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Review

Prospects for p53-based cancer therapy*

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The p53 tumor suppressor plays the role of a cellular hub which gathers stress signals such as damage to DNA or hypoxia and translates them into a complex response. p53 exerts its action mainly as a potent transcription factor. The two major outcomes of p53 activity are highlighted: cell cycle arrest and apoptosis. During malignant transformation p53 or p53-pathway related molecules are disabled extremely often. Mutations in p53 gene are present in every second human tumor. A mutant form of p53 may not only negate the wild type p53 function but may play additional role in tumor progression. Therefore p53 represents a relatively unique and specific target for anticancer drug design. Current approaches include several different molecules able to restore p53 wild-type conformation and activity. Such small molecule drugs hold great promise in treating human tumors with dysfunction of p53 pathway in the near future.

Keywords: p53, tumor suppressor, cancer, stress response, missense mutations, small molecule inhibitors

URGENT NEED FOR EFFECTIVE CANCER THERAPIES

Cancer is a disease resulting from the breakdown of several checkpoints and tumor-suppressing mechanisms (Hanahan & Weinberg, 2000). According to the most recent global statistics available, in 2002 there were 10.9 million new cases of cancer worldwide, 6.7 million deaths, and 24.6 million people who had been diagnosed with cancer in the previous five years (Parkin *et al.*, 2005). World Health Organization predicts that this grim statistic can be even worse and incidence of cancer may increase by 50% in the year 2020. Despite significant advances in cancer diagnostics and therapy there is still little progress in the treatment of advanced disease. Therefore finding effective ways to treat and cure cancer has become the top priority for the modern society (Blagosklonny, 2005). Although global picture revealed in cancer statistics is quite pessimistic it should not hide the basic fact that from the cellular perspective the tumor-suppresing mechanisms are extremely effective, since cancer on average affects every third of us only once in a lifetime, despite the billions of potential targets in our body (Lowe *et al.*, 2004). One of the best known barriers against malignant transformation is a single protein the tumor suppressor p53 (Hofseth *et al.*, 2004).

p53 IS A MODEL TUMOR SUPPRESSOR

p53 is probably the most popular molecule in the field of cellular biology (Koshland, 1993; Vogelstein *et al.*, 2000; Vousden, 2000; Bargonetti & Manfredi, 2002; Levine *et al.* 2004). It receives constantly growing attention; there are more than 31 000 articles in the PubMed with p53 in their title or abstract, with almost 800 already published in the first 3 months of 2005. There are scientific meetings and workshops dedicated solely to p53 and its network. Despite this massive amount of information about p53, we still can not fully translate this knowledge into treatment strategies. Experimental findings from the last two decades have demonstrated the crucial role of wild-type p53 in intrinsic tumor suppression (Levine *et al.*, 2004; Lowe *et al.*, 2004). Therefore the "guardian of the genome" term was coined for p53 (Lane, 1992). p53 has two

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Abbreviations: BCR/ABL, breakpoint cluster region/Abelson leukemia; IARC, International Agency for Research on Cancer; MDM2, mouse double minute 2; NCI, American National Cancer Institute, PRIMA-1; p53 reactivation and induction of massive apoptosis-1.

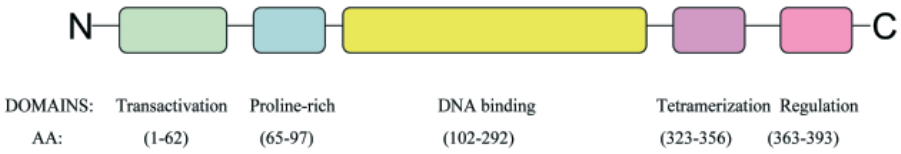

Figure 1. p53 domains.

The p53 gene *TP53* encodes a 393 amino-acid nuclear protein which consists of five functional domains. More than 80% of somatic mutations detected in human tumors affect the DNA-binding domain.

homologues, p63 and p73. Although structurally related, their role is different and is now a subject of intensive studies (Yang *et al.*, 2002; Blandino & Dobbelstein, 2004).

The recently accumulating evidence on the tumor-promoting role of cancer p53 mutants has established a solid background for p53-based treatment strategies. A number of reports have shown that at least *in vitro* it is possible to influence the mutant form of p53 in tumor cells so it regains the wild type conformation and this may prove effective in new drug design (Brachmann, 2004; Haupt & Haupt, 2004, Seeman *et al.*, 2004).

p53 IS A TRANSCRIPTION FACTOR

p53 consists of a N-terminal transactivation domain with a number of phosphorylation sites (including the Ser15, Thr18 and Ser20 sites crucial for protein stabilization), a proline-rich domain, a DNA-binding core domain which is also involved in protein–protein binding, a C-terminal tetramerization domain, and regulatory domain (Fig. 1). p53 functions as a transcription factor which is kept under stringent control and its expression is limited to a short "posttraumatic" period if the cell is challenged with exogenous or endogenous stress factor(s) (Fig. 2). In normal, unstressed cells, p53 is expressed at a very low level; the half-life of the protein does not exceed 20 min. Prolonged p53 expression in normal cells would be detrimental, so there is rapid turnover of p53 mediated by ubiquitin ligases such as Mdm2 (Hdm2 in humans), and the recently discovered Pirh2 (p53-induced protein with a RING-H2 domain) and Cop1 (constitutively photomorphogenic 1), all of which target p53 to proteasomal degradation (Momand *et al.*, 1992; 2000; Kubbutat *et al.*, 1997; Corcoran *et al.*, 2004; Dornan *et al.*, 2004). p53 is involved in autoregulation, it activates transcription of Mdm2 in a negative feedback loop (Barak *et al.*, 1993; Wu *et al.*, 1993). In response to stress, p53 is activated and stabilized through extensive post-translational modifications of two domains: the transactivation and regulation ones, and also through direct interaction with cooperating proteins. These modifications prolong protein's life significantly. Activated p53 binds to target DNA and determines the choice between two main pathways: cell cycle arrest (which can then result in differentiation or senescence) and apoptosis (Fig. 1) (Taylor & Stark, 2001; Bargonetti & Manfredi, 2002; Vousden & Lu, 2002; Haupt *et al.*, 2003). Among the effectors associated with cell cycle arrest, cyclin-dependent kinase inhibitor p21, also known as WAF1 or CIP1 plays a major role (el-Deiry *et al.*, 1993). Other p53 targets involved in cell cycle regulation include: BTG2 (B-cell chronic lymphocytic leukemia translocation gene) (Rouault *et al.*, 1996), 14-3-3-σ (Hermeking *et al.*, 1997), GADD45 (growth arrest and DNA damage) (Hollander *et al.*, 1993), ribonucleotide reductase (p53R2) (Tanaka *et al.*, 2000) and cyclin G (Okamoto & Beach, 1994). On the other hand, p53 is one of the key regulators of both the extrinsic and intrinsic apoptotic pathways. It activates several genes, among them *Fas* and *DR5/KILLER* receptors from the former and *Bax*, *Noxa*, and *PUMA* from the latter pathway (Fridman & Lowe, 2003; Haupt *et al.*, 2003). Interestingly to perform the apoptotic scenario p53 requires cooperation with its homologues, p63 and p73 (Flores *et al.*, 2002). It is still unclear what determines the fate of the cell with activated p53. Several factors have already been identified, including cell type and/or duration and type of the stress signal.

p53 IS MUTATED IN TUMORS

TP53 is of the most commonly mutated gene in human malignancies, with mutations present in roughly every other tumor (Nigro *et al.*, 1989; Sigal & Rotter, 2000). The diversity of mutations is even more impressing, the number of described mutations exceeded 10 000 in 1999. In the latest released version of the IARC TP53 mutation database from July 2004 (R9, http://www-p53.iarc.fr/) there are 19 809 somatic mutations of p53 reported in 1769 original publications (published between 1989 and December 2003) (Olivier *et al.*, 2002). Considering the frequency of p53 mutations, according to the IARC database the top three tumors are esophageal, ovarian and colorectal cancer (between 35 and 75% of tumors have mutations in *TP53*). The majority of mutations in *TP53* gene are missense mutations, 72% and 73% for germline and somatic mutations, respectively. The

Table 1. The most frequent p53 mutations and their effect on protein function (after IARC p53 database)

Codon	Residue Amino acid	Mutant Amino acid	Effect on p53 function
175	Arg	His	Breaks H-bond between L2 and L3 loops
248	Arg	Gln or Trp	Breaks contact with DNA in minor groove
273	Arg	His or Cys	Breaks contact with DNA in major groove
282	Arg	Trp	Destabilizes H2 helix and DNA binding in the major groove and breaks contacts on the β-hairpin

striking feature in the distribution of the mutations is that four out of five are in the DNA-binding domain (residue 102–292) (Fig. 1), only 1% affects the transactivation domain and only 4% the tetramerization and regulation domain. Although there are hundreds of different mutations, the top five alone account for 20% of all p53 core domain mutants and top eight account for 30% (Olivier *et al.*, 2002). The results of these missense mutations affecting the core domain are profound; they partially or completely prevent DNA binding and transcriptional activity (Table 1). However mutant p53 is usually able to form tetramers with the wild type protein through the C-terminal tetramerization domain and may exert a dominant negative effect. This is true usually in early stages of the disease, since loss of heterozygosity is frequently observed in human tumors with mutated p53 (Takahashi *et al.*, 1989) as the disease progresses. Inactivation of p53 in tumors differs from the inactivation of other tumor suppressors in one important feature. Mutant p53 is expressed in significantly higher amounts than wild type p53. This phenomenon has raised the question if mutation of *p53* gene equals loss of p53. Recent studies show that expression of p53 mutants may present more advantage to the tumor than loss of p53. In mice, loss of p53 does not affect the cell cycle or normal development, but strongly predispose to malignant transformation. Mice deficient for both p53 alleles develop normally but succumb to lymphomas at the age of 5 months on average (Donehower *et al.*, 1992). In humans inherited p53 mutations were described in patients from some cancer-prone families (Malkin *et al.*, 1990; Srivastava *et al.*, 1990). This familial syndrome is called Li-Fraumeni syndrome and is a rare autosomal disorder, with frequent mutation of either one or two p53 alleles. Patients are highly susceptible to the development of cancer early in their life, with highest incidence of breast carcinomas, soft-tissue sarcomas, brain tumors, bone sarcomas and adrenocortical carcinomas (Malkin, 1993; Olivier *et al.*, 2003). Mice mimicking human Li-Fraumeni syndrome were recently generated by two groups (Lang *et al.*, 2004; Olive *et al.*, 2004). Although the average life-span of mice expressing mutant p53 was similar to that of mice without p53, the pattern of tumors was remarkably different. One study demonstrated that mice expressing mutant p53 have more carcinomas with aggressive phenotype compared to mice without p53 or with one normal allele (Olive *et al.*, 2004). The second group did not observe any increase in carcinomas, but they used mice of different genetic background. Nevertheless, if carcinomas did develop, their metastatic potential was higher (Lang *et al.*, 2004). Another important message from both studies refers to the role of mutant p53 in tumor cells. If mutated form of p53 would serve simply to "knock-down" the wild type protein from neoplastic cell, then mutant p53 should prevent the lethality of Mdm2 *null* background. The lack of both Mdm2 alleles is lethal during normal development, although such phenotype can be rescued by deletion of p53 (Jones *et al.*, 1995). Heterozygous expression of mutant along with wild type p53 allele did not prevent lethality in Mdm2 *null* mice arguing against inactivation of p53 as a major role of mutant p53. Analysis of tissue expression of mutant p53 in engineered mice was also performed. Expression of the mutant protein was high only in tumor tissue, but low in normal tissues, suggesting a promoting role of mutant p53 in cancer progression. Such "gain-of-function" mutants can accelerate tumor progression (Fig. 3).

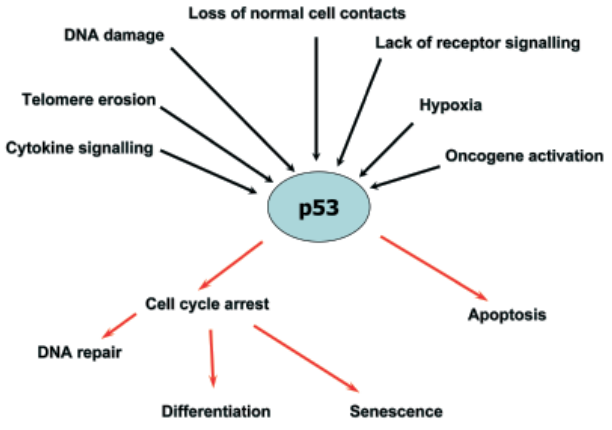

Figure 2. Central role of p53 in cellular response to stress.

p53 determines cell fate upon multiple exogenous and endogenous stress signals.

Contrary to earlier expectations, mutations in *p63* and *p73* genes are rare in human cancer (Blandino & Dobbelstein, 2004).

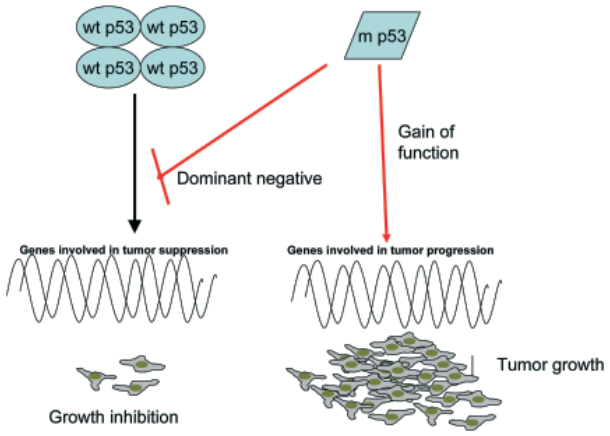

Figure 3. Active role of mutant p53 (m p53) in tumor progression — possible mechanisms.

While wild-type p53 serves as an ultimate tumor suppressor, the mutant form is not just an inactivated antioncogene, but may play active role in the progression of tumor growth.

THERAPEUTIC STRATEGIES

Gene therapy

Reconstitution of wild-type p53. Among the different approaches targeting p53, replacement gene therapies have been explored extensively in recent years. Such a strategy aims at restoration of p53 function in cancer cells by introduction of exogenous p53. Various protocols and vectors have been employed, including retroviruses, adenoviruses and vaccinia-derived vectors. Recent studies focus on adenoviral vectors, with Ad-p53, adenovirus serotype 5 carrying wt p53 gene, as a model example. One of such Ad-p53 vectors has been commercialized by Introgen Therapeutics Inc. under the name Advexin and received the status of an orphan drug from the US Food and Drug Administration in 2003. Advexin has been used in patients with several different types of cancer including head and neck cancer (the most advanced studies — phase III clinical trials), non-metastatic non-small cell lung cancer plus radiation therapy (phase II) and bronchioalveolar lung cancer, ovarian cancer and glioblastoma (phase I) (Swisher *et al.*, 2003; Lang *et al.*, 2003). Although preliminary results were promising, recent data failed to demonstrate antitumor activity in patients and some trials have been discontinued (Zeimet & Marth, 2003). New trials aim at combination of gene transfer with chemotherapy or radiotherapy.

Targeting tumor cells with deficient adenovirus. Adenoviruses avoid apoptosis of infected cell by inactivation of p53 by adenoviral protein E1B. Based on this observation, genetically modified adenovirus ONYX-015 has been engineered. ONYX-015 does not express E1B and is not able to interfere with wt p53. Such a virus induces wt p53 response in normal cells, which halts viral replication and allows the cell to survive. On the other hand, it replicates freely in cells expressing mutant p53, causing cell death (Heise *et al.*, 1997; 2000). Similar lethal effect is observed if wt p53 expression is abrogated by high Mdm2 expression (an indirect way of p53 inactivation in a number of tumors). Adenoviral-mediated targeting of tumors harboring mutant p53 has been already tested in clinical trials — more than 200 patients with different tumor types received ONYX-015 so far. Although ONYX-015 as a single agent did not impress in initial trials, it is actively pursued in combination with chemo- and radiotherapy (Rogulski *et al.*, 2000; Haupt & Haupt, 2004; Galanis *et al.*, 2005).

Restoration of p53 function–targeting mutant p53

A second option is to reactivate mutant p53 in order to restore normal p53 functions. The higher and stable expression of mutant p53 increases the chances that upon restoration of p53 transcriptional activity the apoptotic pathway would predominate. Several compounds with such capability have been identified by random screening and by drug design. Although such chemical compounds differ in structure and in the pattern of susceptible mutants, they form new group of potential drugs — reactivators of p53.

PRIMA-1 represents a model compound from this group. This low molecular mass compound was identified from the NCI chemical library. PRIMA-1 induced apoptosis in human tumor cells with different p53 mutations, among them hot spot mutations (e.g. Arg273His). It activated transcription of several p53 target genes, including *p21*, *Mdm2* and *PUMA* only in cells with mutant p53 and demonstrated antitumor activity in mouse models, with no apparent toxicity (Bykov *et al.*, 2002; 2003). PRIMA-1 does not exert any effect on wild-type p53. However, it is not known if PRIMA-1 directly associates with p53 and, to make it even more puzzling, PRIMA-1 is able to induce apoptosis in the cells without p53. Therefore detailed studies are necessary before further application of PRIMA-1.

Cp-31398 is a styrylquinazoline, another small molecule compound which is able to interfere with mutant p53 and restore wild type function (Foster *et al.*, 1999) Cp-31398 can induce apoptosis not only in cells with mutant p53, it can also interfere with wt p53, increasing the level of the protein and promoting its activity (Takimoto *et al.*, 2002; Wang *et al.*, 2003). However, its mechanism of action remains unresolved since *in vitro* Cp-31398 does not bind to the core domain of p53, either wt or mutated (Bykov *et al.*, 2003; Haupt & Haupt, 2004). One of the hypo-

thetical mechanisms of action might involve stabilization of newly synthesized p53 *via* interaction with p53 tetramers outside the DNA-binding domain. Another proposed mechanism is that Cp-31398 intercalates DNA and stabilizes p53 in a similar way to DNA damaging agents, such as doxorubicin. The lack of N-terminal p53 phosphorylation upon treatment with Cp-31398 argues against such mechanism.

CDB3 is a nine-amino-acid peptide designed rationally by analysis of the interaction and stabilization of p53 by p53-binding protein 2 (53BP2) (Friedler *et al*., 2002; Issaeva *et al*., 2003). It interacts with the DNA-binding domain and corrects some misfolded mutants. This has been shown to induce transcription of several target genes and activation of apoptosis. CDB3 did not activate apoptosis in cells with wt p53.

Curiously, also the C-terminal peptide of p53 is able to restore function of some p53 mutants. Although the mechanism of action is not well understood, data from preclinical studies look promising (Snyder *et al*., 2004).

Another approach targeting cells with mutated p53 employs a chimeric adaptor protein. Such a construct consists of p73 DNA-binding and tetramerization domains fused to p53 oligomerization domain and alone does not exert transcriptional activity because it lacks a transactivation domain. However, in the presence of mutant p53 this construct serves as a link between mutant p53 and target sequences in DNA and activates transcription of target genes. Delivery of such a construct with adenoviral vectors into cells with mutant form of p53 caused activation of p53 target genes and apoptosis (Roth *et al*., 2003).

Wild type p53-directed approaches

Based on the interaction between hdm2 (human analogue of mdm2) and p53 a group of small molecular mass inhibitors of this interaction have been described recently (Vassilev *et al*., 2004). These *cis*-imidazoline analogs, named nutlins, bind to the p53-binding site on the hdm2 molecule, displace p53 from its complexes with hdm2 and prevent hdm2 from targeting p53 to degradation. This leads to accumulation of p53 and activation of p53 target genes. Antitumor effects of nutlins were observed in cells with wild-type p53, but not in cells expressing mutant p53 or without p53 (Klein & Vassilev, 2004}.

Paradoxically, induction of wild type p53 may also serve to protect normal cells from the detrimental effects of chemotherapy or radiotherapy. An example of such cytoprotector is amifostine (WR2721) which causes cell cycle arrest only in cells expressing wt p53 (Seemann *et al*., 2004).

POTENTIAL OBSTACLES

Several unpredictable outcomes resulting from the administration of small molecule activators or inhibitors of p53 pathway need to be considered before planning clinical testing. One involves unpredictable bioavailability and biodistribution. Agents with exquisite selectivity and remarkable potency in *in vitro* studies could be disqualified from further development because of poor solubility, rapid degradation, fast elimination or neutralization by developing immune response. Structural features of small molecules might influence their immunogenicity and production of blocking immune reactions. The same mechanisms might also be responsible for intolerable hypersensitivity reactions. Small molecule drugs, while frequently nonimmunogenic *per se* because of their size, might attach to carrier proteins or cell surface structures and induce immune response. On the other hand, the observed activation of the immune system associated with adenovirus-mediated p53 gene delivery might in part contribute to beneficial response in some patients after replacement gene therapy. All these potential variables are unlikely to be predicted by structure-function studies *in vitro* and could consume both time and money. Therefore, implementation of detailed preclinical studies should be considered as early as possible.

Before we attempt to restore p53 function in tumors, a few more issues need to be resolved. One such important issue is the role of wt p53 in tumors with a lower frequency of p53 mutations. p53 may contribute to the repair of DNA damage after genotoxic stress, but this p53 activity has been generally attributed to normal cells (Taylor & Stark, 2001; Vousden & Lu, 2002; Meek, 2004). However, recent observations have demonstrated that some tumors actually may adapt normal p53 activation in response to genotoxic agents. It has been shown that prostate cancer cells are protected from ionizing radiation-induced DNA damage through activation of p53 (Scott *et al*., 2003). We recently showed that cells transformed with oncogenic tyrosine kinase BCR/ABL may actually benefit from activation of p53 upon DNA damage (Stoklosa *et al*., 2004). p53 induces G2/M delay and allows transformed cells to repair inflicted damage in DNA preventing them from mitotic catastrophe. It has been shown that BCR/ABL can exert a strong antiapoptotic effect in transformed cells by activation of antiapoptotic members of the Bcl2 family (Amarante-Mendes *et al*., 1998; Salomoni *et al*., 2000). Thus in BCR/ABL-transformed cells stabilization of p53 causes rather cell cycle arrest than apoptosis. Such "abuse" of the antioncogene function by a well-known oncogene would suggest that in chronic myelogenous leuke-

mia, a BCR/ABL-positive hematological malignancy, there would be less pressure on inactivation of p53 during leukemogenesis. Indeed almost all patients in the chronic phase of CML retain the wild type form of p53, while in the late stage of CML, called blast crisis, frequency of p53 mutations is relatively low, from 15 to 25% (Neubauer *et al*., 1993; Rovira *et al*., 1995).

It is expected that reactivation of the wild-type conformation of p53 should induce massive apoptosis in tumor cells. Such experimental therapy would probably be combined with classic genotoxic agents to increase the magnitude of apoptosis. One should be cautious with this therapeutic strategy in tumors which are able to recruit p53, such as CML.

CONCLUSION

Recently Andrew von Eschenbach, director of the NCI, announced the year 2015 as a deadline for elimination of suffering and death caused by cancer. Although it is still a remote perspective, we are closer than ever from achieving this goal. The success of imatinib, an inhibitor of c-abl kinase in the treatment of BCR/ABL-positive leukemia has opened a new era of cancer specific small-molecule inhibitors (Sawyers, 2004; Blagosklonny, 2005). Although p53 is not a typical cancer-specific antigen, its central role in the control of cell growth and apoptosis and frequent mutations in tumors make p53 a unique target for cancer therapy. In addition to the strategy of p53 reactivation in tumors, modulation of p53 activity in normal cells may protect them from the side effects of chemotherapy or radiotherapy. Several new compounds targeting p53 enter clinical trials which warrants the hope that p53-oriented therapy will be one of the hottest topics in the coming years even if it turns out to be effective only in a limited spectrum of tumors with p53 malfunction.

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