

*Review***Gain-of-Function Mutations in the Tumor Suppressor Gene *p53***

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**Abstract**

The tumor suppressor protein p53 is a multifunctional transcription factor involved in the control of cell cycle progression, DNA integrity, and cell survival. p53 is mutated in half of all tumors and has a wide spectrum of mutation types. p53 mutants show different degrees of dominance over coexpressed wild-type p53, and loss of the wild-type p53 allele has been observed frequently. Several p53 mutants can exert oncogenic functions beyond their negative domination over the wild-type p53 tumor suppressor functions. These so-called gain-of-function effects, such as enhancement of tumorigenicity and therapy resistance, were investigated in p53-null cells. The possible mechanisms by which p53 mutants exert their gain-of-function effects are reviewed. The existence of functional gains of certain p53 mutants has important ramifications for tumor prognosis and cancer therapies.

**Tumor Suppressor p53**

The key molecular changes in the multistep progression of cancer are still unknown, and a better understanding of this process might lead to more rational therapies and improved survival of patients. The development of tumors is generally accepted to be a multistep process in which alterations in oncogenes and tumor suppressor genes play an important role (1).

The tumor suppressor gene *p53* is mutated in 50% of all tumors (2–4), and it plays a role in the carcinogenesis of many different malignancies. The gene is mutated in more than 90% of head and neck squamous cell carcinomas (5). In contrast, the incidence of p53 mutations is very low in hematological malignancies (6).

The wild-type protein p53 controls cell cycle progression by acting as transcription factor for many genes. All these genes contain a p53 consensus sequence in their promoter region. p53 controls cell cycle arrest and apoptosis via the transcription regulation of genes such as the cyclin-dependent kinase inhibitor p21<sup>Waf1/Cip1</sup>, the protein GADD45, and the apoptosis proteins Bax and Bcl-2. p53 controls its own functionality via

transcription regulation of MDM2, which targets p53 for ubiquitination. The p53 pathways have been reviewed extensively (7–10).

**Mutations in the *p53* Gene**

Loss of p53 activity predisposes cells to the acquisition of oncogenic mutations and may favor genetic instability. Inactivation of p53 occurs mainly through point mutations, although small deletions/insertions in the gene also have been detected. About 10,000 p53 mutations have already been identified in human tumors and are gathered in databases (2–4). The *p53* gene has a wide spectrum of mutations in human tumors (2–4). The great majority of the mutations are clustered (Fig. 1) in the core domain (120–292 bp). This domain is important for DNA-specific binding and is essential for p53 function. Despite the wide mutation spectrum, a few hot spots for mutations are found in the most conserved areas of the gene (2, 3, 11, 12).

In the last decade, many studies, which yielded inconsistent results, have tried to ascribe prognostic significance to the presence of mutated p53 (13). One explanation is that p53 mutations might be missed by analyzing only exons 5–8, by sequencing only genomic DNA, or by using only immunohistochemistry (5, 14). Another explanation for the variable results is that a lot of studies did not included the consequences of different p53 mutations.

**Various Types of p53 Mutations**

The DNA-binding structure of the *p53* gene (Fig. 2) contains a sandwich of two anti-parallel  $\beta$ -sheets that have four and five  $\beta$ -strands and a loop-sheet-helix motif that packs tightly against one end of the  $\beta$ -sandwich. Furthermore, there are two large loops (L2 and L3) that are held together in part by a tetrahedrally coordinated zinc atom (12). Although the  $\beta$ -sandwich comprises a major part of the core domain structure, it is not directly involved in DNA binding. Instead, the core domain uses the loop-sheet-helix motif and one of the two large loops to bind DNA (12).

Several categories of p53 mutation can be distinguished by taking into account the impact of the mutation on either the protein structure/stabilization or interaction with DNA: (a) type I, missense mutations that affect residues of the DNA-binding surface and disrupt the protein-DNA contact points (such as p53-Trp<sup>248</sup> and p53-His<sup>273</sup>); (b) type II, missense mutations that disrupt the protein conformation (such as p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-His<sup>179</sup>, and p53-Gly<sup>281</sup>); and (c) type III, null mutations that completely destroy the functionality of the protein [insertions/deletions (frameshift mutations), nonsense mutations, and splicing junction mutations].

**Loss- and Gain-of-Function p53 Mutants**

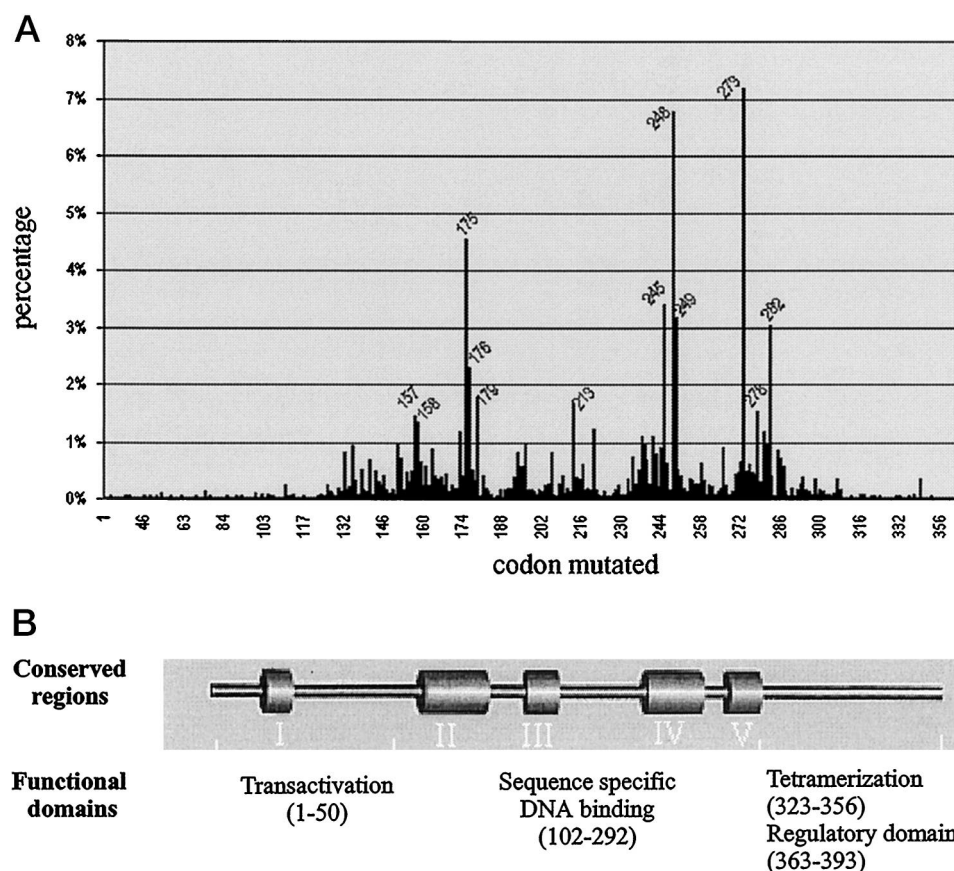
It is generally believed that p53 loses its tumor suppressor function as a consequence of a mutation in *p53*. Most p53 mutants have impaired sequence-specific transactivation activity, which means that p21<sup>Waf1</sup> expression, for example, is not

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**Fig. 1** *p53* gene structure and mutations. **A**, the bars represent the percentage of mutations found in tumors that were primarily investigated for core domain mutations (2, 3). **B**, the *p53* gene consists of 11 exons encoding a protein that comprises a transactivation domain, a core domain/sequence-specific DNA-binding domain, and a COOH-terminal domain with a tetramerization domain and a nonspecific DNA-binding domain (12). Boxes, conserved domains.



up-regulated, and cell cycle arrest or apoptosis after DNA damage will not occur. However, several studies indicate that certain types of p53 mutations, so called gain-of-function mutants, exert functions that the wild-type p53 does not. Known p53 gain-of-function effects are summarized in Table 1 (15–29). Because most p53 mutants exert dominant negative effects on coexpressed wild-type p53 (30), gain-of-function effects of several p53 mutants had to be investigated by introduction of the p53 mutants into cells lacking wild-type p53.

Mutant human p53 alleles (p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, and p53-Gly<sup>281</sup>) expressed in cell lines lacking p53 resulted in either enhanced tumorigenic potential in nude mice or enhanced plating efficiency in agar cell culture (17). In another study, nude mice injected with 10<sup>3</sup> murine fibroblast null cells transfected with the p53 mutant p53-Gly<sup>281</sup> developed tumors in contrast to mice injected with the 10<sup>3</sup> untransfected null cells (27). p53-null, leukemic T cells transfected with certain p53 mutants (p53-His<sup>175</sup>, p53-Gln<sup>213</sup>, and p53-Gln<sup>248</sup>) showed metastatic capacities when they were injected into severe combined immunodeficient mice, in contrast to some other mutants (p53-Cys<sup>273</sup> and p53-His<sup>234</sup>). As a consequence, the mice from the first group showed a shorter survival (29).

A transgenic mouse model was developed with the murine mutant p53-His<sup>172</sup> under the control of human keratin-1-based vector (20). In contrast to the wild-type p53 and p53 knockout

mice, these transgenic mice exhibited increased susceptibility to chemical carcinogenesis, with greatly accelerated benign papilloma formation, malignant conversion, and metastasis. The papillomas in the transgenic mice showed centrosome abnormalities at high frequencies (75% of the cells), whereas the p53-null tumors exhibited abnormal centrosomes less often (4% of the cells; Ref. 20).

These studies show that certain p53 mutants not only lose their tumor suppressor function but gain oncogenic functions.

### Loss of Heterozygosity

Loss of the wild-type p53 allele is frequently detected in tumors (31, 32). The p53 protein functions optimally when it binds to DNA as a wild-type p53 tetramer (33). One mutant p53 protein can disturb a functional tetramer and is therefore able to override the function of three wild-type p53 proteins. However, some biochemical factors and binding regulators can modulate a genotypically mutant p53 into an equilibrium with the wild-type conformation (34).

p53 mutants show different degrees of dominance over wild-type p53 (30, 35). In a study of Li-Fraumeni tumor patients, loss of the wild-type allele was observed in about half of the cases carrying p53 germ-line mutations. This loss apparently was associated with mutation types occurring outside the core domain or truncating the protein (type III; Ref. 36). An associ-

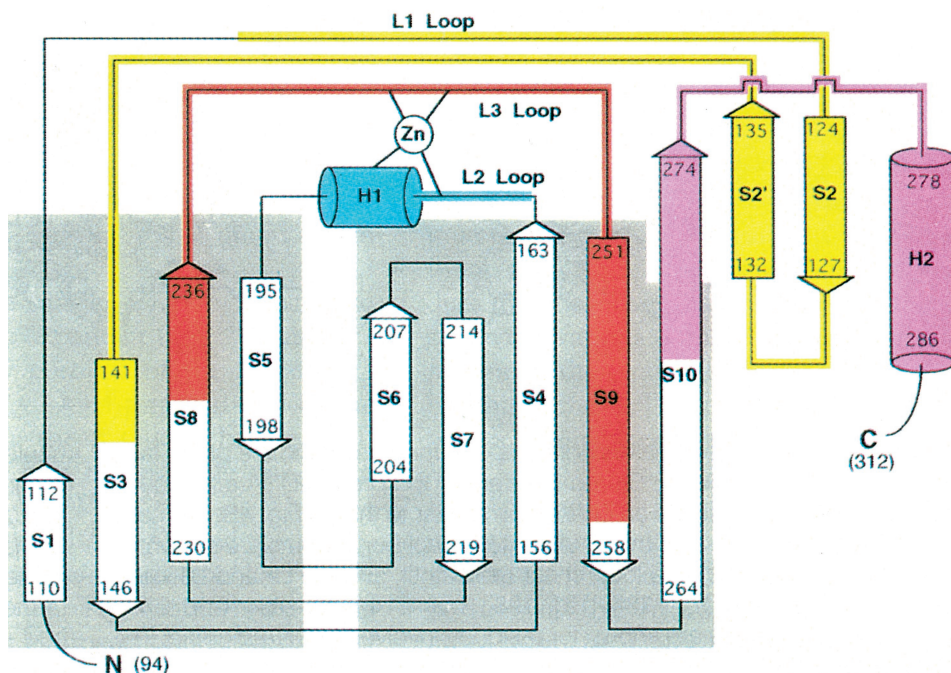


Fig. 2 Topological diagram of the secondary structure elements of p53. The core domain of p53 is depicted. The  $\beta$ -strands (S),  $\alpha$ -helices (H), three of the loops (L), and the zinc atom (Zn) are labeled, and the residues at the beginning and the end of each secondary structure element are indicated. The boundaries of the two  $\beta$ -sheets that make up the  $\beta$ -sandwich are shaded. The conserved regions are colored yellow for region II, blue for region III, orange for region IV, and pink for region V (reprinted from Ref. 12 with permission, copyright 1994, American Association for the Advancement of Science).

Table 1 Several gain-of-function effects of p53

| Result  | Reference no.         |
|---|-----------------------|
| <i>In vitro</i>   |                       |
| Increased proliferation                                   | 15 and 16             |
| Enhanced agar cell culture                                | 17 and 18             |
| Increased growth density                                  | 15                    |
| Disturbed spindle checkpoint and chromosome abnormalities | 19–21                 |
| Antiapoptotic activity                                    | 22 and 23             |
| Enhanced ras-induced morphological transformation         | 24                    |
| Increased therapy resistance                              | 25                    |
| <i>In vivo</i>  |                       |
| Tumorigenicity  | 17, 20, 22, and 26–28 |
| Invasiveness  | 20, 26, and 29        |

Table 2 Genes up-regulated by gain-of function p53 mutants

| Up-Regulated gene                     | Reference no.      |
|---------------------------------------|--------------------|
| Proliferating cell nuclear antigen    | 18, 27, and 38     |
| MDR gene                              | 15, 17, 27, and 39 |
| EGFR                                  | 17, 27, and 40     |
| MDM2 isoforms                         | 41                 |
| VEGF                                  | 42                 |
| Insulin-like growth factor I receptor | 43                 |
| Interleukin-6                         | 44                 |
| bFGF                                  | 45                 |
| c-myc                                 | 39                 |
| c-fos                                 | 18                 |

ation between p53 DNA-contact mutations (type I) and loss of the p53 wild-type allele has been shown in another study that involved head and neck cancer patients (32). Tumors with conformational missense mutations (type II) showed fewer losses of the wild-type allele (32). It has been described that certain p53 mutants (p53-Ser<sup>151</sup>, p53-Ile<sup>247</sup>, p53-Pro<sup>273</sup>, and p53-Leu<sup>273</sup>) can drive the cotranslated wild-type p53 into the mutant phenotypic form (37). These data indicate that the mutation type of the p53 gene may determine whether loss of the remaining wild-type p53 allele is necessary for tumor growth.

### Genes Induced by Gain-of-Function Mutants

There are indications that all of the gain-of-function effects are mediated by indirect and direct increased expression of a diversity of genes (Table 2; Refs. 15, 17, 18, 27, and 38–45). Kawamura *et al.* (18), Lanyi *et al.* (27), and Deb *et al.* (38)

showed that some p53 mutants (p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, p53-Leu<sup>273</sup>, and p53-Gly<sup>281</sup>) activated the proliferating cell nuclear antigen gene.

Gain-of-function mutants can exert mitogenic functions by stimulation of growth factors or growth factor receptors. The *EGFR*<sup>2</sup> gene was found to be up-regulated by certain p53 mutants (p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, and p53-Gly<sup>281</sup>; Refs. 27 and 40). Ludes-Meyers *et al.* (40) showed that the human EGFR promoter is also activated slightly by wild-type p53. However, the EGFR promoter sequence requirements for transactivation by wild-type p53 are different from those for transactivation by certain frequent tumor-derived p53 mutants (p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, and

<sup>2</sup> The abbreviations used are: EGFR, epidermal growth factor receptor; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; MDR, multiple drug resistance; MBP, mutant p53-binding protein.



p53-Gly<sup>281</sup>; Ref. 40). It was shown that wild-type p53 repressed gene expression of the bFGF *in vitro* and that p53-Ala<sup>143</sup> activated *bFGF* gene expression (45). Identical results were found by others for insulin-like growth factor I receptor (43) and interleukin 6 (44) using the following p53 mutants: (a) p53-Ala<sup>143</sup>, p53-Trp<sup>248</sup>, and p53-His<sup>273</sup> (43); and (b) p53-Val<sup>135</sup> and p53-Phe<sup>132</sup> (44), respectively.

In transfection assays, the murine p53 mutant p53-Val<sup>135</sup> and the human mutant p53-His<sup>175</sup> induced expression of VEGF mRNA and protein by activation of protein kinase C (42). VEGF is a pivotal mediator of tumor neoangiogenesis that is a prerequisite for tumor growth (46).

Mutant p53 (p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, and p53-Gly<sup>281</sup>) up-regulated *c-myc* *in vitro*, whereas wild-type p53 repressed *c-myc* expression (39). This study also showed that wild-type p53 served as a dominant negative regulator of this gain of function, and Frazier *et al.* (39) suggested that the wild-type p53 allele must be deleted to exert the full tumor-promoting function of mutant p53. The investigated mutants p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-His<sup>273</sup>, and p53-Leu<sup>273</sup> also up-regulated *c-fos*, whereas wild-type p53 repressed it (18).

Certain MDM2 protein isoforms were overexpressed in lung carcinomas coexpressing MDM2 protein and p53 mutants (41). Several p53 mutants (p53-Phe<sup>157</sup>, p53-Cys<sup>234</sup>, and p53-Thr<sup>209</sup>) were transfected thereafter in the p53-null lung adenocarcinoma cell line H1299 and investigated. DNA-binding experiments revealed that these mutants retained their DNA-binding properties and that the MDM2 isoforms were caused by increased transcription regulated by mutant p53, and not by *MDM2* gene amplification (41). It was suggested that the MDM2 p90-isoform exerts oncogenic functions by either interacting with E2F1 or interacting with the p53 mutant itself (47).

In both p53-lacking human osteosarcoma SAOS-2 cells and murine fibroblast (10<sup>3</sup>) cells, the gain-of-function mutants p53-Ala<sup>143</sup>, p53-His<sup>175</sup>, p53-Trp<sup>248</sup>, p53-Ser<sup>249</sup>, p53-His<sup>273</sup>, and p53-Gly<sup>281</sup> induced the *MDR-1* gene (15, 17, 27, 39), whereas p53-Pro<sup>156</sup> and a p53-Asn<sup>132</sup> did not induce the *MDR-1* gene; they even repressed the *MDR-1* gene (15). The *MDR-1* gene encodes P-glycoproteins that are membrane proteins of the ABC superfamily of transporters that can extrude chemotherapeutic drugs under an ATP-dependent mechanism (48). One can imagine that transcription of the *MDR-1* gene by p53 mutants may lead to chemotherapy resistance. Transfection of the wild-type p53-containing A2780 ovarian carcinoma cell line with mutant p53-Ala<sup>143</sup> resulted in enhanced resistance to irradiation, cisplatin, doxorubicin, and cytarabine (49). Moreover, p53-His<sup>175</sup> as well as p53-His<sup>179</sup> substantially reduced the rate of etoposide-induced apoptosis in p53-null H1299 cells, whereas p53-His<sup>273</sup> induced a higher resistance to cisplatin (25). More data about chemotherapy resistance in tumors with p53 mutants are reviewed by Weller (50) and Peller (51).

Apart from the induction of gene activities, gain-of-function mutants can have other, additional effects. Certain mutants (p53-Trp<sup>248</sup>, p53-Ser<sup>249</sup>, and p53-His<sup>273</sup>) enhanced the frequency of mutations in the T-cell receptor after graded doses of X-radiation compared with p53-null parental and wild-type-p53-expressing normal lymphocytes (52). Iwamoto *et al.* (53) showed that after transfection of lymphocytes with a temperature-sensitive p53 mutant, the T-cell receptor was no longer

expressed on the cell surface by an affected methylation 5' to the T-cell receptor  $\beta$ -chain gene enhancer region.

## Mechanisms for the Gain-of-Function Activity of p53

The investigation of the mechanism for gain-of-function activities by p53 mutants is complex. It has been shown that not all gain-of-function mutants can increase the transcription of the same genes (18, 27). Gain-of-function mutants appear to be promoter and cell-type dependent, probably because of differences in coregulatory proteins (27, 54, 55). Furthermore, p53 is a member of an emerging protein family, so other proteins may compensate for loss of p53 activity in some settings (56). To make the mechanism for gain-of-function even more complex, the function of wild-type p53 may be abrogated by alterations in regulator proteins such as ARF (57), MDM2 (58), or ING1 (59). Nevertheless, several studies have investigated the mechanism of gain-of-function.

The COOH terminus of p53 was shown to be dispensable for wild-type p53 in transactivation and growth suppression but was strictly required for mutant p53 to exert gain of function. Deletion of parts of the COOH terminus of mutant p53-Gly<sup>281</sup> influenced the gain-of-function expression of EGFR (27, 40), MDR-1 (27, 40, 60), and *c-myc* (39). It also inhibited tumor formation in nude mice (27, 60). Deletion of the extreme COOH-terminal part of p53-His<sup>175</sup> interfered with antiapoptotic activity (23). Furthermore, Lin *et al.* (60) showed that in addition to the COOH-terminal domain, two hydrophobic amino acids in the NH<sub>2</sub>-terminal domain of the p53 protein (p53-Gly<sup>281</sup>) were also required to obtain gain-of-function activities.

Moreover, the few p53 mutants with alterations in the tetramerization domain that were detected in tumors were not oncogenic, were not dominant negative, and did not stimulate expression of the *MDR-1* gene (61). Frazier *et al.* (39) showed with p53-Gly<sup>281</sup> that the last 13 COOH-terminal amino acids are dispensable and that amino acids 372–280 are critical to maintain gain-of-function in transcription of *c-myc*. The orientation and position of sequences within exon 1 of *c-myc* were important for the transactivation of *c-myc* by the p53 mutant, and the authors suggested that a RNA-dependent mechanism might be involved in this transactivation.

Four different mechanisms have been suggested for gain-of-function activity of p53 mutants, as shown below.

(a) The half-life of mutant p53 protein is increased, and among others, MDM2 degradation regulation is disturbed (10). Because p53 can also regulate genes by other, less efficient mechanisms than binding to its specific DNA consensus sequence, an increase in p53 protein means that the possibility of gene regulation without such a consensus sequence is increased.

(b) When p53 contains a different conformation caused by certain missense mutations, p53 can interact with several other cellular proteins such as p38, p42, (15), and MBP1, which is a member from the fibulin gene family (62). These interactions might be facilitated by the creation of a novel interaction site by mutation or disruption of an interaction with another cellular protein that normally blocks the interaction. The functions of p38 and p42 are unknown, but when MBP1 is ectopically overexpressed in p53-null H1299 cells transfected with p53-

His<sup>175</sup>, the rate of neoplastic transformation is greatly enhanced (62). MBP1 binds preferentially to p53 mutants of the “structural” rather than “contact” class, with the following order of specificity toward different p53 mutants: p53-His<sup>175</sup> > p53-Gly<sup>281</sup> > p53-His<sup>273</sup> ≥ p53-Trp<sup>248</sup> > wild-type p53. This suggests that the possibility of binding to other proteins plays a role in determining whether a gain-of-function effect is seen with certain mutants, which also explains the fact that some functional gains are cell-type dependent.

(c) Mutant p53 (MethA mutant p53 and other mutants) can bind MAR/SAR DNA elements (16). These elements can regulate gene expression, initiate DNA replication, initiate DNA recombination, and initiate apoptosis (63). The binding of MAR/SAR DNA elements is achieved by the presence of the normal non-specific DNA binding domain and a mutation in the core domain of p53 (16).

(d) Some mutant forms of p53 (p53-Ser<sup>239</sup>, p53-Ser<sup>245</sup>, and p53-His<sup>273</sup>) are able to enhance the activation of human topoisomerase I but are deficient for transcription and growth inhibition (64). They can bind with their COOH-terminal amino acids to topoisomerase I. Topoisomerase I belongs to a group of enzymes involved in the release of topological stress along the DNA double helix. Topoisomerases are necessary for nuclear metabolism and participate in a variety of processes involving the separation and reannealing of DNA including replication, transcription, and DNA repair (65). Activity of topoisomerase I leads to genetic instability by stimulating nonhomologous recombination (66). It was suggested that these gain-of-function p53 mutants contribute to tumorigenicity by stimulating this recombination and transcriptional activity of topoisomerase I (64).

One of these mechanisms may be the most important mechanism *in vivo*, but it is more likely that a combination of these mechanisms plays a role in the existence of functional gains of p53 mutants.

### Relevance for Cancer Research

Apparently, every p53 mutation confers different biological properties that seem to influence the prognostic significance of mutated p53 in tumors. Several studies on prognostic significance and/or therapeutic outcome that have concentrated on the discrimination between types of p53 mutants are discussed below.

In a study on non-small cell lung carcinomas, investigators showed that p53 missense mutations (type I and II) rather than null mutations (type III) were associated with poor prognosis (67). However, another study showed poor outcome for patients with this type of cancer and p53-null mutations rather than the patients with p53 missense mutations (68). Mutations in exon 8, lying in the H2  $\alpha$ -helix (residues 278–286), predicted a worse overall survival for both non-small cell lung adenocarcinoma patients and squamous cell carcinoma patients in a third study (69).

Breast tumors with missense mutations in the L2 and L3 loops from the zinc-binding domain of p53 were found to be associated with decreased disease-free and overall survival of the patients (70). These types of mutations were also found to be associated with resistance to doxorubicin therapy and early

relapse in breast cancer patients (71). Other investigators showed that mutations in residues of p53 that directly contact DNA predict a poor outcome in breast cancer (72). In another study on breast cancer, only p53 mutations in the evolutionary conserved regions II (all deletions) and V (all point mutations) were associated with a significantly worse prognosis. Adjuvant systemic therapy, especially with tamoxifen, along with radiotherapy seemed to have less effect on lymph node-positive tumors with these mutations (73).

A somewhat different kind of observation comes from a study in which BRCA-associated breast cancers exhibit a higher incidence of p53 mutations than sporadic breast cancers. The identified p53 mutants in these BRCA-associated cancers have not been described in other cancers. They have retained the wild-type p53 activities of transactivation, growth suppression, and apoptosis induction; however, they fail to suppress transformation and show enhanced tumorigenicity in rat embryo fibroblasts (74).

In colorectal cancers, it was observed that tumors with p53 mutations in the conserved regions of the gene were poorly differentiated and more aggressive than those with other mutations (75). Another group showed that all colorectal cancer patients with p53 mutations affecting the L3 domain of the protein involved in zinc binding had a shorter cancer-related survival (76).

In contrast to other mutations, p53 DNA contact mutations were shown to result in an accelerated tumor progression and reduced therapeutic responsiveness in head and neck cancer patients (32).

Overall survival for women with ovarian cancer containing p53 mutations in loop 2, loop 3, and the loop-sheet-helix domains together was significantly shorter than the overall survival for women with ovarian cancer containing other p53 mutations (77). In contrast, in another study, it was shown that ovarian cancers with p53-null mutations were more likely to be associated with lymph node metastasis, advanced stage, and high grade at presentation. These tumors progressed with distant metastasis more swiftly than did tumors with either missense mutations or wild-type p53 (78).

From these data, it can be concluded that the type of p53 mutation and its resulting biological function may result in a more aggressive or more treatment-resistant tumor phenotype. Although discrepancies can occur even between correlation studies that do distinguish between different types of p53 mutants, it is evident that discrimination between different types of mutations is necessary to be able to draw conclusions from these kinds of studies.

### Gain-of-Function Mutants and Future p53 Therapy

In addition to prognostic significance, it is also important to know whether tumors with different types of p53 mutations require other therapeutic approaches. The presence of a mutant form of the tumor suppressor gene *p53* has the potential to disrupt the apoptosis pathway and cell cycle arrest after DNA damage, which results in increased radiation resistance and cell survival. Promising studies using wild-type p53 adenoviral or retroviral gene transfer in tumors are ongoing. The transferred wild-type p53 can induce apoptosis or sensitize the tumor cells

to radiotherapy or chemotherapy (79–82). However, because gain-of-function mutants exert additional oncogenic functions, wild-type *p53* gene transfer might not work in these tumors, and the malignant phenotype may be maintained.

A *p53* antisense therapy (83, 84) that blocks the function of the *p53* mutant might be more useful for tumors with gain-of-function mutants. Other promising therapies that might interfere with the different properties of the gain-of-function mutants are structure-based rescue therapies (85).

A synthetic 22-mer peptide (peptide 46) derived from the COOH-terminal domain could restore the growth suppressor function of both DNA contact *p53* mutant proteins (*p53*-Ala<sup>143</sup>, *p53*-His<sup>175</sup>, *p53*-Ser<sup>249</sup>) and structural *p53* mutant proteins (*p53*-Trp<sup>248</sup> and *p53*-His<sup>273</sup>) in human tumor cells by binding within the core and COOH-terminal domains of *p53* (86). The authors suggested that the sequence-specific DNA binding of the tumor-derived *p53* mutant core domain was restored through displacement of the negative regulatory COOH-terminal domain. Furthermore, stabilization of the core domain structure and/or establishment of novel DNA contacts may have contributed to the reactivation of mutant *p53*.

Two other pharmacological compounds, small molecules named CP-257042 and CP31398, have been tested for rescue of the mutant *p53* conformation and its function (87). These compounds promoted conformational stability of the core DNA binding domain of both wild-type *p53* and *p53* mutants (*p53*-His<sup>175</sup>, *p53*-Ser<sup>249</sup>, *p53*-Ala<sup>173</sup>, and *p53*-His<sup>273</sup>) and restored the function of the *p53* mutants. When CP31398 was tested in nude mice, the tumor growth of the colon carcinoma cell line (mutated at *p53* position 241) was completely inhibited, and the growth of the A375.S2 melanoma cell line (mutated at *p53* position 249) was inhibited by 75%.

Anti-*p53* single chain Fv fragments are another novel class of molecules for *p53*-based cancer therapy. Both scFvs Pab421 and 11D3 restored the transcriptional activity of *p53*-His<sup>273</sup> in *p53*-null human tumor cells (88).

To be able to successfully apply any of these novel *p53* therapies in the future, it is necessary to determine the type of *p53* mutation. For transfer of wild-type *p53* into the tumor, it is important that mutants do not possess gain-of-function capacities, and more wild-type *p53* will be necessary if the mutant confers negative domination. Knowledge of the type of *p53* mutation is also important for a therapy that rescues the conformation and function of the mutant *p53*. In contrast to missense mutations, null mutations cannot be rescued. Moreover, recognition of a tumor with a gain-of-function *p53* mutant or a dominant negative mutant might also be helpful in predicting possible chemotherapy or radiotherapy resistance.

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