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REVIEW

Transcription regulation by mutant p53

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In addition to the loss of wild-type p53 activity, a high percentage of tumor cells accumulate mutant p53 protein isoforms. Whereas the hallmark of the wild-type p53 is its tumor suppressor activities, tumor-associated mutant p53 proteins acquire novel functions enabling them to promote a large spectrum of cancer phenotypes. During the last years, it became clear that tumor-associated mutant p53 proteins are not only distinct from the wild-type p53, but they also represent a heterogeneous population of proteins with a variety of structure–function features. One of the major mechanisms underlying mutant p53 gain of function is the ability to regulate gene expression. Although a large number of specific target genes were identified, the molecular basis for this regulation is not fully elucidated. This review describes the present knowledge about the transcriptional activities of mutant p53 and the mechanisms that might underlie its target gene specificity.
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Introduction

The p53 tumor suppressor serves as one of the major cellular barriers against cancer development. Indeed, in almost all human cancers, the p53 pathway is impaired (Vogelstein *et al*., 2000). About half of the tumors sustain mutations in the *TP53* gene itself, whereas the other half maintain a wild-type *TP53* gene but acquire other genetic or epigenetic alterations that compromise the p53 response. Most of the mutations within the *TP53* gene are missense mutations, resulting in the expression of full-length mutant p53 (mutp53) proteins (Hussain and Harris, 1998). This is quite unique as most other tumor suppressor genes are frequently inactivated by frame shift or nonsense mutations leading to either production of truncated proteins or complete elimination of the corresponding gene products. Moreover, the mutated p53 proteins become highly expressed in human cancers, reaching levels far above those observed in normal cells expressing wild-type p53 (wtp53). This suggests the existence of a strong selection for mutant p53 overexpression in carcinogenesis, making the investigation of its contribution to malignant processes crucial to understand better cancer and facilitate the development of novel therapies.

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Discovery of *TP53* mutations in cancer

p53 was first discovered in 1979 as an SV40-binding protein (Chang *et al*., 1979; Kress *et al*., 1979; Lane and Crawford, 1979; Linzer and Levine, 1979). The first reports indicated that the protein is prevalent in many transformed cell lines, unlike normal cells where its expression is low to undetectable (DeLeo *et al*., 1979; Kress *et al*., 1979; Lane and Crawford, 1979; Linzer *et al*., 1979; Rotter *et al*., 1980). This difference was ascribed to the short half-life of p53 in normal cells and its stabilization in transformed cells (Oren *et al*., 1982). The tight positive correlation between p53 protein levels and transformation seemingly supported the notion of p53 as a transformation-associated protein or a proto-oncogene. This notion was further enhanced by subsequent studies that demonstrated upregulation of p53 expression in proliferating cells (Milner and Milner, 1981; Mercer *et al*., 1982; Milner, 1984; Reich and Levine, 1984).

The molecular cloning of the *TP53* gene (Zakut-Houri *et al*., 1983; Zakut-Houri *et al*., 1985) further enabled the understanding of p53 structure and function. Exogenous expression of cloned *TP53* was shown to immortalize cells (Jenkins *et al*., 1984) and endow them with overt tumorigenic potential in mice (Wolf *et al*., 1984; Eliyahu *et al*., 1985). Furthermore, cloned *TP53* could co-operate with Ha-ras to transform normal rat embryonic fibroblasts (Eliyahu *et al*., 1984; Parada *et al*., 1984). All this evidence strengthened the notion that p53 possesses cancer-promoting properties. In parallel, it was reported that p53 proteins from transformed and normal cells react differently with a set of conformation-specific monoclonal antibodies (Milner and Cook, 1986), suggesting that the conformation of p53 might vary between normal and cancer cells, perhaps accounting for the different protein stabilities.

However, elucidation of the sequence of mouse wt *TP53* gene (Eliyahu *et al*., 1988; Finlay *et al*., 1988) revealed that all the experiments discussed above, utilizing cloned *TP53*, actually employed mutant variants of the protein. Such mutations endowed the

protein with the abilities to immortalize cells, bind the heat shock protein hsp70 and react with specific monoclonal antibodies (Jenkins *et al*., 1985; Hinds *et al*., 1987; Ben David *et al*., 1988; Finlay *et al*., 1988). Thus, the available data did not provide any answer to the question whether authentic wtp53 indeed contributes positively to cancer. In parallel, evidence was obtained showing inactivation of the *TP53* gene in transformed lymphoid cell lines induced by Ab-MuLV virus. Likewise, *TP53* gene inactivation and frequent rearrangements were shown in mouse spleen tumors induced by the Friend erythroleukaemia virus (Mowat *et al*., 1985; Chow *et al*., 1987; Munroe *et al*., 1988), strongly suggesting that p53 inactivation, rather than excessive p53 activation, might promote transformation.

The suggestion was confirmed when it was shown that the wtp53 protein is devoid of any transforming activity, and that such activity is exhibited only by plasmids encoding mutant, tumor-derived forms of p53 (Eliyahu *et al*., 1988; Finlay *et al*., 1988; Hinds *et al*., 1989). Importantly, it could be demonstrated that wtp53 not only failed to transform cells, but actually actively suppressed oncogene-mediated transformation (Eliyahu *et al*., 1989; Finlay *et al*., 1989). This, along with data emanating from the study of human tumor specimens (Baker *et al*., 1989), firmly established wtp53 as a bona fide tumor suppressor rather than an oncogene.

Equipped with this novel understanding, many experiments performed during the first years of p53 research could be reinterpreted, establishing the basis for elucidation of the various roles of p53 in carcinogenesis. Several cancer-related processes could be discerned: (1) p53 loss of function – wtp53 can exert growth-inhibitory effects on cancer cells, effects that are abrogated when the *TP53* gene undergoes mutations or complete loss in the course of tumor development. (2) Mutp53 Dominant-negative function – Mutp53 expression in cells harboring wtp53 may enable the mutant protein to inactivate the endogenous wtp53 and overcome its tumor-suppressive functions, therefore promoting tumorigenesis. (3) Mutp53 gain of function (GOF) – expression of mutp53 in cells lacking wtp53 enhances their tumorigenic potential (Wolf *et al*., 1984), whereas its downregulation (e.g. by antisense RNA) results in a slower rate of DNA synthesis (Shohat *et al*., 1987), indicating a wtp53-independent tumor-promoting activity of mutp53.

Properties of cancer-associated mutant p53 proteins

Analysis of a large number of human cancer cases has established that about half of all these cases harbor p53 mutations, about 80% of which are missense mutations; of those, close to 97% occur within the sequence-specific DNA binding (SSDB) domain of p53. Practically, each of the amino acids comprising this domain can serve as a site for mutation in cancer. However, the distribution is not random: 6 mutational hot spots could be identified, which together account for about 40% of all *TP53* missense mutations. Essentially, all tumor-associated DBD mutations compromise the ability of p53 to bind with high affinity to specific DNA sequences. Structurally, these mutations can be roughly divided into two main classes: those that alter amino acid residues responsible for forming sequence-specific contacts with DNA (DNA contact mutants), and those that disrupt the global conformation of p53 (conformational or structural mutants). The diversity of *TP53* mutations found in human cancer is intriguing, and may be explained by the flexibility of the DNA-binding core domain of p53, which can adopt at least five thermodynamic states (Bullock and Fersht, 2001). Functionally, *TP53* mutations result in loss of wtp53 tumor suppressor activities, acquisition of an ability to suppress the function of the remaining wild-type *TP53* allele via a dominant-negative mechanism, and at least in some cases also wtp53-independent gain of oncogenic function. Different mutations may vary with regard to their contribution to each of these three outcomes. Generally, the more the mutation disrupts the original wild-type conformation, the less wtp53 activity will be retained, and the more likely it is that new oncogenic functions will prevail. Furthermore, as wtp53 has very diverse activities, including some that may help cell survival through increased capacity to cope with stress, mutants that retain only those particular activities of wtp53 while having lost the other ones may also contribute to cancer. The heterogeneity of p53 mutants is discussed extensively in a review by Soussi and Lozano (2005).

During the process of carcinogenesis, *TP53* mutations mostly arise sporadically in one allele, resulting in cells expressing both wild-type and mutp53, where the latter might suppress the tumor suppressor activities of the former by oligomerizing with it through the C-terminal tetramerization domain (reviewed in Sigal and Rotter, 2000). Eventually, in the course of tumor progression, the remaining wt *TP53* allele is often lost (mostly by deletion), further enhancing tumorigenesis. Analysis of Li-Fraumeni syndrome family members, heterozygous for germline *TP53* mutations, as well as mouse models trying to mimic this syndrome, have revealed a high propensity to develop a broad spectrum of tumors at an early age. Most of the arising tumors were found to express the mutant allele only, implying a selection towards losing the remaining wt *TP53* allele in the process (Trkova *et al*., 2003; Olive *et al*., 2004). This situation prevails also in most mutp53-expressing sporadic human tumors analysed.

This review focuses primarily on mutp53 gain of new functions, as determined in experimental systems where mutp53 is expressed on a p53-null background, where clear wtp53-independent mutp53 activities can be demonstrated.

Oncogenic activities of mutp53

The first evidence for mutp53 GOF was obtained in experiments, where expression of mutp53 in p53-null murine L-12 pre-B cells endowed them with the ability

to elicit lethal tumors in syngeneic mice (Wolf *et al*., 1984). Several cancer-associated mutp53 isoforms were later shown to confer augmented tumorigenic potential in mice when overexpressed in a variety of cell types, such as p53-null mouse fibroblasts, p53-null human osteosarcoma cells (Dittmer *et al*., 1993; Lanyi *et al*., 1998), and T-cell acute lymphoblastic leukemia cells (Hsiao *et al*., 1994). Analysis of L-12-derived tumors revealed that mutp53 interfered with cell differentiation (Shaulsky *et al*., 1991), a phenomenon closely associated with tumor progression. Analysis of additional cell systems exogenously expressing mutp53 further demonstrated the ability of mutp53 to promote cancer-supporting phenotypes. Mutp53 was shown to increase genomic instability in Li-Fraumeni syndrome-derived fibroblasts in conjunction with disruption of the mitotic spindle checkpoint (Gualberto *et al*., 1998), in Jurkat cells following X-irradiation as measured by altered T-cell receptor surface expression (Iwamoto *et al*., 1996), in mammary mouse fibroblasts following ultraviolet (UV) and ionizing radiation (IR) as reflected by aberrant centrosome numbers (Murphy *et al*., 2000) and in Saos-2 human osteosarcoma cells as assessed by gene amplification (El-Hizawi *et al*., 2002). Mutp53 was also shown to enhance colony formation when overexpressed in p53-null mouse fibroblasts and human lung cancer-derived cells (Murphy *et al*., 2000; Deb *et al*., 2002; Weisz *et al*., 2004; Scian *et al*., 2004a). Furthermore, exogenous mutp53 was found to enhance the growth rate of such cells (Deb *et al*., 2002; Scian *et al*., 2004b). Particular attention was devoted to the ability of mutp53 to impinge upon apoptotic pathways, primarily as this might be important in the context of efficient killing of tumor cells by chemotherapy and radiotherapy. Here, mutp53 gain of oncogenic function was manifested as the ability of various p53 mutants to interfere with apoptotic cell death upon treatment with various stress inducers, including growth factor deprivation and genotoxic agents such as IR, UV radiation, cisplatin, etoposide, doxorubicin, and α-amanitin (Peled *et al*., 1996; Li *et al*., 1998; Blandino *et al*., 1999; Murphy *et al*., 2000; Matas *et al*., 2001; Sigal *et al*., 2001; Yap *et al*., 2004). Mutp53 was also reported to protect hepatocytes from a combination of HBV-X protein and TNFα (Lee *et al*., 2000). Moreover, Li-Fraumeni syndrome-derived fibroblasts, endogenously expressing mutp53, exhibited increased resistance to apoptosis in response to mitomycin C, UV and IR, relative to p53-null fibroblasts (Gualberto *et al*., 1998).

More recently, the advent of RNA interference technology enabled a critical assessment of the role of endogenous mutp53 in tumor-derived cells harboring natural p53 mutations, presumably due to positive selection during the course of tumor progression. Such cancer cells could now be analysed for the relevance of their endogenous mutp53 to cancerous phenotypes. Indeed, consistent with the conclusions drawn from the analysis of transfected mutp53, it could be shown that cancer-derived cells depend on their mutp53 expression for enhanced proliferation, basal and stress-induced survival (Olive *et al*., 2004; Weisz *et al*., 2004; Bossi *et al*., 2006; Di Agostino *et al*., 2006), increased DNA-damage-induced DNA synthesis (Di Agostino *et al*., 2006), and the ability to form tumors in mice (Bossi *et al*., 2006). These studies were significantly strengthened by establishing mouse models where mutp53 is expressed from its endogenous locus. Knock-in mice expressing p53 mutant displayed an altered tumor spectrum as compared to p53-null mice, with increased incidence of more aggressive and metastatic tumors (Lang *et al*., 2004; Olive *et al*., 2004). Analysis of mouse embryonic fibroblasts (MEFs) from these mice revealed an enhanced growth rate of mutp53-expressing cells, relative to p53-null cells. Moreover, MEFs from mutp53-expressing mice were more readily transformed by oncogenic Ras than MEFs from p53-null mice (Lang *et al*., 2004). In agreement with the observed survival advantage conferred by mutp53 upon cancer cells in experimental *in vitro* and *in vivo* models, analysis of breast and colon cancer clinical data has shown that patients whose tumors harbor certain missense p53 mutations tend to have a poorer prognosis and higher resistance to chemotherapy than those that do not express any p53 protein (Soussi and Beroud, 2001).

Together, all these studies are indicative of a positive contribution of cancer-associated p53 mutants to carcinogenesis. This generalization agrees with the high prevalence of mutp53 expression in cancer and the theory of cells retaining an oncogenic protein that confers a survival advantage to the developing tumor. However, one must bear in mind that these conclusions are largely based on experimental models analyzing only several common mutations. Even though this small set of hot-spot mutations contains both structural and DNA contact mutants, it is still possible that they do not represent all cancer-associated p53 mutants. This becomes particularly important if one tries to use mutp53 status as a guideline for personalized cancer therapy. Therefore, it will eventually be important to extend the functional analysis to a larger and more diverse set of cancer-associated p53 mutants, and particularly to identify mutants that vary with regard to their impact on tumor biology.

Mechanisms of p53 GOF

Two optional models for mutp53 GOF have been proposed. One suggests that mutp53 proteins gain new functions due to their inhibitory interactions with p53 family members, p63 and p73. These interactions were demonstrated to inhibit various tumor-suppressive activities of p63 and p73, thereby promoting carcinogenesis (Moll *et al*., 2001; Irwin *et al*., 2003; Lang *et al*., 2004; Olive *et al*., 2004). The other model assumes a more direct oncogenic role of mutp53, through regulation of the expression of a specific set of genes. Indeed, the first to be identified was the MDR-1 gene whose promoter was shown to be upregulated by mutp53 (Chin *et al*., 1992). This finding introduced the idea of

transcriptional activation as a mechanism for mutp53 oncogenic function, as MDR-1 was known to promote cancer chemoresistance. Subsequently, it was shown that the p53 281G mutant harboring additional mutations in two N-terminal amino acids (leu-22 and trp-23) required for the transcriptional activity of the wtp53 protein, was not capable anymore of transactivating the MDR-1 gene or conferring tumorigenicity to cells (Lin *et al*., 1995). These findings implied a coupling between transcriptional activation by mutp53 and its pro-oncogenic effects, suggesting that a transcriptional regulation mechanism underlies mutp53 oncogenic GOF. Further support for a role of mutp53 in gene regulation was provided by the observation that treating cells with actinomycin D, a potent transcriptional inhibitor, abolished mutp53 GOF as reflected in resistance of L12 cells to DNA damage-induced apoptosis (Li *et al*., 1998). Many subsequent studies have since then confirmed that a variety of p53 mutants can upregulate the expression of genes involved in various cellular processes implicated in cancerous progression, including growth regulation, metabolism, angiogenesis, drug resistance and genomic instability. For instance, the143A, 175H, 248W, 273H and 281G p53 mutants were shown to elevate the expression of EGFR (Ludes-Meyers *et al*., 1996), the 143A, 248W and 273H mutants were found to increase IGF-I-R expression (Werner *et al*., 1996), PCNA was reported to be transactivated by the 281G mutant (Lanyi *et al*., 1998) and c-myc by mutant 143A (Frazier *et al*., 1998; Matas *et al*., 2001), whereas 248W upregulated L37, RPP-1, and S2 ribosomal protein gene expression (Loging and Reisman, 1999), 174Y transactivated c-fos (Preuss *et al*., 2000), 125A, 248W and 249T transactivated the IGF-II gene (Lee *et al*., 2000), DUTPase was activated by 248W and 175H (Pugacheva *et al*., 2002), and 281G upregulated hsMAD1 and NFKB2 (Deb *et al*., 2002; Iwanaga and Jeang, 2002). In some of those cases, the requirement of an intact N terminus for the transcriptional and oncogenic activities of mutp53 could be demonstrated (Lanyi *et al*., 1998; Matas *et al*., 2001; Pugacheva *et al*., 2002), and in several instances, a partial contribution of the C terminus of mutp53 was also reported (Frazier *et al*., 1998; Lanyi *et al*., 1998; Deb *et al*., 2002). A functional correlation between gene regulation by mutp53 and its oncogenic activities was widely assumed, but not proven directly in these experiments. More recently, it was shown that the CD95/Fas/Apo1 gene, encoding a death receptor implicated in a variety of apoptotic responses, could be negatively regulated by mutp53 (Zalcenstein *et al*., 2003), demonstrating that mutp53 can not only transactivate genes but also repress the transcription of other genes. The downregulation of CD95 expression by mutp53 might partially account for its documented antiapoptotic effects.

The advent of expression microarrays at the beginning of the 21st century provided the possibility to perform global gene expression analysis; this, along with the development of supporting bioinformatics tools, enabled for the first time a comprehensive examination of the effects of mutp53 on the cell transcriptome. A number of studies employed this method in systems where mutp53 was exogenously expressed, including Li-Fraumeni syndrome-derived 041 fibroblasts expressing p53–175 H (Knaup and Roemer, 2004), H1299 lung cancer cells expressing p53–175H, p53–273H and p53–281G (Weisz *et al*., 2004; Scian *et al*., 2004a, b), p53-deficient HCT116 colorectal cancer cells overexpressing p53–138P and p53–175 H (O'Farrell *et al*., 2004), and U20S osteosarcoma cells harboring p53–157F, p53–175 H and p53–248Q (Mizuarai *et al*., 2006). The long lists of mutp53-regulated genes obtained by these studies included some that had already been reported previously, along with many novel mutp53 targets, unraveling the potential involvement of mutp53 in additional cellular processes such as transcriptional and translational regulation, signal transduction, cell motility, DNA repair, proteolysis and more. For some of those genes, regulation by mutp53 was confirmed also at the protein level and functional oncogenic relevance of this regulation by mutp53 was assigned; these include Cam2 (Knaup and Roemer, 2004), ASNS and hTERT (Scian *et al*., 2004a), EGR1 (Weisz *et al*., 2004), MSP (Zalcenstein *et al*., 2006), GEF-H1 (Mizuarai *et al*., 2006) and ATF3 (Buganim *et al*., 2006). These studies also allowed the comparison of the transcriptional programs of different p53 mutants, confirming that individual p53 mutants share some but not all transcriptional targets, compatible with the notion that they may possess distinct GOF phenotypes.

Some of the mutp53-regulated genes identified by these studies provided a substantial insight into the molecular mechanisms that underlie the reported biological activities of tumor-associated p53 mutants. One area in which such understanding has been gained is the antiapoptotic role of mutp53. As mentioned above, mutp53 was found to repress the CD95 promoter (Zalcenstein *et al*., 2003). As expected, this conferred increased resistance to killing by the CD95 ligand, when such ligand was experimentally added to the culture. However, downregulation of CD95 may also have broader antiapoptotic effects, as the CD95 pathway has been implicated also in maximizing death upon treatment with a variety of chemotherapy agents. Further work has revealed that downregulation of the MSP/Mst1 gene by mutp53 may also similarly contribute to increased antiapoptotic capacity. In fact, RNAi-mediated knockdown of endogenous MSP, aimed to mimic the repression of that gene by mutp53, rendered H1299 lung cancer cells more resistant to killing by DNA damaging chemotherapy (Zalcenstein *et al*., 2006). Interestingly, analysis of gene expression profiles in several types of cancer revealed lower levels of MSP RNA in the tumor as compared with the corresponding normal tissue; this was also seen in large cell lung carcinoma, the tumor type from which H1299 had been derived (Zalcenstein *et al*., 2006). Genes that are upregulated by mutp53 are also likely to play a role in conferring resistance to an apoptotic stimuli. One such example is EGR1: knockdown of EGR1 attenuated the antiapoptotic effect of mutp53 overexpression, suggesting that the elevated levels of EGR1 induced by

this overexpressed mutp53 are required for maximal protection against apoptosis in that experimental setting (Weisz *et al*., 2004).

Of particular interest is the effect of mutp53 on the activity of NF-κB. This transcription factor plays an important role in conferring an antiapoptotic state under a wide variety of conditions, including in many types of human cancers. Expression microarray analysis identified the NFKB2 gene as a target for positive transcriptional regulation by mutp53 (Scian *et al*., 2004b). This gene encodes the p100/p52 subunit of NF-κB. In line with this finding, overexpression of mutp53 was shown to increase NF-κB activity and protect cells against chemotherapy-induced death (Scian *et al*., 2005). More recently, it was found that mutp53 can also augment and prolong the activation of NF-κB by tumor necrosis factor alpha (TNF-α), in this case acting via the canonical p50-p65 NF-κB complex (L Weisz and A Damalas, unpublished). This observation is of great interest because activation of NF-κB by TNF-α has been strongly implicated in tumorigenesis, particularly in the context of chronic inflammation. Of note, a significant correlation could be demonstrated between the presence of endogenous mutp53 protein and constitutive NF-κB activation in human tumor specimens, derived from non-small lung cancer and head and neck squamous cell carcinoma (V Gorgoulis, unpublished). This, along with a growing number of additional findings, provides an example for how the transcriptional profiling of mutp53 is beginning to provide explanations for the biology of mutp53 GOF.

Mechanisms of transcriptional regulation by mutp53

The existing knowledge regarding the molecular mechanisms whereby mutp53 regulates gene expression is still lacking. One possibility is that mutp53's impact on its target gene promoters could be a consequence of its ability to bind and inactivate transcription-competent (TA) forms of the p53 family members, p63 and p73. While this may indeed hold for some promoters, it is unlikely to be the only explanation. For instance, a dominant p73-derived polypeptide, which interferes with the activity of both p73 and p63 (Y Daniely and M Oren, data not shown) had no effect on the ability of mutp53 to downregulate the CD95 gene promoter (Zalcenstein *et al*., 2003). In addition, regulation of gene expression by TA forms of p63 and p73 is expected to rely in part on the interaction of those proteins with canonical p53 response elements (p53RE); yet, many of the mutp53 target genes do not harbor recognizable p53RE. This argues in favor of a mechanism that relies on other molecular properties of mutp53, distinct from its dominant negative effects on TA isoforms of p63 and p73.

Thus, mutp53 may act as a transcription factor, capable of directly regulating specific gene expression. Such mechanism predicts that mutp53 should be found associated with at least some of its target gene promoters. Indeed, a physical interaction was shown to occur between mutp53 and the promoters of several genes reported to be regulated by it, including the genes encoding CD95, EGR1 and MSP (Zalcenstein *et al*., 2003, 2006; Weisz *et al*., 2004). Such specific interactions could be shown to occur within living cells with no need of any external inducers, implying that mutp53 is constitutively complexed with these specific promoter regions. Yet, various signals may alter the association of mutp53 with different promoters, thereby influencing selectively its biological activity; this was demonstrated for the promoters of the Cdk1 and CyclinB2 genes, where enhanced mutp53 binding was detected upon exposure to DNA damage (Di Agostino *et al*., 2006).

The question remains as to how specificity of gene regulation is achieved by mutp53 proteins that have lost their ability to bind DNA in a sequence-specific manner. At least two alternatives may be considered; the first is that mutp53 recognizes directly specific elements within the DNA; a second possibility is that mutp53 is tethered to chromatin through protein–protein interactions with one or more SSDB proteins.

So far, attempts to delineate a specific mutp53 DNA-binding sequence distinct from those regulated by wtp53 have failed, mainly owing to lack of sequence similarity between different mutp53-responsive promoters, an observation strengthened by large-scale expression micro-array experiments identifying dozens of p53 target genes that enabled statistically improved promoter sequence analysis. However, mutp53 has been reported to bind a wide range of DNA secondary structures. For example, mutp53 was shown to preferentially bind matrix attachment regions (MARs) with a high potential of base unpairing *in vitro* (Will *et al*., 1998). MARs anchor chromatin fibers to the nuclear matrix, generating chromatin domains that may enhance or repress transcription (Will *et al*., 1998). Recently, the same group analysed the *in vitro* binding of mutp53 to non-B DNA and showed that this binding is solely dependent on the stereo-specific configuration of the DNA and not on DNA sequence (Gohler *et al*., 2005). Thus, although presently but not directly associated with identified mutp53-specific target genes, it is possible that this stereo-specific binding rather than the sequence-specific binding is a mechanistic basis for the interaction of mutp53 with MAR elements. Furthermore, this might imply that chromatin remodeling may be involved in transcriptional activities mediated by mutp53.

An alternative explanation how promoter-specific activation can be mediated by mutp53 proteins is that mutp53 target promoters can be bound by other, SSDB proteins, which recruit mutp53 through protein–protein interactions. Indeed, mutp53 was shown to interact with several sequence-specific transcription factors, which possess binding sites within genes that are responsive to mutp53. One such example is Sp1 (Gualberto and Baldwin, 1995; Chicas *et al*., 2000); the interaction of mutp53 with Sp1 was shown to elicit cooperative effects and amplify the activating effects of Sp1 on transcription. Similarly, activation of the MDR-1 promoter by mutp53 was shown to require its association with

transcription factors of the Ets family (Sampath *et al*., 2001). The notion that mutp53 is tethered to specific chromatin regions via interactions with sequence-specific transcription factors is reinforced by the work of Di-Agostino *et al*. (2006) demonstrating a functionally significant interaction of mutp53 with NF-Y, an important transcription factor involved in cell-cycle progression, which binds specifically to CCAAT box elements in the DNA. In response to DNA damage, the transcriptional coactivator p300 is recruited to mutp53, leading to dismissal of histone deacetylase (HDAC)1 from mutp53-NF-Y complexes present on NF-Y target promoters, and eventually upregulation of mutp53 target genes. This is accompanied by global increase in histone acetylation and decrease in histone methylation, further suggesting that mutp53 interactions with proteins such as chromatin remodeling factors underlie transcriptional facilitation. The requirement for DNA damage in order to elicit molecular events that rely on mutp53 activity is in line with the functional experiments discussed earlier, where stress was needed in order to induce or enhance mutp53 GOF phenotypes. Additionally, it implies that at least some of the activities of mutp53 may be related to the cellular response to stress.

Owing to the complex nature of transcriptional activation by mutp53, it is conceivable that both stereo-specific DNA recognition and protein–protein interactions, along with other yet uncharacterized additional mechanisms, may underlie transcriptional regulation by mutp53.

Mutp53: role of residual wtp53 activity?

When considering the biochemical features of mutp53 that underlie GOF mechanisms, a most intuitive assumption is that they are at least partially based on those of the wtp53 protein from which the mutant isoforms are derived. The vast majority of cancer-associated p53 mutations occur within the central DBD, which mediates SSDB to p53-responsive elements (p53-RE). This results in conformational alteration of the DBD, typically leading to loss of the most prominent biochemical activity of wtp53, namely its ability to trigger sequence-specific transcriptional activation, which underlies much of wild-type p53's tumor suppressor functions. Different p53 mutants can display different degrees of impairment of their transactivation potential, reflecting the nonidentical impact of individual mutations on the overall structure of the mutant protein (Pan and Haines, 2000; Inga *et al*., 2002; Kato *et al*., 2003). Retention of residual SSDB capacity in a particular p53 mutant might result in p53-RE-dependent activation of some promoters but not others. Thus, in addition to the newly acquired ability of mutp53 to modulate the expression of genes that are not regulated at all by wtp53, imbalanced transactivation of canonical wtp53-target genes might sometimes also contribute to the GOF activity of particular p53 mutants. This is exemplified by the p53R231Q mutant, which can induce mdm2 gene expression while being defective in activating transcription of the *p21* and *PIG3* genes (Pan and Haines, 2000). The impact of such an imbalance is likely to be augmented by the relatively high abundance of the mutp53 proteins in the tumor cells, as compared to the low abundance of the wild-type protein in the corresponding normal cells. Notably, some tumors that do not harbor *TP53* gene mutations exhibit rather high levels of wtp53 protein. It is conceivable that in such tumors the wtp53 is somehow biased towards a transcriptional profile that is beneficial, rather than inhibitory, to tumor progression. A similar imbalanced phenotype might be permanently fixed by some of the cancer-associated *TP53* mutations, further enforcing the notion that residual wtp53 functions may underlie some of the biochemical properties of mutp53. This is strengthened by expression microarray analysis comparing wt and mutp53 transcriptional profiles in a human tumor-derived cell line, where a significant degree of shared regulatory activities could be detected (O'Farrell *et al*., 2004).

It is expected that residual wtp53 activity in the mutant protein is largely mediated by its N- and C-terminal domains, which remain intact at least in terms of sequence. Although significant work has been done determining the three-dimensional structure of tumor-associated mutp53 proteins DBD (Joerger *et al*., 2005, 2006), there is presently insufficient information regarding whether the DBD alterations elicit global conformational changes within the mutp53 protein, which may also affect the structure–function properties of the N- and C-terminal domains.

The p53 C terminus is responsible for non-SSDB, which is greatly DNA structure-dependent. This includes high affinity binding to double- and single-stranded DNA, secondary DNA structures, and aberrant sites in the DNA such as mismatched bases and DNA bulges (Yakovleva *et al*., 2001; Kim and Deppert, 2003). Thus it is possible that the nonSSDB activity of the p53 C terminus is also regulating the specificity of mutp53 DNA binding. This idea is supported by the finding that mutp53 DNA binding requires the same domains involved in wtp53-SSDB to non-linear DNA, namely the core domain and the C terminus (Muller *et al*., 1996; Will *et al*., 1998), as well as by the finding that these domains were necessary for the transactivation of some promoters by mutp53 (Frazier *et al*., 1998; Lanyi *et al*., 1998) and for some of mutp53 oncogenic functions (Sigal *et al*., 2001). Further support for this notion is offered by studies showing that post-translational alterations of the C terminus affects mutp53 transcriptional activities (Yap *et al*., 2004; Di Agostino *et al*., 2006). The latter may support a role for the stereo-specific DNA recognition as a molecular mechanism for the transcriptional effects of mutp53. In addition to the contribution of the C terminus to interaction with DNA, it may also play a role in mutp53 transcriptional activity through residual protein–protein interactions that are shared with the wtp53 protein.

A similar situation may also pertain with regard to the N-terminal domain of mutp53. As described above, one

of the fundamental observations leading to the notion that mutp53 acts as a transcription factor was that mutations at positions 22 and 23 of the mutp53 N terminus result in attenuation of its transcriptional activity (Lin *et al*., 1995; Matas *et al*., 2001). In the case of wtp53, it has been shown that this attenuation is because of the disruption of p53's interaction with several components of the transcriptional machinery proteins (Lin *et al*., 1994). It appears most likely that functional interactions of the wtp53 N-terminal domain with the basal transcriptional machinery are also retained in mutp53 and are required for full transcriptional potency. Indeed, several of the proteins reported to interact with mutp53 and to affect its function are known to interact also with wtp53; these include Sp1 (Bargonetti *et al*., 1997; Pastorcic and Das, 2000; Kim and Deppert, 2003), Ets-1 (Sampath *et al*., 2001), and NF-Y (Di Agostino *et al*., 2006). Interestingly, the interactions of these proteins with wtp53 often result in opposite transcriptional outcomes than those observed with mutp53. Although wtp53 inhibits Sp1-dependent activation, presumably by interfering with DNA binding by Sp1 (Bargonetti *et al*., 1997), mutp53 proteins cooperate with Sp1 and amplify its activating effects on transcription. Similarly, the physical association between wtp53 and Ets-1 is inhibitory to Ets-1 activity (Pastorcic and Das, 2000) whereas the mutp53/Ets-1 interaction potentiates Ets-1-mediated transcription (Sampath *et al*., 2001). A similar picture emerges with regard to NF-Y. Here the molecular mechanism was further elucidated, showing that upon DNA damage, binding of wtp53 to NF-Y on its target promoters leads to the recruitment of HDACs and release of histone acetyltransferases (HATs) resulting in transcriptional repression, whereas the opposite happens when mutp53 is in the complex (Di Agostino *et al*., 2006). Interestingly, unlike the mechanisms discussed earlier, transactivation by the mutp53-NF-Y complex does not require the N terminus of mutp53, implying that this activity of mutp53 utilizes other domains of the protein. In support of this, wtp53 interacts with NF-Y, p300 and HDAC1 through its C-terminal domain (Imbriano *et al*., 2005).

There are also cases where wtp53 and mutp53 employ very different interactions when regulating the same gene. For example, transcriptional regulation of the MDR-1 promoter by wtp53 and by mutp53 is mediated by different promoter regions (Sampath *et al*., 2001) and similar observations were demonstrated for CD95 (Zalcenstein *et al*., 2003), suggesting that the assembly of functionally distinct complexes may be determined by different mechanisms. Either way, it seems that the functional consequence of mutp53 presence in the complex is tumor promotion while the presence of wtp53 results in tumor suppression. This is further illustrated by the relationship of EGR1 with p53, where the presence of mutp53 or wtp53 may affect EGR1 transcriptional activities differently, resulting in shifting cell fate towards survival or death, respectively (Weisz *et al*., 2004). As increasing evidence emerges regarding mutp53 activities, it is becoming clearer that mutp53 and wtp53 are often involved in overlapping biochemical pathways and biological processes, but often they exert opposite effects on the same pathway. This might encourage the search for oncogenic mutp53 functions in pathways where wtp53 is involved as a tumor suppressor. These might include effects on chromatin remodelling, as well as the recently discovered direct apoptotic activity of wtp53 in the mitochondria.

Concluding remarks

Although significant progress has been made in identifying mutp53-regulated genes, the exact mechanism by which mutp53 affects gene expression patterns still remains to be fully elucidated, and probably involves an amalgamation of mechanisms including altered sequence specificity, protein–protein interactions and differential affinity to particular DNA structures. In view of the growing evidence for a role of mutp53 in human cancer, it will be important to gain additional insights into the molecular mode of action of tumor-associated mutp53 proteins. This may enable better targeting of mutp53 by novel anti-cancer agents, and may help identify additional new targets for therapy among those genes whose aberrant regulation by mutp53 contributes to its GOF.

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