the complementary strand by replication. By use of special test systems it could be demonstrated that p53 controls the accuracy of recombination processes, that is, it reduces the frequency of erroneous exchange events by one to two orders of magnitude.[9] In summary, p53 guarantees the maintenance of genomic stability during natural proliferation phases and during cellular stress situations by activities in recombinative DNA repair as well as by switching on growth regulatory molecules.[10]

[*] Dr. L. Wiesmüller
Heinrich-Pette-Institut für Experimentelle Virologie und Immunologie an der Universität Hamburg
Martinistrasse 52, 20251 Hamburg (Germany)
Fax: (+49) 40-48051-117
E-mail: wiesmuel@hpi.uni-hamburg.de

  0570-0833/00/3910-1768 $ 17.50+.50/0

Due to this central, but also extremely complex, role of p53 as a tumor suppressor, this protein has become the most important object for cancer research since its discovery 20 years ago. In the field of diagnosis, the main goal today is to establish the functional status of p53 in tumors in order to improve the rate of curing by individual cancer therapies.[4] For this purpose methods from gene technology, microscopy and biochemistry are applied. The importance of individual therapies is exemplified by the fact that p53-deficient colon carcinoma cells are resistant to an inhibitor of DNA synthesis, 5-fluorouracil, whereas they respond extremely sensitively towards irradiation. Taken together, it seems that, as a consequence of the individual p53 mutation and of the specific anticancer treatment applied, the impairment of DNA repair and the inability to execute apoptosis produce different cellular responses. For the development of new therapeutic strategies, scientists from basic and clinical research try to enhance or reconstitute the p53-dependent activities, which mediate apoptosis in tumor cells. To this end, scientists test the applicability of synthetic peptides for blocking the attack of p53 by the cellular protein degradation machinery or catalyzing the refolding of mutant p53 into wild-type p53.[11, 12] According to another approach, wild-type p53 is introduced into p53-deficient cells with adenoviral or retroviral gene vehicles.[13] The most promising strategy today (clinical trials at stages I and II) was developed by ONYX Pharmaceuticals in Richmond, California. It comprises the targeted destruction of tumor cells lacking functional wild-type p53 by viral lysis.[14] For this purpose the adenoviral genome was manipulated in such a way that the virus can only reproduce in p53-deficient cells and, therefore, actively kills the corresponding cancer cells.

Theoretically, we would expect that the same functions which enable wild-type p53 to eliminate tumor cells, can conversely be suppressed so that normal tissues are protected from the destructive effect of genotoxic stress. With the aim of developing a corresponding strategy for alleviation of the side effects of radio- and chemotherapies with patients carrying tumors devoid of functional wild-type p53, Komarov and colleagues, in their recently published work, searched a library of 10000 synthetic chemicals for inhibitors of wild-type p53.[15] The selection procedure chosen by the authors relies on the murine fibroblast cell line ConA, which carries wild-type p53. Additionally, the bacterial gene coding for β-galactosidase (*lacZ*) was introduced into ConA cells in combination with a synthetic DNA-recognition sequence for p53. This combination of DNA elements allows enhanced activity of cellular p53 protein as a transcriptional transactivator to be demonsrated 14 hours after treatment of the cells with the cytostatic agent doxorubicin. When the β-galactosidase substrate X-Gal turns blue in the cell cultures plated in 96-well plates, this indicates the activation of the *lacZ* gene by p53. When this colour change cannot be detected, this indicates a reduction of the transcriptional activity by the chemical added to the corresponding well. A water-soluble compound with a molecular mass of 367 was influencing this p53-dependent activity most prominently and was consequently named "p fifty three inhibitor" Pifitrin α (PFTα; Figure 2). At concentrations of 10 μM, PFTα suppresses the p53-dependent gene activation by more than 90% after application of doxorubicin ($\leq$0.4 μg mL^{-1}) or UV irradiation ($\leq$25 J m^{-2}). As would be expected from this, PFTα also inhibits transcriptional transactivation of chromosomal genes with p53-recognition sequences and downstream biological processes, such as the induction of cell cycle arrest or apoptosis, according to the cell and DNA damage type. Comparison of cells which differ in their p53 status, ascertained that, in each case, the PFTα effects were p53-dependent. The anti-apoptotic effect of PFTα could be demonstrated for appropriate tumorigenic cells (C8) by use of a staining technique, which indicates cellular vitality, and by subsequent quantitative evaluation with a microplate reader. After UV irradiation or application of doxorubicin up to 90% of the cells were protected from cell death by the concomitant addition of PFTα. For the chemotherapeutic agents etoposide, taxol, and Ara C, a maximally twofold protective effect was observed. The PFTα-mediated reduction of p53's ability to induce cell cycle arrest at the G1 stage after treatment with γ-rays was demonstrated by fluorescence-activated cell sorting (FACS) analysis of ConA-fibroblasts. When the cellular DNA is stained with a fluorescent dye, the DNA content in a single cell can be measured cytometrically by this method. Thus, within a certain population the cellular distribution into different stages can be established before and after DNA synthesis.

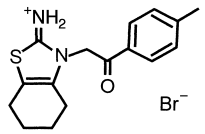

Figure 2. Chemical structure of pifitrin α, 2-(2-imino-4,5,6,7-tetrahydrobenzothiazol-3-yl)-1-*p*-tolylethanone hydrobromide.

To clarify the stage at which PFTα acts within the DNA damage-induced signal transduction pathway, wild-type p53 was produced in cells without endogenous p53 (Saos-2) by introduction of an appropriate gene vehicle. As PFTα still suppressed p53-induced apoptosis under these conditions, as compared to the experimental setup when preexisting wild-type p53 becomes damage-induced, the inhibitor must act downstream of p53. From the results obtained by separation of p53 proteins with gel electrophoresis in two dimensions by charge and mass, an alteration of p53 activities by a distinct pattern of modifications could be excluded.[6] Furthermore, the interaction with certain recognition sequences, underlying the transcriptional transactivation of p53 target genes, was not impaired by PFTα, as the formation of high molecular mass complexes of radioactively labeled DNA fragments and p53 was detected equally well after polyacrylamide gel electrophoresis of proteins from untreated as well as from PFTα-treated cells. The only change which the authors noticed after the addition of PFTα was the decrease of cellular p53 amounts, especially in nuclei, as measured by immunoblot analysis (detection of proteins with specific antibodies after gel electrophoresis and subsequent transfer to a membrane). So far it remained unclear whether this phenomenon can be traced back to diminished de novo synthesis of p53, to increased instability of the protein, or to interference with the nuclear import.

To examine the applicability of PFTα to treatments which protect against the toxic side effects of cancer therapies,

  0570-0833/00/3910-1769 $ 17.50+.50/0

experiments with living animals were performed as the next step. Mice from two different breeding strains were injected intraperitoneally with 2.2 mg kg^{-1} body weight of PFTα and subsequently treated with 8 Gy ionising radiation. The survival rate, body weight, and changes in tissue morphology and apoptosis were monitored for 100 days. Within this time period full protection from irradiation-induced death by PFTα was observed. At the same time the loss of body weight was smaller than without pretreatment. The latter correlated with reduced apoptosis in irradiated tissues, which was shown by a staining technique for the microscopic visualization of chromosome breaks (TUNEL assay). Interestingly, in these mice no tumors were found by the authors up to seven months after irradiation and concomitant drug-mediated inactivation of p53.

It is generally agreed that p53 represents a central factor for the maintenance of genome stability, and, consequently, for the suppression of cancer.[3] From this, the promising data presented by Komarov and colleagues appear almost unbelievable: Why does the loss of p53 function in mice which were nullizygous for p53 cause the formation of fatal tumors within six months, while the inactivation of p53 by PFTα does not involve unrestrained cell growth, although these animals were additionally exposed to ionising radiation?[15, 16] Were the tested animals not observed long enough or was the number of mice (30) too small? Long term studies with several thousands of animals seem to be unavoidable, an approach which brings to mind comparable efforts made after the lively discussion about contradictory results for possible side effects of a snowdrop gene in genetically modified potatoes.[17] However, the apparent discrepancy regarding the PFTα-mediated p53 inactivation without any increase in cancer susceptibilities could be broken up by the multifunctionality of the tumor suppressor. As shown in this work, PFTα suppresses the growth regulatory functions of p53. Nevertheless, the possibility exists that this compound does not influence the surveillance of DNA-repair processes by p53. By analogy, certain p53 mutants still show separate functions in proliferation and DNA-repair.[10, 18] Indeed, the lack of tumors in mice which were nullizygous for p21, as well as in a fraction of mice which were transgenic for the transcriptionally inactive mutant p53(135Val), already indicated that alternative mechanisms to growth control must contribute to tumor suppression by p53.[19, 20] If DNA-repair studies and thoroughly executed DNA mutation and rearrangement analyses of tissues after combined PFTα and radiation exposure substantiate the theory that PFTα reduces the p53-dependent suicide program after chemo- or radiotherapeutic treatments without permitting the destabilization of the genome, then indeed, this discovery could help to improve the quality of life for cancer patients in the future.

[1] B. Vogelstein, K. W. Kinzler, *Trends Genet.* **1993**, *9*, 138 – 141.
[2] M. S. Greenblat, W. P. Bennett, M. Hollstein, C. C. Harris, *Cancer Res.* **1994**, *54*, 4855 – 4878.
[3] D. P. Lane, *Nature* **1992**, *358*, 15 – 16.
[4] G. McGilland, D. E. Fisher, *J. Clin. Invest.* **1999**, *104*, 223 – 225.
[5] T. Jacks, R. A. Weinberg, *Nature* **1996**, *381*, 643 – 644.
[6] D. W. Meek, L. E. Campbell, L. J. Jardine, U. Knippschild, L. McKendrick, D. M. Milne, *Biochem. Soc. Trans.* **1997**, *25*, 416 – 419.
[7] A. Levine, *Cell* **1997**, *88*, 323 – 331.
[8] F. Janus, N. Albrechtsen, I. Dornreiter, L. Wiesmüller, F. Grosse, W. Deppert, *Cell. Mol. Life Sci.* **1999**, *55*, 12 – 27.
[9] C. Dudenhöffer, G. Rohaly, K. Will, W. Deppert, L. Wiesmüller, *Mol. Cell. Biol.* **1998**, *18*, 5332 – 5342.
[10] C. Dudenhöffer, M. Kurth, F. Janus, W. Deppert, L. Wiesmüller, *Oncogene* **1999**, *18*, 5773 – 5784.
[11] A. Bottger, V. Bottger, A. Sparks, W. L. Liu, S. F. Howard, D. P. Lane, *Curr. Biol.* **1997**, *7*, 860 – 869.
[12] G. Selivanova, L. Ryabchenko, E. Jansson, V. Iotsova, K. G. Wiman, *Mol. Cell. Biol.* **1999**, *19*, 3395 – 3402.
[13] M. Favrot, J. L. Coll, N. Louis, A. Negoescu, *Gene Ther.* **1998**, *5*, 728 – 739.
[14] J. R. Bischoff, D. H. Kirn, A. Williams, C. Heise, S. Horn, M. Muna, L. Ng, J. A. Nye, A. Sampson-Johannes, A. Fattaey, F. McCormick, *Science* **1996**, *274*, 373 – 432.
[15] P. G. Komarov, E. A. Komarova, R. V. Kondratov, K. Christov-Tselkov, J. S. Coon, M. V. Chernov, A. V. Gudkov, *Science* **1999**, *85*, 1733 – 1737.
[16] L. A. Donehower, M. Harvey, B. L. Sladge, M. J. McArthur, C. A. Montgomery, J. S. Butel, A. Bradley, *Nature* **1992**, *356*, 215 – 221.
[17] S. W. Ewen, A. Pusztai, *Lancet* **1999**, *354*, 1353 – 1354.
[18] Y. Saintigny, D. Rouillard, B. Chaput, T. Soussi, B. S. Lopez, *Oncogene* **1999**, *18*, 3553 – 3565.
[19] C. Deng, P. Zhang, J. W. Harper, S. J. Elledge, P. Leder, *Cell* **1995**, *82*, 675 – 684.
[20] D. L. Schaffner, P. Chevez-Barrios, S. L. Huang, R. Barrios, B. F. Dickey, M. R. Shaker, S. Rajagopalan, G. M. Habib, R. M. Lebovitz, M. W. Lieberman, *Lab. Invest.* **1996**, *74*, 1005 – 1011.

  0570-0833/00/3910-1770 $ 17.50+.50/0