p53: Balancing tumour suppression and implications for the clinic

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Abstract

It is well accepted today that cancer develops through a multi-step process that involves normal cells being led by well-defined phases into cancer cells. Along this process cells lose their natural cancer defence system that is mediated by tumour suppressor genes and accumulate genetic instabilities that permit the expression of the specific oncogenic networks. Remarkable is the p53 tumour suppressor which is mutated in more than 50% of human cancers. In turn, various mutant p53 proteins with an oncogenic activity are accumulated in the cells and contribute to malignancy. This chapter overviews the p53 field with respect to the history behind the discovery of the p53 tumour suppressor, the structure and function of p53, the oncogenic activities of the various p53 mutants and the clinical significance of a tailor-made p53-based gene therapy.

The history of the p53 field

In the early 1970s, scientists already understood that the malignant transformation process involved accumulation of mutations within the coding DNA that altered gene functions which were important for maintaining normal cellular function. During this process, cells lose their natural cancer defence systems mediated by tumour suppressor genes and accumulate genetic modifications that permit the onset of specific oncogenic networks. The tumour suppressor gene family consists of several members that vary in their structures and cell specificities. The phosphoprotein p53 was initially discovered in 1979 as it was immunoprecipitated, along with the tumour promoting simian virus 40 (SV-40) large T and small t antigens, using sera from mice bearing SV40-induced tumours and from uninfected murine embryonal carcinoma cell lines [1,2]. In parallel, p53 was identified using sera prepared against BALB/c Meth A sarcoma in syngeneic or compatible F1 mice [3]. These studies and others mischaracterised the cellular p53 as a transformation-related antigen because it was found to be accumulated at a high concentration in the nucleus of neoplastic transformed cell lines and to increase proliferation, whereas in normal cells it was almost undetectable [4–7]. In accordance with these observations, ectopic expression of p53 was shown to cooperate with oncogenic H-Ras^{V12} to induce transformation of normal embryonic cells and to immortalise cells with a finite lifespan [8–10]. A few years later several findings raised the possibility that p53 might act as a tumour suppressor gene rather than as an oncogene. First, it was shown that expression of mouse p53 results in a substantial and selective inhibition of SV-40 origin-dependent DNA replication. In addition, p53 was demonstrated to compete with the DNA polymerase alpha for binding to SV-40 T antigen [11–13]. Second, the coding region of the p53 gene was found to be altered by viruses such as Moloney murine leukaemia and was shown to be deleted in several human cancers [14–19]. Third, the cloning of the p53 gene in 1983 [20] enabled scientists to identify mutations within the coding region of p53 in many transformed cell lines [21–24]. It was the study of Finlay and colleagues in 1988 that raised the questions of what the correct p53 wild-type sequence is and whether a wild-type p53 gene can transform cells in culture [25]. The authors showed that one of the p53 cDNA clones (11-4 p53 cDNA clone) failed to transform primary rat fibroblasts when co-transfected with the Ras oncogene. Mutating the amino acid 158 or 215 resulted in the activation of the 11-4 p53 cDNA clone for transformation with Ras. In addition, these mutant cDNA clones produced a p53 protein that preferentially bound to a heat shock protein, hsc70, a protein that was shown to interact only with p53 proteins that could cooperate with Ras to induce transformation [24,26]. By 1989 p53 was formally crowned as a tumour suppressor gene when DNA clones of the wild-type p53 protein were able to inhibit the capability of E1A and Ras or mutant p53 and Ras-activated oncogenes to transform primary rat embryo fibroblasts. The rare clones of transformed foci that resulted from E1A and Ras plus wild-type p53 triple transfections contained mutation within

the p53 coding sequence, similar to the observed transformation-activated p53 proteins and to the p53 mutants selected in human tumours [27,28]. These findings led to the understanding that the DNA tumour viruses have evolved mechanisms to interact with the p53 negative regulator of cellular growth in order to enhance their own replication in growing cells. SV-40 and adenovirus type 5 produce viral encoded oncoproteins (large T, small t antigens and E1A) that also form oligomeric protein complexes with p53, thereby inactivating its functions [29]. Over the years, numerous publications have demonstrated diverse DNA rearrangements and hot spot mutations within the coding region of p53 in many human tumours such as colorectal carcinoma, lung, breast, oesophagus, head and neck, haematopoietic and leukaemia [21,23,30–35]. One of the most important indications for the role of p53 in cancer prevention was the observation that a germ-line transmission of a mutated p53 allele was found in a cancer-prone family bearing the Li–Fraumeni syndrome. Later on, this inherited p53 mutation was confirmed to predispose the members of the family to increased susceptibility to cancer [36–38]. The next step was to address the role of p53 in cancer prevention. A first clue for the role of p53 in maintaining genomic integrity came from the work of Remvikos and colleagues [39] as they showed that colorectal adenocarcinoma tumours that contained aneuploid cell subpopulations were also more frequently (65%) positive for mutant p53 overexpression than diploid ones (20%). This suggests a role for wild-type p53 in maintaining normal genome diplody. Another role for p53 in inhibiting cell growth was illustrated in a study describing a temperature-sensitive behaviour of a particular p53 mutant, $p53^{V135}$. This p53 mutant elicited transformation at 37.5ºC; however, at 32.5ºC it suppressed the proliferation of transformed cells, recapitulating the behaviour of wild-type p53 [40]. These data demonstrated that the ability of wild-type p53 to suppress transformation is due to induction of growth arrest. In accordance with this study, human colorectal carcinoma cell lines transfected with wild-type p53 gene formed five- to ten-fold less colonies compared to cells transfected with a mutant p53 gene. Immunocytochemical analysis demonstrated that the carcinoma cells expressing wild-type p53 gene did not progress through the cell cycle, as evident by their failure to incorporate thymidine into DNA [41]. All in all, these studies showed that the wild-type gene can specifically suppress the growth of human tumours. A third role for wild-type p53 in preventing cancer was suggested by Yonish-Rouach and colleagues [42] as they utilised the temperature-sensitive p53 mutant system to demonstrate that conversion of the mutant p53 form into a wild-type p53 conformation in a murine myeloid leukaemic cell line that normally lacks p53 resulted in rapid loss of cell viability in a way characteristic of apoptosis. The ability to eliminate cancer cells by inducing programmed cell death is one of the hallmarks of tumour suppressors.

In an attempt to decipher the manner by which p53 exerts its tumour suppressive activities, Fields and Jang, and Raycroft and colleagues, showed that a hybrid protein containing the DNA binding domain of yeast GAL4 and portions of p53 can activate transcription in both yeast and mammalian cells, indicating that p53 protein contains a transcription-activating sequence and suggesting that p53 might activate the transcription of genes that suppress cell proliferation [43,44]. Indeed, a year later p53 was shown to activate the muscle-specific creatine kinase (MCK) gene via a responsive element that resided within MCK enhancer [45]. Additionally, p53 was shown to repress the expression of genes that are needed for ongoing cell proliferation such as c-fos [46] supporting the notion that p53 is a transcription factor that provokes its anti-cancerous function by regulating gene expression.

Since then, numerous studies have implicated p53 as a conductor of various cell processes such as differentiation, development, metabolism, senescence and DNA repair [47–51]. The scientific community acknowledged the merits of p53 as it was given the prestigious title “guardian of the genome” in 1992 followed by its election as the “molecule of the year” in 1993 [52,53]. Today, p53 is considered as one of the most important tumour suppressor genes in human cancer and an attractive candidate for therapeutic design for cancer therapy.

Wild-type p53 as tumour eradicator – structure and function

The human p53 gene is located at chromosome 17p13.1 and encodes a 393 amino acid nuclear protein. The protein consists of four central domains which permit its function as a transcription factor: a loosely folded N-terminal transactivation domain (TAD), a DNA-binding domain (DBD), an oligomerisation domain (OD) and a C-terminal regulatory domain (CRD). In addition to these distinct domains, the protein contains a proline-rich domain (PRD), two nuclear export signals (NES) and three nuclear localisation signals (NLS) (Fig. 1).

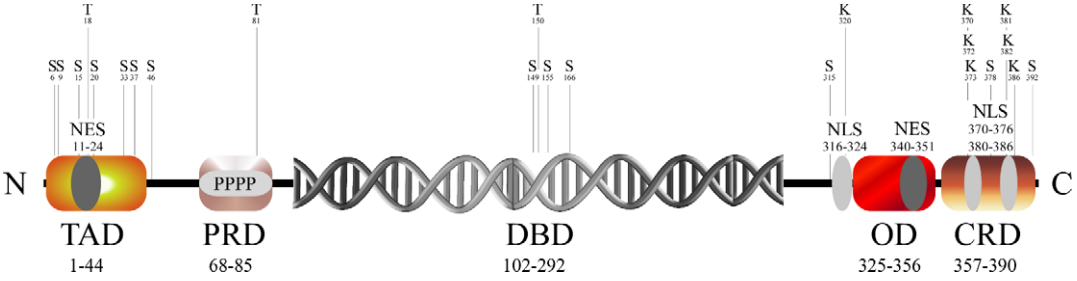

Fig. 1. A schematic representation of the p53 protein architecture. The p53 protein contains four central domains: the transactivation domain (TAD), the DNA binding domain (DBD), the oligomerisation domain (OD) and the C-terminal regulatory domain (CRD). These domains together with a proline rich domain (PRD), two nuclear export signals (NES) and three nuclear localisation signals (NLS) facilitate p53 function as transcription factor and tumour eradicator. These domains are subjected to various post translational modifications such as phosphorylation, acetylations, ubiquitinations, sumoylation, methylations and neddylations that stabilise and activate the p53 protein. Numbers indicate position in amino acids. S- Serine, T- Threonine, K- Lysine and P- Proline.

The TAD (residues 1–44) is required for both p53 transactivation capability and protein-protein interaction. This region binds basal transcriptional components from several groups: the TATA-box binding protein associated factors TAFII40, TAFII60, p300/CBP, p62 TFIIH, the p53 regulators Hdm2, SETD2, Mdmx and other proteins such as HmtSSB [54–61]. Together with the adjacent PRD (68–85 residues) TAD plays a role in p53 stability, which is regulated by the p53 negative effector Hdm2 [54]. TAD contains several phosphorylation sites at positions 6, 9, 15, 20, 33 and 37 that are modified by several protein kinases such as DNA-PK, Raf-1, p38 (MAPK), GSK3, ATM, FAK and CK1 [62–70]. These phosphorylations switch the largely folded transactivation domain to a more open conformation that interacts with transcription factors, leading to enhancement of gene expression [71]. In addition, increase in p53 stability, DNA binding capability and induction of apoptosis are related to the phosphorylated status of p53. Mutational analysis of the TAD revealed an important role for amino acids Leu-22 and Trp-23 in facilitating both protein-protein interactions and gene activation/repression [72,73]. Interestingly, although a mutant p53 bearing these mutations ($p53^{L22Q/W23S}$) is defective in transcriptional activation of numerous p53 target genes, it can induce the expression of pro-apoptotic targets including PIDD and AIP1 [74], implying the existence of several mechanisms for p53-mediated gene activation, whereby the amino acids Leu-22 and Trp-23 are essential only for specific p53 target genes. The importance of the TAD can be seen in one of the p53 isoforms, p53DeltaN. p53DeltaN is produced by internal initiation of translation at codon 40 and lacking the N-terminal first transactivation domain. The p53DeltaN is impaired in its transcriptional activation capacity and is unable to form a complex with the p53 regulatory protein Hdm2. Furthermore, p53DeltaN oligomerises with p53 and negatively regulates its transcriptional and growth-suppressive activities [75].

DBD (residues 102–292), a 191-amino-acid region that corresponds to the central portion of the p53 protein, binds DNA in a sequence specific manner [76]. Crystal structural analysis and nuclear magnetic resonance (NMR) of the p53 core domain revealed a structure of a beta sandwich that serves as a scaffold for two large loops and a loop-sheet-helix motif. The two loops are held together in part by a tetrahedrally coordinated zinc atom, and the loop-sheet-helix motif forms the DNA binding surface of p53 [77]. Structurally, the zinc ion coordinates the motions among the different protein structural elements which could also be important for optimal binding and core packing. The influence of zinc on protein stability is mainly localised to the L2 loop [78]. The DBD binds a specific consensus sequence that contains two copies of a 10-bp motif 5′-PuPuPuC(A/T)-(T/A)GPyPyPy-3′, separated by 0–13 bps (Pu and Py stand for purine and pyrimidine, respectively) [79]. However, many other non-canonical binding sites and several half sites are found to be responsive to wild-type p53 following various circumstances [80–82]. The symmetry of the two copies and the structure of the tetramerisation domain [83] have led to the understanding that p53 is a dimer of dimers.

Similar to the TAD, the DBD is also subjected to modifications such as phosphorylations, acetylations and ubiquitinations. Several proteins, among them PKA, WCE, CKIIbeta, Plk1, vIRF1 and Hdm2, elicit these modifications [84–87]. In addition to DBD modifications that improve p53 binding, cellular and viral proteins interact with the DBD of p53 and modulate its expression, binding affinity and activity. Such proteins are PARP, HIF-1α, 53BP1, 53BP2, hTRbeta1, ZBP-89, NPM, ASPP, Tip60, MAML1, Hzf, PC4, NM23-H1,

STRAP, MIF and Twist [88–100]. Reactive oxygen intermediates (ROI) regulate the DNA-binding activity of p53 as well, by modulating the redox status of a critical set of cysteines in the DNA-binding domain, involved in the coordination of zinc [101]. Interestingly, the DBD of p53 can also interact with the cytoplasmic proteins BclXL and Bcl2, targeting them to the mitochondria [102]. These data implicate p53–Bcl2 complexes in the direct mitochondrial p53 pathway of apoptosis and further support the notion that the DBD of p53 is a dual function domain, mediating both its transactivation function and its direct mitochondrial apoptotic function.

The OD (residues 325–356) is responsible for the creation of a tetramer that is found to be the predominant form of p53 in binding to the DNA [103]. Each p53 subunit recognises five nucleotides of the 20 nucleotide-long DNA binding site [104]. The domain forms a 20-kilodalton symmetric tetramer with a topology made up from a dimer of dimers. The two primary dimers each comprised of two anti-parallel helices linked by an anti-parallel beta sheet, one beta strand and one helix, are contributed from each monomer. The interface between the two dimers forming the tetramer is mediated solely by helix-helix contacts. Moreover, hydrophobic interactions within the protein core and by a number of electrostatic interactions stabilise the tetrameric structure [83,105]. The binding of Hdm2 to p53 is significantly impaired by the loss of the quaternary structure [106], further supporting the notion that the regulation of p53 activity is mainly related to the tetrameric structure. The phosphorylation state of p53, specifically at serine S392 and to a lesser extent serines S315 and S378, is important to the tetrameric creation [107,108]. Several proteins interact with the OD of p53 and affect its tetrameric structure including the S100B Ca^{2+}-binding protein, PARP, HMGA1 and TDG [97,109–111]. In addition, deletion or mutation of p53's OD markedly impairs the ability of p53 to oligomerise with BAK. Mouse embryo fibroblasts from BAK null mice have greatly reduced mitochondrial p53 compared to wild-type fibroblasts, highlighting the importance of the p53–BAK interaction in the localisation of p53 to mitochondria [112]. The contribution of the OD to cancer suppression is reinforced by the fact that Li–Fraumeni families with a germ-line mutation in the p53 gene, which may result in a mutated OD or its entire loss, are segregated with the cancer phenotype [113,114].

The CRD (residues 357–390) contains various posttranslational modification sites, such as phosphorylation, ubiquitination, acetylation, sumoylation and methylation, which control p53 stabilisation and activation. For example, casein kinase II phosphorylates the C-terminal serine of p53 (residue 389 for murine p53) and hence increases the capability of p53 to suppress cell growth [115]. The co-factors p300/CBP/PCAF acetylate the CRD and stimulate p53 sequence-specific DNA-binding activity, possibly as a result of an acetylation-induced conformational change [116–119]. S100B inhibits phosphorylation of the p53 CRD in a Ca^{2+}-dependent manner and thereby regulates its activity [120]. Several other proteins are suggested to bind to the CRD and to regulate p53 responses; these include Ck2, Mts1, PIAS1, nucleolin, hGTSE-1, GSK3β, ERα and JAZ [121–128]. Interaction of CRD with DNA ends generated after DNA damage causes activation of sequence-specific p53 DNA binding and may thus provide a molecular link between DNA damage and p53-mediated growth arrest and apoptosis [129]. In addition, the C-terminal tail of p53 is also considered as a negative regulator of p53 activity because it can lock the DBD and the TAD in a latent conformation. The overlap of the regulatory region in CRD, which is rich in basic amino acids, with the second DNA-binding site in the p53 molecule, is the key feature of the steric model [130,131]. Upon CRD posttranslational modifications the DBD is released from the C-terminal tail and becomes active [132–134]. Additionally, non-specific DNA sequences do not directly inhibit the binding of the p53 consensus DNA elements to the DBD of p53 but rather bind to the CRD and thereby inhibit p53 activity [135,136]. This data support that notion that the CRD plays an essential role in mediating p53 transcriptional activity.

p53 is considered to be one of the most powerful tumour eradicators because of its involvement in numerous anti-cancerous processes (Fig. 2). This includes cell cycle arrest, apoptosis, differentiation, senescence and DNA repair [42,47,49,50,137,138]. Moreover, the fact that p53 is stabilised and activated upon diverse stress signals induced by DNA damage, hypoxia, oncogenic stress, deprivation of nutrients and angiogenesis accentuates the role of p53 as a critical guardian of cell stability [47,137,139–142]. Accordingly, the role of p53 in cancer prevention is mainly related to its ability to transactivate a great number of specific targets involved in several gene networks. For example, the well studied p53 target genes $p21^{WAF1}$, *GADD45* and *14,3,3 sigma* are activated transcriptionally by p53 in response to DNA damage and regulate cell cycle checkpoints [143–145]. Alternatively, p53 can transactivate different targets such as *BAX*, *PUMA*, *Noxa*, and *DR5* leading

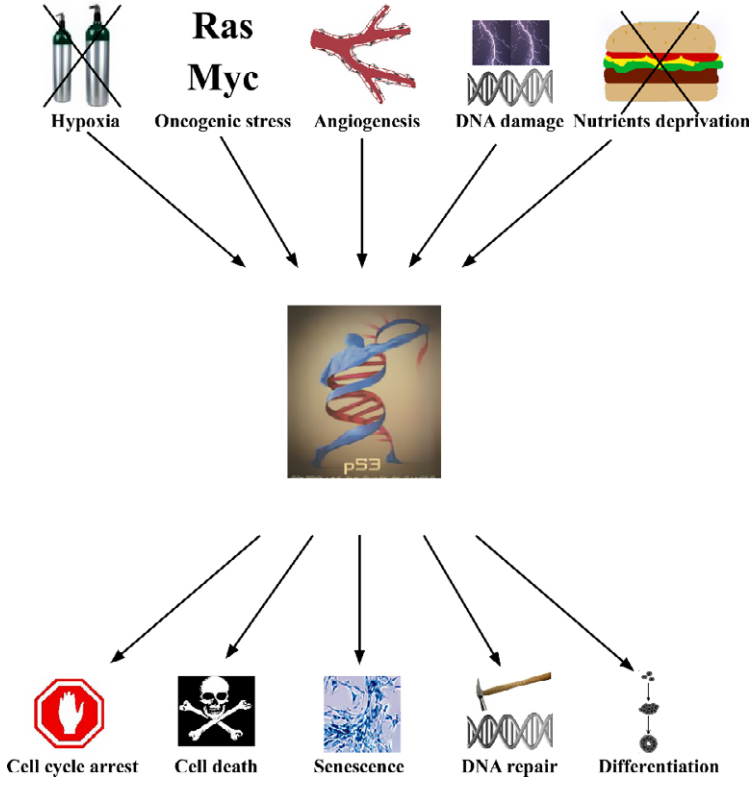

Fig. 2. The p53 activation signals and their p53-dependent outcomes. The tumour suppressor p53 is stabilised and activated by several stress pathways (upper panel). This includes lack of oxygen (hypoxia), activation of oncogenes such as myc and Ras (oncogenic stress), massive blood vessel creation (angiogenesis), DNA mutagenic agents such as cisplatinum and doxorubicin (DNA damage) and lack of nutrients (nutrients deprivation). The outcomes of p53 activation are diverse (lower panel) and are dependent on the activation of its downstream target genes.

to induction of apoptosis [146–149]. The function of p53 is mostly ascribed to its capability to regulate its target genes either through direct interaction with chromatin, via its specific consensus sequence, or via interaction with regulators of transcription. However, evidence exists that p53 also has an extra-nuclear and transcription independent role in the induction of apoptosis by releasing pro-apoptotic Bcl-2 proteins sequestered by Bcl-xL [150]. Interactions between Bcl-xL, cytoplasmic p53, and PUMA coordinate these distinct p53 functions. Following genotoxic stress, Bcl-xL sequesters cytoplasmic p53, which in turn elevates the expression of PUMA. Subsequently, PUMA displaces p53 from Bcl-xL, allowing p53 to induce mitochondrial permeabilisation [151]. Taken together, the fact that p53 eradicates tumour cells by various pathways makes it a strong candidate for silencing in cancer cells. Indeed, the majority of human cancers exhibit a high incidence of p53 dysfunction, manifested by alterations in p53 expression, mutation of the p53 protein or by indirect modifications of other components of the p53 pathway.

The dark side of p53 - mutant p53 gain of function

p53 is inactivated in about 50% of human primary tumours [30]. Notably, the predominant mode of p53 inactivation is by point mutation rather than by deletion or truncation. Mutational analysis of the p53 gene using the International Agency for Research of Cancer (IARC) database reveals that almost all the amino acids comprising the p53 protein can be mutated in human tumours with several hot spot mutations residing within the DBD of p53 (Fig. 3). Crystal structural analysis of the various hot spot p53 mutations has classified these mutations into two categories at large: a group of DNA-contact mutations (for example R248, R273) which represents mutations within amino acids that directly interact with the DNA while retaining the wild-type p53 conformation, and a group of p53 conformational mutations (for example R175, H179) which represents mutations that alter the scaffold that orients the structure of the DNA-binding interface [77]. Several studies showed that mutations residing within one of the p53 alleles are

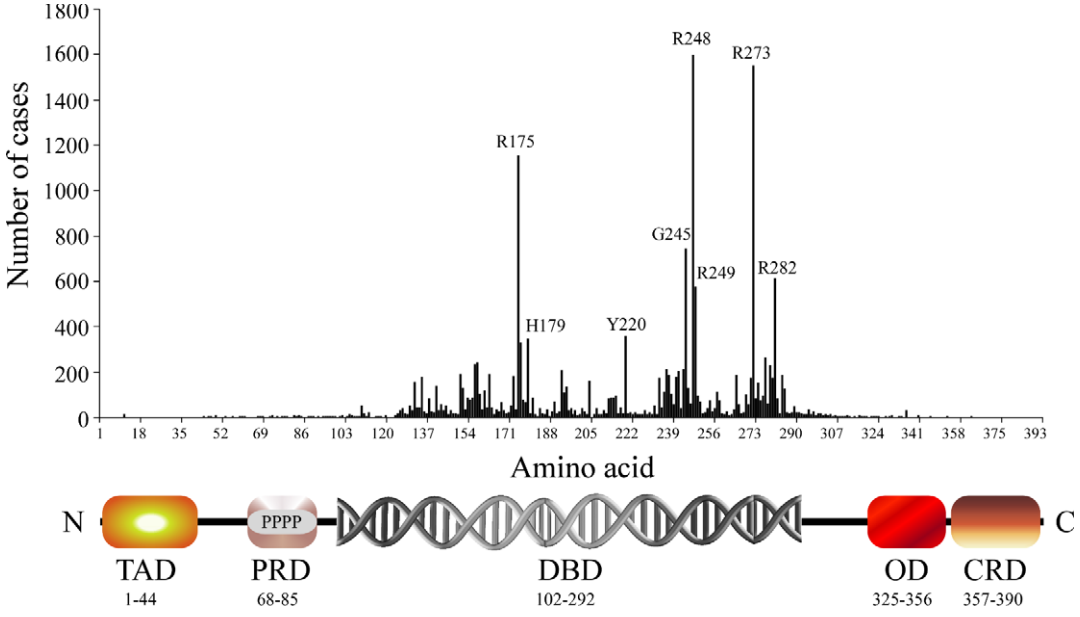

Fig. 3. The frequency of the various missense mutations within p53 in human tumours. Mutational analysis of the p53 gene using the IARC database (http://www-p53.iarc.fr/) reveals that almost all the amino acids comprising the p53 protein can be mutated in human tumours with several hot spot mutations residing within the DBD of p53.

able to oligomerise with the remaining intact wild-type allele and to inhibit its transcriptional capability by a dominant negative mechanism [152–155]. In addition, it has been suggested that mutant p53 can drive the wild-type p53 allele into a mutant conformation thereby blocking its activity [153]. However, when Chan and colleagues examined how many mutant subunits a wild-type p53 tetramer can tolerate, they found that the p53 mutants, $p53^{R249S}$ and $p53^{R273H}$, are very ineffective in impairing the transcriptional activity of wild-type p53, as at least three mutant subunits are required to inactivate a tetramer. In contrast, the endogenous p53 isoform p53DeltaN is a very potent inhibitor of p53 as one p53DeltaN manages to abolish the wild-type transcriptional activity of one tetramer [156]. In accordance with that, Jõers and colleagues showed that mutant p53 can suppress wild-type p53 activity without oligomerising with the wild-type protein. They suggested that a specific cofactor is needed for p53-dependent transcription and by depleting it, mutant p53 inactivates wild-type transcriptional activity [157]. The fact that mutant p53 is generally highly overexpressed in tumours and therefore might interact with various cellular components in both wild-type p53 dependent and independent manners has led to the hypothesis that mutant p53 might possess additional oncogenic activities that confer tumour cells with significant survival advantages. Indeed, some p53 mutations not only result in loss of wild-type activity or act in a dominant negative manner but also acquire additional functions, termed “Gain of Function”. It was already shown in the late 1980s that p53 mutants gain a pronounced transforming potential compared to their p53 null counterparts [158–162]. In recent years it became very clear that mutant p53 expressing cells are less sensitive to a variety of cell responses associated with tumour elimination such as apoptosis, autophagy, chemotherapy, radiation and cell cycle arrest and more prone to metastasis, invasiveness and genomic aberrations [163–171]. *In vivo* studies using mice with endogenous mutant p53 allele showed that mutant p53 bearing mice display allele-specific tumour spectra, higher metastatic frequency, enhanced cell proliferation and higher transformation potential compared to their p53-null counterparts [172–174]. One explanation for the observed activities lies in the fact that mutant p53 can transactivate specific gene networks that mediate its oncogenic activities. In this case mutant p53, which lost its ability to bind to a p53 responsive element, interacts with regulatory elements (promoter, intronic region and 5’/3’ UTR) within genes via different mediators and transactivates or represses a long list of targets, some of which are involved in cell death (*CD-95, ATF3, procaspase-3, EGR, BAG-1),* proliferation (*c-myc, GEF-H1, hs-MAD1*, *fos*, *IGF-II, ASNS, ANGPT1, Id2, Cyclin B2, NARS, L37, API5, AMSH, RPP-1, S2*, *RNA pol IIE, EGFR*, *CDC25A, H2 relaxin),* survival (*MST1, bFGF, HSP70, NFκB),* drug resistance (*MDR1, dUTPase*) and metastasis (*rhoGAP, TGF-βRII*, *IL-6, h15-LO*)

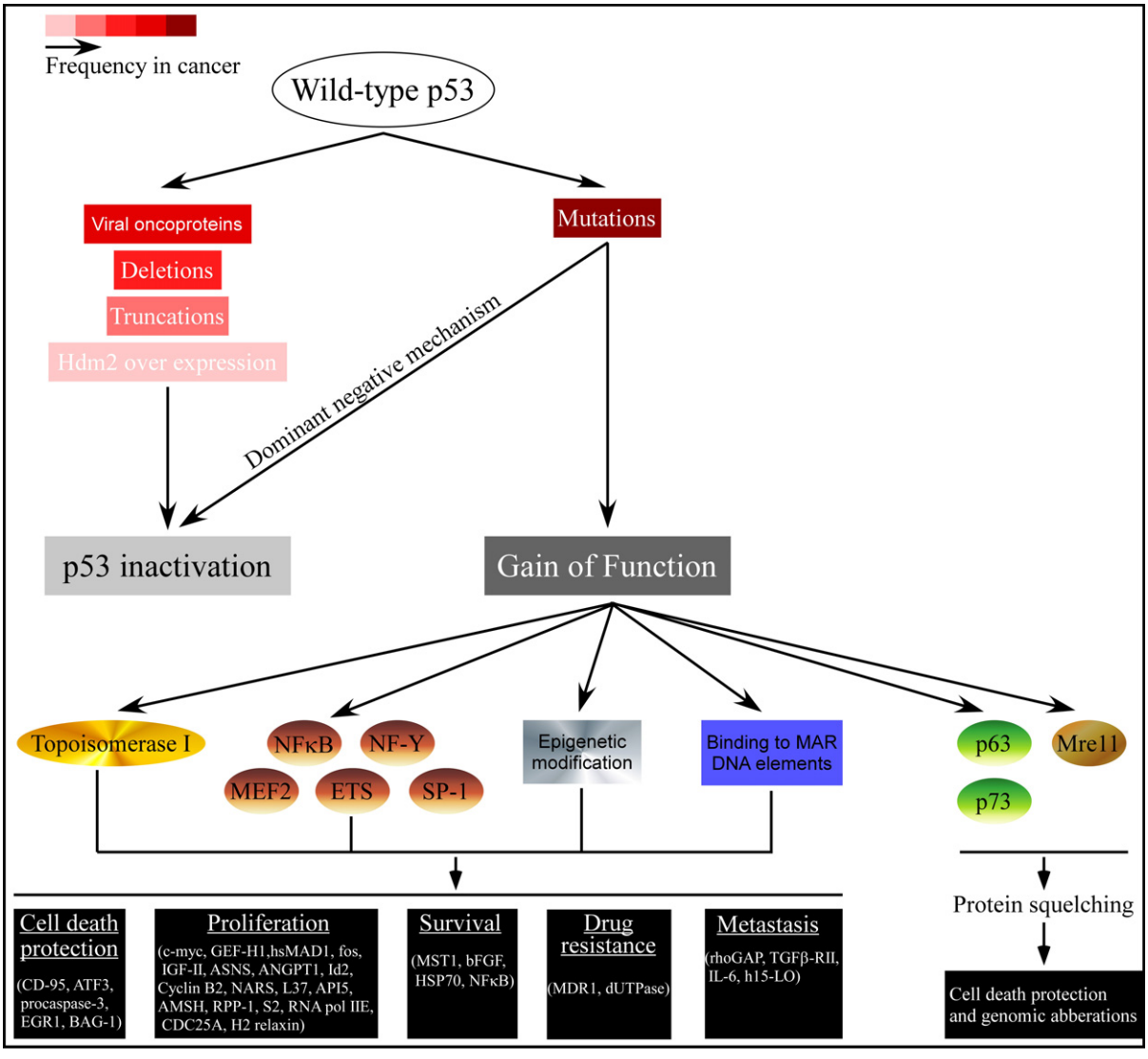

Fig. 4. The commonly occurring alteration in p53 in human cancers and mutant p53 Gain of Function mechanisms. p53 is inactivated in about 50% of human primary tumours. The predominant mode of p53 inactivation is by point mutation rather than by viral oncoproteins, deletion, truncation, or Hdm2 overexpression (upper panel). Mutations within one p53 allele can either act by a dominant negative manner or by a Gain of Function mechanism. Several possibilities have been proposed for the manner by which mutant p53 exerts its oncogenic activity. This includes activation of specific gene networks via several mediators that facilitate its DNA binding or by squelching out several proteins that are essential for tumour prevention (lower panel).

[160,175–193]. The fact that mutations within the transactivation domain of mutant p53 (L22Q/W23S) abolish its Gain of Function activity implies that mutant p53 needs to transactivate genes in order to elicit its oncogenic activities [194,195]. In addition to its ability to modulate gene expression under basal condition, mutant p53 can affect gene expression following various signalling pathways as well. For example, mutant p53 can elevate the transcription of the viral gene human T-leukaemia/lymphoma virus (HTLV)-I LTR following 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment [196] and protect cells from death induced by TPA by attenuating the expression of ATF3 [197]. Additionally, mutant p53 can enhance the activity of the NFκB transcription factor following tumour necrosis factor alpha (TNF-α) treatment [198] and diminish the transforming growth factor (TGF-β) signalling by repressing the TGF-βRII [199].

Several mechanisms have been proposed regarding the manner by which mutant p53 transactivates gene expression (Fig. 4). One theory proclaims that mutant p53 protein interacts with topoisomerase I thus promoting gene amplification at large independently of mutant p53 capacity to interact with other pro-

teins [200]. Others suggest that mutant p53 interacts with various binding sites via several transcription factors and leads to induction of transcription. Among them are ETS-1, SP-1, and MEF2 [196,197,201]. Recently, the transcription factor NF-Y has been suggested to be a pivotal mediator of mutant p53 promoter binding in response to DNA damage [202]. The expression of cyclin A, cyclin B1, cdk1, and cdc25C and cdk1-associated kinase activities is upregulated after DNA damage, due to histone acetylation which is induced by the complex formation of NF-Y, mutant p53 and p300.

A third possibility is that mutant p53 can induce epigenetic changes resulting in altered gene expression. Evidence supporting this is the observation that treatment of histone deacetylase inhibitor, trichostatin-A, in mutant p53 expressing cells results in relief of mutant p53-mediated genes suppression. This suggests a mechanism by which mutant p53 suppresses gene expression by inducing hypo-acetylation of target gene promoters [203].

A different perspective for the manner by which mutant p53 transactivates genes came from the work of Muller and colleagues who showed that mutant p53 specifically recognises and binds the nuclear matrix attachment region (MAR) DNA elements. These elements constitute important higher order regulatory elements of chromatin structure and function [204, 205]. The observation that mutant $p53^{G245S}$ binds to repetitive DNA elements associated with MAR *in vivo* supports this hypothesis. Furthermore, this could imply that by interacting with key structural components of the nucleus, mutant p53 exerts its oncogenic activities through perturbation of nuclear structure and function [206].

In addition to its ability to transactivate genes, mutant p53 has been proposed to exert its Gain of Function activities by squelching out and distorting the physiological function of several cellular proteins. Concordantly, mutant p53 binds to p63 and p73, other p53 family members, and impedes their function and thereby gains oncogenic functions such as apoptosis and DNA damage resistance [207]. In addition to its ability to interact with p63, mutant p53 activates the promoter of the p63DeltaN gene, an endogenous dominant negative isoform of p63, further disrupting the critical balance of expression between these two related isoforms [208].

A second protein that is affected by the high expression of the various hot spot p53 mutants is the nuclease Mre11 gene. Song and colleagues illustrated that the protein-protein interaction between mutant p53 and Mre11 suppresses the binding of the –Rad50–NBS1 (MRN) complex to double-stranded DNA, thus impairing the activation of the ATM gene which leads to disruption of a critical DNA damage-response pathway [209]. These oncogenic activities of mutant p53 strongly encourage scientists to develop drugs that will either restore the wild-type p53 activities to the mutant p53 forms or will eliminate tumour cells bearing mutant p53.

Clinical significance: p53-based gene therapy

Data accumulated in the field for the last 30 years can be now translated into the clinic for both better prognosis and for p53-based therapy. Several epidemiological cancers have by now accumulated large databases pertaining to the distribution of the variety of mutant p53 proteins in different tumour types. This information serves as a solid statistical basis for evaluating the significant contribution of the individual mutant p53 expressed in a given tumour. Indeed, several studies showed that various p53 mutants can be graded as to their oncogenic power and thus can serve as predictors of cancer prognosis. Accordingly, this information, coupled with the observation that the various mutants exhibit variations in their chemotherapy resistance, could be central for developing p53-based tailor-made therapy [210,211]. The understanding that wild-type p53 plays a crucial role in cancer prevention, and the fact that it is inactivated in the majority of human cancers, strongly encourages scientists to develop p53-specific-based cancer therapies. It is accepted that tumour progression involves several well-defined changes in the genetic status of p53. Some tumours exhibit an intact wild-type p53 gene structure. In such cases deregulation of the up-stream or down-stream pathways should be the therapeutic targets. In other cases, p53 is deleted or inactivated by viruses; in these cases introduction of the full length wild-type p53 should be the preferred direction. However, in the vast majority of cases, p53 is mutated in both alleles due to a loss of heterozygocity process, a process where, following the occurrence of a mutation on one p53 allele, the remaining wild-type allele is lost by deletion or the mutated allele is duplicated. In such cases the mutated protein, which is also an oncogene, must be the target of the therapy. Unravelling the mechanisms that underlie the activity of wild-type p53 in normal cells and understanding the nature of the oncogenic mutant p53 activity is expected to pave the way for the ultimate p53-based therapy.

To date, p53-based genetic therapy is considering three main different directions (Fig. 5). The first direction involves the case where p53 is intact in the tumour

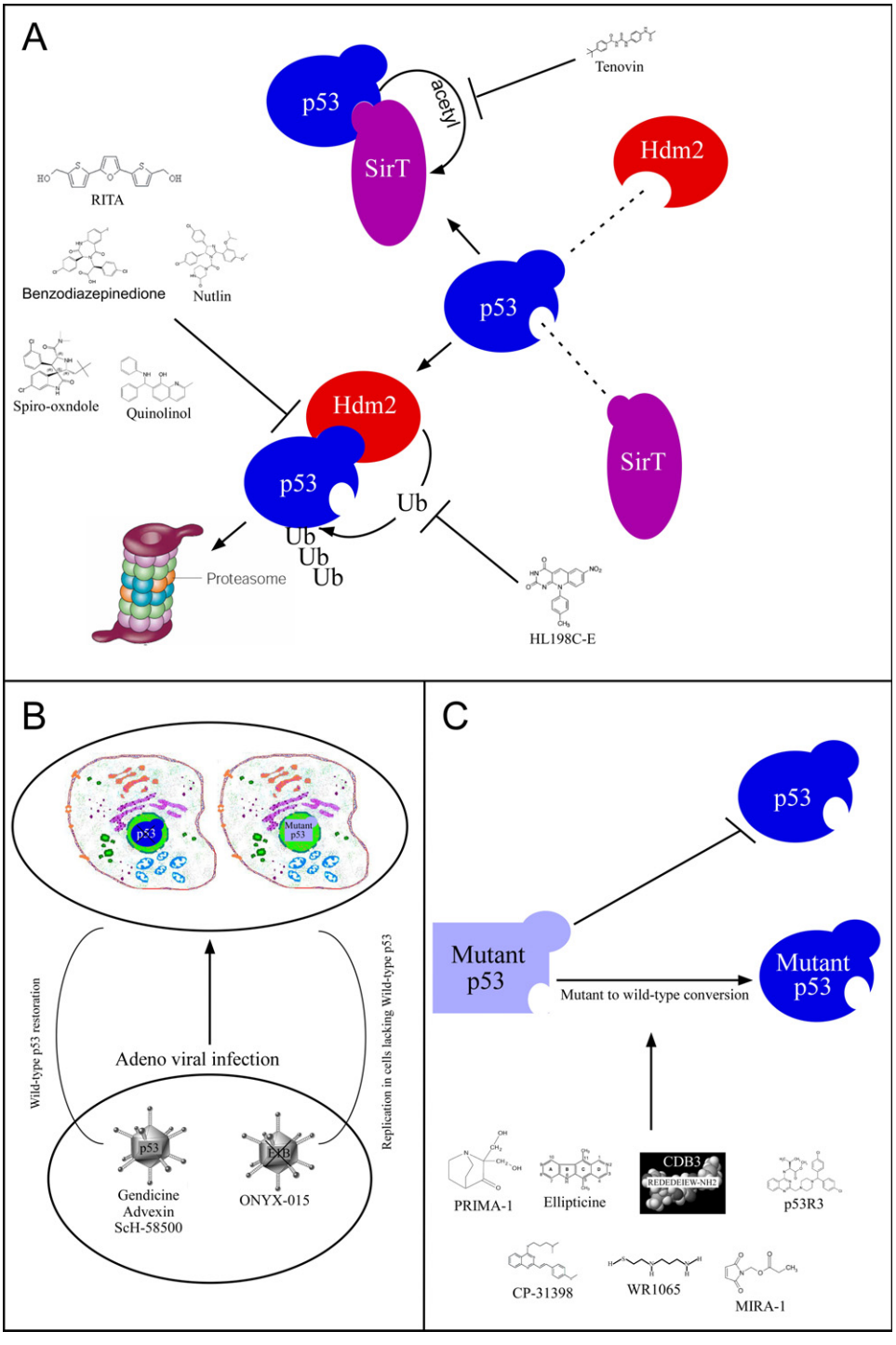

Fig. 5. p53-based gene therapy. p53-based genetic therapy is considering three main different directions. The first direction involves the case where p53 is intact in the tumour but less effective due to over expression of its negative regulator Hdm2 or due to the absence of its stabilisers ARF or ATM. In this case, re-activation of the wild-type p53 protein by its stabilisation, using small molecules that target the interaction between p53 and Hdm2 or by stabilising its posttranslational state, is the main focus (A). The second direction involves the case where p53 is absent from the tumour cells because of allelic deletion or truncation. Under these circumstances, full length wild-type p53 protein must be introduced into the tumour cells in order to re-activate the p53-mediated anti-cancerous processes. In this way, wild-type p53 is forcibly introduced into the tumour cells by adenovirus infection (B). The third direction is to eliminate mutant p53 bearing tumours by restoring the wild-type p53 activity to mutant p53 by small molecules and peptides (C) or by targeting these tumours with viruses that are specific to mutant p53 bearing tumours (B).

but less effective due to over expression of its negative regulator Hdm2 or due to the absence of its stabilisers ARF or ATM [212,213]. In this case, re-activation of the wild-type p53 protein by its stabilisation, using small molecules that target the interaction between p53 and Hdm2, thus preventing p53 degradation, is the main focus. To identify compounds that could inhibit p53–Hdm2 binding, Vassilev and colleagues screened a diverse library of synthetic chemicals and found that one class of cis-imidazoline analogues (Nutlins) could displace recombinant p53 protein from its complex with Hdm2 with median inhibitory concentration (IC50) values in the 100 to 300 nM range [214]. The crystal structure of the Hdm2–Nutlin-2 complex verifies that the inhibitor binds to the p53 binding site on Hdm2. Importantly, Nutlin compounds can induce both growth arrest and apoptosis in a p53-dependent manner in many tested cell lines [215,216], further supporting the specificity and the sensitivity of Nutlins in preventing both wild-type p53 degradation and tumour progression. Another compound that interferes with the p53–Hdm2 complex is RITA (Reactivation of p53 and Induction of Tumour cell Apoptosis) [217]. Different from the Nutlin compounds that bind to Hdm2, RITA has been proposed to bind to the p53 transactivation domain-binding cleft which mediates Hdm2 binding which thereby prevents the formation of p53–Hdm2 complex which leads to p53 stabilisation and activation [218]. In contrast, a NMR *in vitro* study showed that RITA does not block the binding of Hdm2 to p53 and suggested an alternative mechanism for p53 accumulation by RITA [219]. Several more compounds that interfere with the Hdm2–p53 complex and induce p53 stabilisation and activation have been discovered such as benzodiazepines [220–222], spiro-oxindoles [223] and quinolinols [224]. In parallel, a family of 5-deazaflavin derivatives [1–3 (HL198C-E)] has been found to act as low molecular weight inhibitors of the E3 activity of Hdm2. The inhibition of the ubiquitination process by this compound leads to a blockage of the Hdm2 mediated p53 degradation which results in p53 stabilisation and accumulation [225].

Another mechanism to activate the wild-type p53 protein is by affecting its acetylation status. The small compound tenovin stabilises wild-type p53 in tumours and suppresses tumour growth through the inhibition of the protein-deacetylating activities of SirT1 and SirT2, two important members of the sirtuin family [226].

The second direction involves the case where p53 is absent from the tumour cells because of allelic deletion or truncation. Under these circumstances, full length wild-type p53 protein must be introduced into the tumour cells in order to re-activate the p53-mediated anti-cancerous processes. In this way, wild-type p53 is forcibly introduced into the tumour cells by adenovirus serotype 5-mediated infection (rAd-p53). At least three p53-based gene therapy products are in use in clinical trails: these include Gendicine [227], Advexin [228] and ScH-58500 [229]. More than 20 kinds of cancer indications have been treated with the rAd-p53, such as head and neck squamous cell carcinoma (HNSCC), lung cancer, breast cancer and liver cancers. Importantly, this treatment is more effective when coupled with chemotherapy [230].

The third and most challenging prospect is to restore the wild-type p53 activity to mutant p53 bearing tumours by reverting mutant p53 conformation into a wild-type conformation. This concept was raised following the observation that PAb241 antibody against the carboxy-terminus of p53 or the presence of ME1, a mouse single chain Fv fragment (scFv) against the common epitope of mutant p53, can restore specific DNA-binding capability to several p53 mutants [231,232]. In addition, the extensive studies of suppressor mutations (mutations that revert the mutant conformation to a wild-type conformation [233–235]), together with the fact that p53 is a very elastic molecule, make this concept feasible. This approach uses small molecules and peptides that change the structure of the mutant p53 protein and enable it to interact partially with DNA.

High throughput screening of chemical libraries has led to the identification of a group of small synthetic molecules such as CP-31398 which can restore p53 function to mutant p53 by stabilising the active conformation of the protein that is destabilised in many mutants [236]. CP-31398 elevates the p53 targets $p21^{WAF1}$ and Bax, alters mitochondrial membrane potential causing the release of cytochrome c, and induces the cleavage of caspases-9 and -3 [237]. These activities suppress tumour growth both by arresting the cells and by subjecting them to apoptosis.

PRIMA-1 (P53 Reactivation and Induction of Massive Apoptosis) is a molecule that has been identified following a screen for a library of low-molecular-weight compounds that are capable of inducing apoptosis in human tumour cells through restoration of the transcriptional transactivation function of mutant p53. PRIMA-1 succeeds in renovating the DNA binding capability of DNA-contact mutants and conformational mutants both *in vitro* and in living cells. PRIMA-1 restores the p53–Hsp90α interaction, enhances the translocation of the p53–Hsp90α complex and thereby reactivates p53 transcriptional activity [238]. *In vivo*

studies in mice revealed an anti-tumour effect with no apparent toxicity [239]. Additionally, PRIMA-1 inhibits the growth of cell lines derived from various human tumour types in a mutant p53-dependent manner [240].

Another compound that restores wild-type p53 activity to mutant p53 forms is P53R3. P53R3 restores sequence-specific DNA binding of the endogenously expressed $p53^{R175H}$ and $p53^{R273H}$ mutants in gel-shift assays. Overexpression of the hot spot p53 mutants $p53^{R175H}$, $p53^{R248W}$ and $p53^{R273H}$ in the p53 null glioma cell line LN-308 reveals that P53R3 induces p53-dependent antiproliferative effects with much higher specificity and over a wider range of concentrations than the p53 rescue drug PRIMA-1 [241].

Ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) is one of the simplest naturally occurring alkaloids with a planar structure. It was isolated from the leaves of the evergreen tree *Ochrosia elliptica* Labill (Apocynaceae), which grows wild in Oceania. Interestingly, several ellipticine derivatives have potent anti-cancer activity and seemed to convert the mutant p53 proteins into wild-type structure [242].

The aminothiol WR1065 is an active metabolite of the cytoprotector amifostine. It is an anti-mutagenic agent that scavenges free-radicals. In addition to its ability to activate the wild-type p53 in MCF-7 cells and to induce G1 arrest, it activates mutant p53 by modulating protein conformation. WR1065 at concentrations of 0.5–4 mM partially restores wild-type conformation of $p53^{V272M}$, stimulates DNA binding activity, and increases the expression of p53 target genes $p21^{WAF-1}$, *GADD45*, and *Hdm2*, leading to cell-cycle arrest [243].

The maleimide-derived molecule MIRA-1 restores transcriptional transactivation to mutant p53 in living cells. It reactivates DNA binding and induces a conformational change of the mutant p53 protein to its active wild-type conformation. As a result, MIRA-1 induces mutant p53-dependent cell death in different human tumour cells. The structural analogue MIRA-3 shows anti-tumour activity *in vivo* against human mutant p53-carrying tumour xenografts in SCID mice [244].

CDB3 is a nine-residue peptide that is derived from the p53 sequence. It binds to the p53 core domain and stabilises it *in vitro* [245]. Moreover, CDB3 is able to raise the apparent melting temperatures of the core domain of p53 mutant $p53^{R249S}$, thus suggesting it acts as a "chaperone" that maintains destabilised p53 mutants in a native conformation.

By taking a different approach, Bischoff and colleagues showed that a mutant adenovirus that does not express the human adenovirus E1B gene and encodes a 55-kilodalton protein (ONYX-015) that inactivates the cellular tumour suppressor protein p53, can replicate in and lyse p53-deficient human tumour cells but not cells with functional p53. When the 55-kilodalton E1B protein is ectopically expressed in p53 functional cells it renders them sensitive to infection with the mutant virus. Importantly, injection of the mutant virus into p53-deficient human cervical carcinomas grown in nude mice causes a significant reduction in tumour size and causes complete regression of 60% of the tumours [246]. Although there is a strong contradiction in the literature regarding the specificity of the virus and whether the absence of functional p53 is necessary for virus replication [247], it is clear that this virus can destroy mutant p53 bearing tumours [248,249].

p53-based gene therapy is a promising prospect and is intensively investigated by leading drug companies (Fig. 6). Understanding the characteristics of each individual mutant p53 and the regulation of wild-type

Drug	Way of action	Clinical stage	Types of cancer in clinic
Nutlin	p53-Hdm2 complex inhibitor	Pre-clinical	
RITA	p53-Hdm2 complex inhibitor	Pre-clinical	
Benzodiaz -epinedione	p53-Hdm2 complex inhibitor	Pre-clinical	
Quinolinol	p53-Hdm2 complex inhibitor	Pre-clinical	
Spiro-oxndole	p53-Hdm2 complex inhibitor	Pre-clinical	
HL198C-E	Hdm2 E3 ligase inhibitor	Pre-clinical	
Tenovin	SirT deacetylase inhibitor	Pre-clinical	
Gendicine	p53 encoding adenovirus	Approval in China	Head & Neck
Advexin	p53 encoding adenovirus	Phase I-III	Mouth, Squamous cell carcinoma, Breast, Bladder, Liver and Head & Neck
SCH-58500	p53 encoding adenovirus	Phase I-III	Brain, CNS, Fallopian tube, Ovarian and Peritoneal Cavity
CP-31398	Mutant p53 conversion and reactivation	Pre-clinical	
PRIMA-1	Mutant p53 conversion and reactivation	Pre-clinical	
Ellipticine	Mutant p53 conversion and reactivation	Pre-clinical	
WR1065	Mutant p53 conversion and reactivation	Pre-clinical	
MIRA-1	Mutant p53 conversion and reactivation	Pre-clinical	
p53R3	Mutant p53 conversion and reactivation	Pre-clinical	
CDB3	Mutant p53 conversion and reactivation	Pre-clinical	
ONYX-015	E1B deficient adenovirus	Phase I-II	Sarcomas in combination with MAP chemotherapy.

Fig. 6. Drugs that modulate p53 function via diverse mechanisms. A summary of the method of action and the clinical phase of various drugs that are being developed to modify the p53 status or drugs that are selective to mutant p53 bearing tumours.

p53 in the various cell types will facilitate a specific tailor-made gene therapy for each individual tumour.

Conflict of interest statement

None declared.

References

1 Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. *Nature* 1979;**278**(5701):261–3.
2 Linzer DI, Levine AJ. Characterization of a 54K dalton cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell* 1979;**17**(1):43–52.
3 DeLeo AB, Jay G, Appella E, Dubois GC, Law LW, Old LJ. Detection of a transformation-related antigen in chemically induced sarcomas and other transformed cells of the mouse. *Proc Natl Acad Sci USA* 1979;**76**(5):2420–4.
4 Crawford LV, Pim DC, Gurney EG, Goodfellow P, Taylor-Papadimitriou J. Detection of a common feature in several human tumor cell lines – a 53,000-dalton protein. *Proc Natl Acad Sci USA* 1981;**78**(1):41–5.
5 Dippold WG, Jay G, DeLeo AB, Khoury G, Old LJ. p53 transformation-related protein: detection by monoclonal antibody in mouse and human cells. *Proc Natl Acad Sci USA* 1981;**78**(3):1695–9.
6 Crawford LV, Pim DC, Bulbrook RD. Detection of antibodies against the cellular protein p53 in sera from patients with breast cancer. *Int J Cancer* 1982;**30**(4):403–8.
7 Rotter V. p53, a transformation-related cellular-encoded protein, can be used as a biochemical marker for the detection of primary mouse tumor cells. *Proc Natl Acad Sci USA* 1983; **80**(9):2613–7.
8 Eliyahu D, Raz A, Gruss P, Givol D, Oren M. Participation of p53 cellular tumour antigen in transformation of normal embryonic cells. *Nature* 1984;**312**(5995):646–9.
9 Jenkins JR, Rudge K, Currie GA. Cellular immortalization by a cDNA clone encoding the transformation-associated phosphoprotein p53. *Nature* 1984;**312**(5995):651–4.
10 Parada LF, Land H, Weinberg RA, Wolf D, Rotter V. Cooperation between gene encoding p53 tumour antigen and ras in cellular transformation. *Nature* 1984;**312**(5995):649–51.
11 Braithwaite AW, Sturzbecher HW, Addison C, Palmer C, Rudge K, Jenkins JR. Mouse p53 inhibits SV40 origin-dependent DNA replication. *Nature* 1987;**329**(6138):458–60.
12 Gannon JV, Lane DP. p53 and DNA polymerase alpha compete for binding to SV40 T antigen. *Nature* 1987;**329**(6138):456–8.
13 Sturzbecher HW, Brain R, Maimets T, Addison C, Rudge K, Jenkins JR. Mouse p53 blocks SV40 DNA replication in vitro and downregulates T antigen DNA helicase activity. *Oncogene* 1988;**3**(4):405–13.
14 Ben David Y, Prideaux VR, Chow V, Benchimol S, Bernstein A. Inactivation of the p53 oncogene by internal deletion or retroviral integration in erythroleukemic cell lines induced by Friend leukemia virus. *Oncogene* 1988;**3**(2):179–85.
15 Rovinski B, Munroe D, Peacock J, Mowat M, Bernstein A, Benchimol S. Deletion of 5'-coding sequences of the cellular p53 gene in mouse erythroleukemia: a novel mechanism of oncogene regulation. *Mol Cell Biol* 1987;**7**(2):847–53.
16 Wolf D, Admon S, Oren M, Rotter V. Abelson murine leukemia virus-transformed cells that lack p53 protein synthesis express aberrant p53 mRNA species. *Mol Cell Biol* 1984;**4**(3):552–8.
17 Wolf D, Rotter V. Inactivation of p53 gene expression by an insertion of Moloney murine leukemia virus-like DNA sequences. *Mol Cell Biol* 1984;**4**(7):1402–10.
18 Wolf D, Rotter V. Major deletions in the gene encoding the p53 tumor antigen cause lack of p53 expression in HL-60 cells. *Proc Natl Acad Sci USA* 1985;**82**(3):790–4.
19 Mowat M, Cheng A, Kimura N, Bernstein A, Benchimol S. Rearrangements of the cellular p53 gene in erythroleukaemic cells transformed by Friend virus. *Nature* 1985;**314**(6012): 633–6.
20 Oren M, Levine AJ. Molecular cloning of a cDNA specific for the murine p53 cellular tumor antigen. *Proc Natl Acad Sci USA* 1983;**80**(1):56–9.
21 Baker SJ, Fearon ER, Nigro JM, et al. Chromosome 17 deletions and p53 gene mutations in colorectal carcinomas. *Science* 1989; **244**(4901):217–21.
22 Jenkins JR, Rudge K, Chumakov P, Currie GA. The cellular oncogene p53 can be activated by mutagenesis. *Nature* 1985; **317**(6040):816–8.
23 Nigro JM, Baker SJ, Preisinger AC, et al. Mutations in the p53 gene occur in diverse human tumour types. *Nature* 1989; **342**(6250):705–8.
24 Sturzbecher HW, Chumakov P, Welch WJ, Jenkins JR. Mutant p53 proteins bind hsp 72/73 cellular heat shock-related proteins in SV40-transformed monkey cells. *Oncogene* 1987;**1**(2):201–11.
25 Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc70–p53 complex with an altered half-life. *Mol Cell Biol* 1988;**8**(2):531–9.
26 Pinhasi-Kimhi O, Michalovitz D, Ben-Zeev A, Oren M. Specific interaction between the p53 cellular tumour antigen and major heat shock proteins. *Nature* 1986;**320**(6058):182–4.
27 Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. *Cell* 1989;**57**(7): 1083–93.
28 Levine AJ. The p53 tumor suppressor gene and gene product. *Princess Takamatsu Symp* 1989;**20**:221–30.
29 Levine AJ. Tumor suppressor genes. *Bioessays* 1990;**12**(2): 60–6.
30 Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991;**253**(5015):49–53.
31 Hollstein MC, Peri L, Mandard AM, et al. Genetic analysis of human esophageal tumors from two high incidence geographic areas: frequent p53 base substitutions and absence of ras mutations. *Cancer Res* 1991;**51**(15):4102–6.
32 Fenaux P, Collyn d'Hooghe M, Jonveaux P, et al. Rearrangement and expression of the p53 gene in myelodysplastic syndrome and acute myeloid leukemia. *Nouv Rev Fr Hematol* 1990;**32**(5): 341–4.
33 Takahashi T, Nau MM, Chiba I, et al. p53: a frequent target for genetic abnormalities in lung cancer. *Science* 1989;**246**(4929): 491–4.
34 Kelman Z, Prokocimer M, Peller S, et al. Rearrangements in the p53 gene in Philadelphia chromosome positive chronic myelogenous leukemia. *Blood* 1989;**74**(7):2318–24.
35 Thompson AM, Steel CM, Chetty U, et al. p53 gene mRNA expression and chromosome 17p allele loss in breast cancer. *Br J Cancer* 1990;**61**(1):74–8.

36 Malkin D, Li FP, Strong LC, et al. Germ line p53 mutations in a familial syndrome of breast cancer, sarcomas, and other neoplasms. *Science* 1990;**250**(4985):1233–8.

37 Srivastava S, Zou ZQ, Pirollo K, Blattner W, Chang EH. Germline transmission of a mutated p53 gene in a cancer-prone family with Li-Fraumeni syndrome. *Nature* 1990;**348**(6303): 747–9.

38 Santibanez-Koref MF, Birch JM, Hartley AL, et al. p53 germline mutations in Li-Fraumeni syndrome. *Lancet* 1991;**338**(8781):1490–1.

39 Remvikos Y, Laurent-Puig P, Salmon RJ, Frelat G, Dutrillaux B, Thomas G. Simultaneous monitoring of P53 protein and DNA content of colorectal adenocarcinomas by flow cytometry. *Int J Cancer* 1990;**45**(3):450–6.

40 Michalovitz D, Halevy O, Oren M. Conditional inhibition of transformation and of cell proliferation by a temperature-sensitive mutant of p53. *Cell* 1990;**62**(4):671–80.

41 Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. *Science* 1990;**249**(4971):912–5.

42 Yonish-Rouach E, Resnitzky D, Lotem J, Sachs L, Kimchi A, Oren M. Wild-type p53 induces apoptosis of myeloid leukaemic cells that is inhibited by interleukin-6. *Nature* 1991;**352**(6333): 345–7.

43 Fields S, Jang SK. Presence of a potent transcription activating sequence in the p53 protein. *Science* 1990;**249**(4972):1046–9.

44 Raycroft L, Wu HY, Lozano G. Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. *Science* 1990;**249**(4972):1049–51.

45 Weintraub H, Hauschka S, Tapscott SJ. The MCK enhancer contains a p53 responsive element. *Proc Natl Acad Sci USA* 1991;**88**(11):4570–1.

46 Ginsberg D, Mechta F, Yaniv M, Oren M. Wild-type p53 can down-modulate the activity of various promoters. *Proc Natl Acad Sci USA* 1991;**88**(22):9979–83.

47 Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991;**51**(23 Pt 1):6304–11.

48 Schmid P, Lorenz A, Hameister H, Montenarh M. Expression of p53 during mouse embryogenesis. *Development* 1991;**113**(3): 857–65.

49 Shaulsky G, Goldfinger N, Peled A, Rotter V. Involvement of wild-type p53 in pre-B-cell differentiation in vitro. *Proc Natl Acad Sci USA* 1991;**88**(20):8982–6.

50 Shay JW, Pereira-Smith OM, Wright WE. A role for both RB and p53 in the regulation of human cellular senescence. *Exp Cell Res* 1991;**196**(1):33–9.

51 Sherley JL. Guanine nucleotide biosynthesis is regulated by the cellular p53 concentration. *J Biol Chem* 1991;**266**(36): 24815–28.

52 Lane DP. Cancer. p53, guardian of the genome. *Nature* 1992; **358**(6381):15–6.

53 Beijnen JH. p53 selected as molecule of the year 1993. *Pharm World Sci* 1994;**16**(1):1.

54 Chen J, Marechal V, Levine AJ. Mapping of the p53 and mdm-2 interaction domains. *Mol Cell Biol* 1993;**13**(7):4107–14.

55 Teodoro JG, Halliday T, Whalen SG, Takayesu D, Graham FL, Branton PE. Phosphorylation at the carboxy terminus of the 55-kilodalton adenovirus type 5 E1B protein regulates transforming activity. *J Virol* 1994;**68**(2):776–86.

56 Thut CJ, Chen JL, Klemm R, Tjian R. p53 transcriptional activation mediated by coactivators TAFII40 and TAFII60. *Science* 1995;**267**(5194):100–4.

57 Gu W, Shi XL, Roeder RG. Synergistic activation of transcription by CBP and p53. *Nature* 1997;**387**(6635): 819–23.

58 Di Lello P, Jenkins LM, Jones TN, et al. Structure of the Tfb1/p53 complex: Insights into the interaction between the p62/Tfb1 subunit of TFIIH and the activation domain of p53. *Mol Cell* 2006;**22**(6):731–40.

59 Xie P, Tian C, An L, et al. Histone methyltransferase protein SETD2 interacts with p53 and selectively regulates its downstream genes. *Cell Signal* 2008;**20**(9):1671–8.

60 Popowicz GM, Czarna A, Holak TA. Structure of the human Mdmx protein bound to the p53 tumor suppressor transactivation domain. *Cell Cycle* 2008;**7**(15):2441–3.

61 Wong TS, Rajagopalan S, Townsley FM, et al. Physical and functional interactions between human mitochondrial single-stranded DNA-binding protein and tumour suppressor p53. *Nucleic Acids Res* 2009;**37**(2):568–81.

62 Lees-Miller SP, Sakaguchi K, Ullrich SJ, Appella E, Anderson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol Cell Biol* 1992;**12**(11):5041–9.

63 Ullrich SJ, Sakaguchi K, Lees-Miller SP, et al. Phosphorylation at Ser-15 and Ser-392 in mutant p53 molecules from human tumors is altered compared to wild-type p53. *Proc Natl Acad Sci USA* 1993;**90**(13):5954–8.

64 Jamal S, Ziff EB. Raf phosphorylates p53 in vitro and potentiates p53-dependent transcriptional transactivation in vivo. *Oncogene* 1995;**10**(11):2095–101.

65 Unger T, Sionov RV, Moallem E, et al. Mutations in serines 15 and 20 of human p53 impair its apoptotic activity. *Oncogene* 1999;**18**(21):3205–12.

66 Sanchez-Prieto R, Rojas JM, Taya Y, Gutkind JS. A role for the p38 mitogen-acitvated protein kinase pathway in the transcriptional activation of p53 on genotoxic stress by chemotherapeutic agents. *Cancer Res* 2000;**60**(9):2464–72.

67 Turenne GA, Price BD. Glycogen synthase kinase3 beta phosphorylates serine 33 of p53 and activates p53's transcriptional activity. *BMC Cell Biol* 2001;**2**:12.

68 Turenne GA, Paul P, Laflair L, Price BD. Activation of p53 transcriptional activity requires ATM's kinase domain and multiple N-terminal serine residues of p53. *Oncogene* 2001;**20**(37):5100–10.

69 Golubovskaya VM, Finch R, Cance WG. Direct interaction of the N-terminal domain of focal adhesion kinase with the N-terminal transactivation domain of p53. *J Biol Chem* 2005; **280**(26):25008–21.

70 MacLaine NJ, Oster B, Bundgaard B, et al. A central role for CK1 in catalyzing phosphorylation of the p53 transactivation domain at serine 20 after HHV-6B viral infection. *J Biol Chem* 2008;**283**(42):28563–73.

71 Kar S, Sakaguchi K, Shimohigashi Y, et al. Effect of phosphorylation on the structure and fold of transactivation domain of p53. *J Biol Chem* 2002;**277**(18):15579–85.

72 Lin J, Chen J, Elenbaas B, Levine AJ. Several hydrophobic amino acids in the p53 amino-terminal domain are required for transcriptional activation, binding to mdm-2 and the adenovirus 5 E1B 55-kD protein. *Genes Dev* 1994;**8**(10):1235–46.

73 Roemer K, Mueller-Lantzsch N. p53 transactivation domain mutant Q22, S23 is impaired for repression of promoters and mediation of apoptosis. *Oncogene* 1996;**12**(10):2069–79.

74 Baptiste-Okoh N, Barsotti AM, Prives C. A role for caspase 2 and PIDD in the process of p53-mediated apoptosis. *Proc Natl Acad Sci USA* 2008;**105**(6):1937–42.
75 Courtois S, Verhaegh G, North S, et al. DeltaN-p53, a natural isoform of p53 lacking the first transactivation domain, counteracts growth suppression by wild-type p53. *Oncogene* 2002;**21**(44):6722–8.
76 Pavletich NP, Chambers KA, Pabo CO. The DNA-binding domain of p53 contains the four conserved regions and the major mutation hot spots. *Genes Dev* 1993;**7**(12B):2556–64.
77 Cho Y, Gorina S, Jeffrey PD, Pavletich NP. Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. *Science* 1994;**265**(5170):346–55.
78 Duan J, Nilsson L. Effect of Zn2+ on DNA recognition and stability of the p53 DNA-binding domain. *Biochemistry* 2006; **45**(24):7483–92.
79 el-Deiry WS, Kern SE, Pietenpol JA, Kinzler KW, Vogelstein B. Definition of a consensus binding site for p53. *Nat Genet* 1992;**1**(1):45–9.
80 Hearnes JM, Mays DJ, Schavolt KL, Tang L, Jiang X, Pietenpol JA. Chromatin immunoprecipitation-based screen to identify functional genomic binding sites for sequence-specific transactivators. *Mol Cell Biol* 2005;**25**(22):10148–58.
81 Jegga AG, Inga A, Menendez D, Aronow BJ, Resnick MA. Functional evolution of the p53 regulatory network through its target response elements. *Proc Natl Acad Sci USA* 2008; **105**(3):944–9.
82 Jordan JJ, Menendez D, Inga A, Nourredine M, Bell D, Resnick MA. Noncanonical DNA motifs as transactivation targets by wild type and mutant p53. *PLoS Genet* 2008;**4**(6):e1000104.
83 Jeffrey PD, Gorina S, Pavletich NP. Crystal structure of the tetramerization domain of the p53 tumor suppressor at 1.7 angstroms. *Science* 1995;**267**(5203):1498–502.
84 Shin YC, Nakamura H, Liang X, et al. Inhibition of the ATM/p53 signal transduction pathway by Kaposi's sarcoma-associated herpesvirus interferon regulatory factor 1. *J Virol* 2006;**80**(5):2257–66.
85 Chen J, Dai G, Wang YQ, et al. Polo-like kinase 1 regulates mitotic arrest after UV irradiation through dephosphorylation of p53 and inducing p53 degradation. *FEBS Lett* 2006;**580**(15): 3624–30.
86 Chan WM, Mak MC, Fung TK, Lau A, Siu WY, Poon RY. Ubiquitination of p53 at multiple sites in the DNA-binding domain. *Mol Cancer Res* 2006;**4**(1):15–25.
87 Adler V, Pincus MR, Minamoto T, et al. Conformation-dependent phosphorylation of p53. *Proc Natl Acad Sci USA* 1997;**94**(5):1686–91.
88 Jung H, Seong HA, Ha H. Critical role of cysteine residue 81 of macrophage migration inhibitory factor (MIF) in MIF-induced inhibition of p53 activity. *J Biol Chem* 2008;**283**(29): 20383–96.
89 Iwabuchi K, Li B, Massa HF, Trask BJ, Date T, Fields S. Stimulation of p53-mediated transcriptional activation by the p53-binding proteins, 53BP1 and 53BP2. *J Biol Chem* 1998; **273**(40):26061–8.
90 Jung H, Seong HA, Ha H. NM23-H1 tumor suppressor and its interacting partner STRAP activate p53 function. *J Biol Chem* 2007;**282**(48):35293–307.
91 Blagosklonny MV, An WG, Romanova LY, Trepel J, Fojo T, Neckers L. p53 inhibits hypoxia-inducible factor-stimulated transcription. *J Biol Chem* 1998;**273**(20):11995–8.
92 Bai L, Merchant JL. ZBP-89 promotes growth arrest through stabilization of p53. *Mol Cell Biol* 2001;**21**(14):4670–83.
93 Bergamaschi D, Samuels Y, Sullivan A, et al. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. *Nat Genet* 2006;**38**(10):1133–41.
94 Barrera-Hernandez G, Zhan Q, Wong R, Cheng SY. Thyroid hormone receptor is a negative regulator in p53-mediated signaling pathways. *DNA Cell Biol* 1998;**17**(9):743–50.
95 Batta K, Kundu TK. Activation of p53 function by human transcriptional coactivator PC4: role of protein-protein interaction, DNA bending, and posttranslational modifications. *Mol Cell Biol* 2007;**27**(21):7603–14.
96 Das S, Raj L, Zhao B, et al. Hzf Determines cell survival upon genotoxic stress by modulating p53 transactivation. *Cell* 2007;**130**(4):624–37.
97 Malanga M, Pleschke JM, Kleczkowska HE, Althaus FR. Poly(ADP-ribose) binds to specific domains of p53 and alters its DNA binding functions. *J Biol Chem* 1998;**273**(19): 11839–43.
98 Shiota M, Izumi H, Onitsuka T, et al. Twist and p53 reciprocally regulate target genes via direct interaction. *Oncogene* 2008.
99 Tang Y, Luo J, Zhang W, Gu W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. *Mol Cell* 2006;**24**(6):827–39.
100 Zhao Y, Katzman RB, Delmolino LM, et al. The notch regulator MAML1 interacts with p53 and functions as a coactivator. *J Biol Chem* 2007;**282**(16):11969–81.
101 Meplan C, Richard MJ, Hainaut P. Redox signalling and transition metals in the control of the p53 pathway. *Biochem Pharmacol* 2000;**59**(1):25–33.
102 Tomita Y, Marchenko N, Erster S, et al. WT p53, but not tumor-derived mutants, bind to Bcl2 via the DNA binding domain and induce mitochondrial permeabilization. *J Biol Chem* 2006; **281**(13):8600–6.
103 Friedman PN, Chen X, Bargonetti J, Prives C. The p53 protein is an unusually shaped tetramer that binds directly to DNA. *Proc Natl Acad Sci USA* 1993;**90**(8):3319–23.
104 Halazonetis TD, Kandil AN. Conformational shifts propagate from the oligomerization domain of p53 to its tetrameric DNA binding domain and restore DNA binding to select p53 mutants. *Embo J* 1993;**12**(13):5057–64.
105 Clore GM, Omichinski JG, Sakaguchi K, et al. High-resolution structure of the oligomerization domain of p53 by multidimensional NMR. *Science* 1994;**265**(5170):386–91.
106 Marston NJ, Jenkins JR, Vousden KH. Oligomerisation of full length p53 contributes to the interaction with mdm2 but not HPV E6. *Oncogene* 1995;**10**(9):1709–15.
107 Sakaguchi K, Sakamoto H, Xie D, et al. Effect of phosphorylation on tetramerization of the tumor suppressor protein p53. *J Protein Chem* 1997;**16**(5):553–6.
108 Sakaguchi K, Sakamoto H, Lewis MS, et al. Phosphorylation of serine 392 stabilizes the tetramer formation of tumor suppressor protein p53. *Biochemistry* 1997;**36**(33):10117–24.
109 Frasca F, Rustighi A, Malaguarnera R, et al. HMGA1 inhibits the function of p53 family members in thyroid cancer cells. *Cancer Res* 2006;**66**(6):2980–9.
110 Lin J, Blake M, Tang C, et al. Inhibition of p53 transcriptional activity by the S100B calcium-binding protein. *J Biol Chem* 2001;**276**(37):35037–41.
111 Kim EJ, Um SJ. Thymine-DNA glycosylase interacts with and functions as a coactivator of p53 family proteins. *Biochem Biophys Res Commun* 2008;**377**(3):838–42.
112 Pietsch EC, Leu JI, Frank A, Dumont P, George DL, Murphy ME. The tetramerization domain of p53 is

required for efficient BAK oligomerization. *Cancer Biol Ther* 2007;**6**(10):1576–83.
113 Lomax ME, Barnes DM, Hupp TR, Picksley SM, Camplejohn RS. Characterization of p53 oligomerization domain mutations isolated from Li-Fraumeni and Li-Fraumeni like family members. *Oncogene* 1998;**17**(5):643–9.
114 Plummer SJ, Santibanez-Koref M, Kurosaki T, et al. A germline 2.35 kb deletion of p53 genomic DNA creating a specific loss of the oligomerization domain inherited in a Li-Fraumeni syndrome family. *Oncogene* 1994;**9**(11):3273–80.
115 Rolley N, Milner J. Specific DNA binding by p53 is independent of mutation at serine 389, the casein kinase II site. *Oncogene* 1994;**9**(10):3067–70.
116 Albrechtsen N, Dornreiter I, Grosse F, Kim E, Wiesmuller L, Deppert W. Maintenance of genomic integrity by p53: complementary roles for activated and non-activated p53. *Oncogene* 1999;**18**(53):7706–17.
117 Meek DW. Mechanisms of switching on p53: a role for covalent modification? *Oncogene* 1999;**18**(53):7666–75.
118 Oren M. Regulation of the p53 tumor suppressor protein. *J Biol Chem* 1999;**274**(51):36031–4.
119 Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. *Cell* 1997;**90**(4):595–606.
120 Rustandi RR, Baldisseri DM, Weber DJ. Structure of the negative regulatory domain of p53 bound to S100B(betabeta). *Nat Struct Biol* 2000;**7**(7):570–4.
121 Watcharasit P, Bijur GN, Song L, Zhu J, Chen X, Jope RS. Glycogen synthase kinase-3beta (GSK3beta) binds to and promotes the actions of p53. *J Biol Chem* 2003;**278**(49):48872–9.
122 Daniely Y, Dimitrova DD, Borowiec JA. Stress-dependent nucleolin mobilization mediated by p53–nucleolin complex formation. *Mol Cell Biol* 2002;**22**(16):6014–22.
123 Gotz C, Scholtes P, Prowald A, Schuster N, Nastainczyk W, Montenarh M. Protein kinase CK2 interacts with a multi-protein binding domain of p53. *Mol Cell Biochem* 1999;**191**(1–2): 111–20.
124 Monte M, Benetti R, Buscemi G, Sandy P, Del Sal G, Schneider C. The cell cycle-regulated protein human GTSE-1 controls DNA damage-induced apoptosis by affecting p53 function. *J Biol Chem* 2003;**278**(32):30356–64.
125 Megidish T, Xu JH, Xu CW. Activation of p53 by protein inhibitor of activated Stat1 (PIAS1). *J Biol Chem* 2002;**277**(10): 8255–9.
126 Grigorian M, Andresen S, Tulchinsky E, et al. Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein: functional consequences of their interaction. *J Biol Chem* 2001;**276**(25):22699–708.
127 Liu W, Konduri SD, Bansal S, et al. Estrogen receptor-alpha binds p53 tumor suppressor protein directly and represses its function. *J Biol Chem* 2006;**281**(15):9837–40.
128 Yang M, Wu S, Su X, May WS. JAZ mediates G1 cell-cycle arrest and apoptosis by positively regulating p53 transcriptional activity. *Blood* 2006;**108**(13):4136–45.
129 Selivanova G, Iotsova V, Kiseleva E, et al. The single-stranded DNA end binding site of p53 coincides with the C-terminal regulatory region. *Nucleic Acids Res* 1996;**24**(18):3560–7.
130 Anderson ME, Woelker B, Reed M, Wang P, Tegtmeyer P. Reciprocal interference between the sequence-specific core and nonspecific C-terminal DNA binding domains of p53: implications for regulation. *Mol Cell Biol* 1997;**17**(11): 6255–64.
131 Bayle JH, Elenbaas B, Levine AJ. The carboxyl-terminal domain of the p53 protein regulates sequence-specific DNA binding through its nonspecific nucleic acid-binding activity. *Proc Natl Acad Sci USA* 1995;**92**(12):5729–33.
132 Hupp TR, Sparks A, Lane DP. Small peptides activate the latent sequence-specific DNA binding function of p53. *Cell* 1995;**83**(2):237–45.
133 Yakovleva T, Pramanik A, Kawasaki T, et al. p53 Latency. C-terminal domain prevents binding of p53 core to target but not to nonspecific DNA sequences. *J Biol Chem* 2001;**276**(19): 15650–8.
134 Sakamoto H, Kodama H, Higashimoto Y, et al. Chemical synthesis of phosphorylated peptides of the carboxy-terminal domain of human p53 by a segment condensation method. *Int J Pept Protein Res* 1996;**48**(5):429–42.
135 Friedler A, Veprintsev DB, Freund SM, von Glos KI, Fersht AR. Modulation of binding of DNA to the C-terminal domain of p53 by acetylation. *Structure* 2005;**13**(4):629–36.
136 Weinberg RL, Freund SM, Veprintsev DB, Bycroft M, Fersht AR. Regulation of DNA binding of p53 by its C-terminal domain. *J Mol Biol* 2004;**342**(3):801–11.
137 Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nat Rev Cancer* 2002;**2**(8):594–604.
138 Service R. Slow DNA repair implicated in mutations found in tumors. *Science* 1994;**263**(5152):1374.
139 Dameron KM, Volpert OV, Tainsky MA, Bouck N. The p53 tumor suppressor gene inhibits angiogenesis by stimulating the production of thrombospondin. *Cold Spring Harb Symp Quant Biol* 1994;**59**:483–9.
140 Graeber TG, Peterson JF, Tsai M, Monica K, Fornace AJ, Jr., Giaccia AJ. Hypoxia induces accumulation of p53 protein, but activation of a G1-phase checkpoint by low-oxygen conditions is independent of p53 status. *Mol Cell Biol* 1994;**14**(9):6264–77.
141 Hursting SD, Perkins SN, Phang JM, Barrett JC. Diet and cancer prevention studies in p53-deficient mice. *J Nutr* 2001;**131**(11 Suppl):3092S–3094S.
142 Tolbert D, Lu X, Yin C, Tantama M, Van Dyke T. p19(ARF) is dispensable for oncogenic stress-induced p53-mediated apoptosis and tumor suppression in vivo. *Mol Cell Biol* 2002;**22**(1):370–7.
143 Hermeking H, Lengauer C, Polyak K, et al. 14-3-3 sigma is a p53-regulated inhibitor of G2/M progression. *Mol Cell* 1997;**1**(1):3–11.
144 Kastan MB, Zhan Q, el-Deiry WS, et al. A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell* 1992;**71**(4):587–97.
145 el-Deiry WS, Tokino T, Velculescu VE, et al. WAF1, a potential mediator of p53 tumor suppression. *Cell* 1993;**75**(4):817–25.
146 Miyashita T, Reed JC. Tumor suppressor p53 is a direct transcriptional activator of the human bax gene. *Cell* 1995;**80**(2):293–9.
147 Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. *Mol Cell* 2001;**7**(3):683–94.
148 Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. *Science* 2000;**288**(5468):1053–8.
149 Wu GS, Burns TF, McDonald ER, 3rd, et al. KILLER/DR5 is a DNA damage-inducible p53-regulated death receptor gene. *Nat Genet* 1997;**17**(2):141–3.
150 Chipuk JE, Green DR. Cytoplasmic p53: bax and forward. *Cell Cycle* 2004;**3**(4):429–31.

151 Chipuk JE, Bouchier-Hayes L, Kuwana T, Newmeyer DD, Green DR. PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. *Science* 2005;**309**(5741): 1732–5.
152 Gannon JV, Greaves R, Iggo R, Lane DP. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. *Embo J* 1990;**9**(5):1595–602.
153 Milner J, Medcalf EA. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. *Cell* 1991;**65**(5):765–74.
154 Milner J, Medcalf EA, Cook AC. Tumor suppressor p53: analysis of wild-type and mutant p53 complexes. *Mol Cell Biol* 1991;**11**(1):12–9.
155 Munroe DG, Peacock JW, Benchimol S. Inactivation of the cellular p53 gene is a common feature of Friend virus-induced erythroleukemia: relationship of inactivation to dominant transforming alleles. *Mol Cell Biol* 1990;**10**(7):3307–13.
156 Chan WM, Siu WY, Lau A, Poon RY. How many mutant p53 molecules are needed to inactivate a tetramer? *Mol Cell Biol* 2004;**24**(8):3536–51.
157 Joers A, Kristjuhan A, Kadaja L, Maimets T. Tumour associated mutants of p53 can inhibit transcriptional activity of p53 without heterooligomerization. *Oncogene* 1998;**17**(18):2351–8.
158 Halevy O, Michalovitz D, Oren M. Different tumor-derived p53 mutants exhibit distinct biological activities. *Science* 1990; **250**(4977):113–6.
159 Bhatia K, Goldschmidts W, Gutierrez M, Gaidano G, Dalla-Favera R, Magrath I. Hemi- or homozygosity: a requirement for some but not other p53 mutant proteins to accumulate and exert a pathogenetic effect. *Faseb J* 1993;**7**(10):951–6.
160 Dittmer D, Pati S, Zambetti G, et al. Gain of function mutations in p53. *Nat Genet* 1993;**4**(1):42–6.
161 Hsiao M, Low J, Dorn E, et al. Gain-of-function mutations of the p53 gene induce lymphohematopoietic metastatic potential and tissue invasiveness. *Am J Pathol* 1994;**145**(3):702–14.
162 Sun Y, Nakamura K, Wendel E, Colburn N. Progression toward tumor cell phenotype is enhanced by overexpression of a mutant p53 tumor-suppressor gene isolated from nasopharyngeal carcinoma. *Proc Natl Acad Sci USA* 1993;**90**(7):2827–31.
163 Morselli E, Tasdemir E, Maiuri MC, et al. Mutant p53 protein localized in the cytoplasm inhibits autophagy. *Cell Cycle* 2008; **7**(19):3056–61.
164 Agapova LS, Ilyinskaya GV, Turovets NA, Ivanov AV, Chumakov PM, Kopnin BP. Chromosome changes caused by alterations of p53 expression. *Mutat Res* 1996;**354**(1):129–38.
165 Gualberto A, Aldape K, Kozakiewicz K, Tlsty TD. An oncogenic form of p53 confers a dominant, gain-of-function phenotype that disrupts spindle checkpoint control. *Proc Natl Acad Sci USA* 1998;**95**(9):5166–71.
166 Li R, Sutphin PD, Schwartz D, et al. Mutant p53 protein expression interferes with p53-independent apoptotic pathways. *Oncogene* 1998;**16**(25):3269–77.
167 Blandino G, Levine AJ, Oren M. Mutant p53 gain of function: differential effects of different p53 mutants on resistance of cultured cells to chemotherapy. *Oncogene* 1999;**18**(2):477–85.
168 Hixon ML, Flores A, Wagner M, Gualberto A. Gain of function properties of mutant p53 proteins at the mitotic spindle cell cycle checkpoint. *Histol Histopathol* 2000;**15**(2):551–6.
169 Murphy KL, Dennis AP, Rosen JM. A gain of function p53 mutant promotes both genomic instability and cell survival in a novel p53-null mammary epithelial cell model. *Faseb J* 2000;**14**(14):2291–302.
170 Bristow RG, Peacock J, Jang A, Kim J, Hill RP, Benchimol S. Resistance to DNA-damaging agents is discordant from experimental metastatic capacity in MEF ras-transformants-expressing gain of function MTp53. *Oncogene* 2003;**22**(19): 2960–6.
171 Bossi G, Lapi E, Strano S, Rinaldo C, Blandino G, Sacchi A. Mutant p53 gain of function: reduction of tumor malignancy of human cancer cell lines through abrogation of mutant p53 expression. *Oncogene* 2006;**25**(2):304–9.
172 Liu G, McDonnell TJ, Montes de Oca Luna R, et al. High metastatic potential in mice inheriting a targeted p53 missense mutation. *Proc Natl Acad Sci USA* 2000;**97**(8):4174–9.
173 Lang GA, Iwakuma T, Suh YA, et al. Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. *Cell* 2004;**119**(6):861–72.
174 Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. *Cell* 2004;**119**(6):847–60.
175 Frazier MW, He X, Wang J, Gu Z, Cleveland JL, Zambetti GP. Activation of c-myc gene expression by tumor-derived p53 mutants requires a discrete C-terminal domain. *Mol Cell Biol* 1998;**18**(7):3735–43.
176 Kelavkar UP, Badr KF. Effects of mutant p53 expression on human 15-lipoxygenase-promoter activity and murine 12/15-lipoxygenase gene expression: evidence that 15-lipoxygenase is a mutator gene. *Proc Natl Acad Sci USA* 1999;**96**(8):4378–83.
177 Yang X, Pater A, Tang SC. Cloning and characterization of the human BAG-1 gene promoter: upregulation by tumor-derived p53 mutants. *Oncogene* 1999;**18**(32):4546–53.
178 Loging WT, Reisman D. Elevated expression of ribosomal protein genes L37, RPP-1, and S2 in the presence of mutant p53. *Cancer Epidemiol Biomarkers Prev* 1999;**8**(11):1011–6.
179 Lee YI, Lee S, Das GC, Park US, Park SM, Lee YI. Activation of the insulin-like growth factor II transcription by aflatoxin B1 induced p53 mutant 249 is caused by activation of transcription complexes; implications for a gain-of-function during the formation of hepatocellular carcinoma. *Oncogene* 2000;**19**(33):3717–26.
180 Preuss U, Kreutzfeld R, Scheidtmann KH. Tumor-derived p53 mutant C174Y is a gain-of-function mutant which activates the fos promoter and enhances colony formation. *Int J Cancer* 2000;**88**(2):162–71.
181 Iwanaga Y, Jeang KT. Expression of mitotic spindle checkpoint protein hsMAD1 correlates with cellular proliferation and is activated by a gain-of-function p53 mutant. *Cancer Res* 2002; **62**(9):2618–24.
182 Pugacheva EN, Ivanov AV, Kravchenko JE, Kopnin BP, Levine AJ, Chumakov PM. Novel gain of function activity of p53 mutants: activation of the dUTPase gene expression leading to resistance to 5-fluorouracil. *Oncogene* 2002;**21**(30):4595–600.
183 Zalcenstein A, Stambolsky P, Weisz L, et al. Mutant p53 gain of function: repression of CD95(Fas/APO-1) gene expression by tumor-associated p53 mutants. *Oncogene* 2003;**22**(36):5667–76.
184 Scian MJ, Stagliano KE, Ellis MA, et al. Modulation of gene expression by tumor-derived p53 mutants. *Cancer Res* 2004; **64**(20):7447–54.
185 Weisz L, Zalcenstein A, Stambolsky P, et al. Transactivation of the EGR1 gene contributes to mutant p53 gain of function. *Cancer Res* 2004;**64**(22):8318–27.

186 Zalcenstein A, Weisz L, Stambolsky P, Bar J, Rotter V, Oren M. Repression of the MSP/MST-1 gene contributes to the antiapoptotic gain of function of mutant p53. *Oncogene* 2006; **25**(3):359–69.
187 Vinall RL, Tepper CG, Shi XB, Xue LA, Gandour-Edwards R, de Vere White RW. The R273H p53 mutation can facilitate the androgen-independent growth of LNCaP by a mechanism that involves H2 relaxin and its cognate receptor LGR7. *Oncogene* 2006;**25**(14):2082–93.
188 Mizuarai S, Yamanaka K, Kotani H. Mutant p53 induces the GEF-H1 oncogene, a guanine nucleotide exchange factor-H1 for RhoA, resulting in accelerated cell proliferation in tumor cells. *Cancer Res* 2006;**66**(12):6319–26.
189 Wong RP, Tsang WP, Chau PY, Co NN, Tsang TY, Kwok TT. p53-R273H gains new function in induction of drug resistance through down-regulation of procaspase-3. *Mol Cancer Ther* 2007;**6**(3):1054–61.
190 Yan W, Liu G, Scoumanne A, Chen X. Suppression of inhibitor of differentiation 2, a target of mutant p53, is required for gain-of-function mutations. *Cancer Res* 2008;**68**(16):6789–96.
191 Ludes-Meyers JH, Subler MA, Shivakumar CV, et al. Transcriptional activation of the human epidermal growth factor receptor promoter by human p53. *Mol Cell Biol* 1996;**16**(11):6009–19.
192 Angelo LS, Talpaz M, Kurzrock R. Autocrine interleukin-6 production in renal cell carcinoma: evidence for the involvement of p53. *Cancer Res* 2002;**62**(3):932–40.
193 Tsutsumi-Ishii Y, Tadokoro K, Hanaoka F, Tsuchida N. Response of heat shock element within the human HSP70 promoter to mutated p53 genes. *Cell Growth Differ* 1995;**6**(1):1–8.
194 Lin J, Teresky AK, Levine AJ. Two critical hydrophobic amino acids in the N-terminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. *Oncogene* 1995;**10**(12):2387–90.
195 Matas D, Sigal A, Stambolsky P, et al. Integrity of the N-terminal transcription domain of p53 is required for mutant p53 interference with drug-induced apoptosis. *Embo J* 2001; **20**(15):4163–72.
196 Torgeman A, Mor-Vaknin N, Zelin E, et al. Sp1-p53 heterocomplex mediates activation of HTLV-I long terminal repeat by 12-O-tetradecanoylphorbol-13-acetate that is antagonized by protein kinase C. *Virology* 2001;**281**(1): 10–20.
197 Buganim Y, Kalo E, Brosh R, et al. Mutant p53 protects cells from 12-O-tetradecanoylphorbol-13-acetate-induced death by attenuating activating transcription factor 3 induction. *Cancer Res* 2006;**66**(22):10750–9.
198 Weisz L, Damalas A, Liontos M, et al. Mutant p53 enhances nuclear factor kappaB activation by tumor necrosis factor alpha in cancer cells. *Cancer Res* 2007;**67**(6):2396–401.
199 Kalo E, Buganim Y, Shapira KE, et al. Mutant p53 attenuates the SMAD-dependent transforming growth factor beta1 (TGF-beta1) signaling pathway by repressing the expression of TGF-beta receptor type II. *Mol Cell Biol* 2007;**27**(23):8228–42.
200 El-Hizawi S, Lagowski JP, Kulesz-Martin M, Albor A. Induction of gene amplification as a gain-of-function phenotype of mutant p53 proteins. *Cancer Res* 2002;**62**(11):3264–70.
201 Sampath J, Sun D, Kidd VJ, et al. Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. *J Biol Chem* 2001;**276**(42):39359–67.
202 Di Agostino S, Strano S, Emiliozzi V, et al. Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. *Cancer Cell* 2006;**10**(3):191–202.
203 Vikhanskaya F, Lee MK, Mazzoletti M, Broggini M, Sabapathy K. Cancer-derived p53 mutants suppress p53-target gene expression – potential mechanism for gain of function of mutant p53. *Nucleic Acids Res* 2007;**35**(6):2093–104.
204 Muller BF, Paulsen D, Deppert W. Specific binding of MAR/SAR DNA-elements by mutant p53. *Oncogene* 1996;**12**(9):1941–52.
205 Deppert W. Binding of MAR-DNA elements by mutant p53: possible implications for its oncogenic functions. *J Cell Biochem* 1996;**62**(2):172–80.
206 Deppert W, Gohler T, Koga H, Kim E. Mutant p53: "gain of function" through perturbation of nuclear structure and function? *J Cell Biochem* 2000;Suppl 35:115–22.
207 Strano S, Munarriz E, Rossi M, et al. Physical and functional interaction between p53 mutants and different isoforms of p73. *J Biol Chem* 2000;**275**(38):29503–12.
208 Lanza M, Marinari B, Papoutsaki M, et al. Cross-talks in the p53 family: deltaNp63 is an anti-apoptotic target for deltaNp73alpha and p53 gain-of-function mutants. *Cell Cycle* 2006;**5**(17):1996–2004.
209 Song H, Hollstein M, Xu Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. *Nat Cell Biol* 2007;**9**(5):573–80.
210 Bergamaschi D, Gasco M, Hiller L, et al. p53 polymorphism influences response in cancer chemotherapy via modulation of p73-dependent apoptosis. *Cancer Cell* 2003;**3**(4):387–402.
211 Morgan SE, Kim R, Wang PC, et al. Differences in mutant p53 protein stability and functional activity in teniposide-sensitive and -resistant human leukemic CEM cells. *Oncogene* 2000; **19**(43):5010–9.
212 Freedman DA, Wu L, Levine AJ. Functions of the MDM2 oncoprotein. *Cell Mol Life Sci* 1999;**55**(1):96–107.
213 Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. *Nucleic Acids Res* 1998;**26**(15):3453–9.
214 Vassilev LT, Vu BT, Graves B, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* 2004;**303**(5659):844–8.
215 Kojima K, Konopleva M, Samudio IJ, et al. MDM2 antagonists induce p53-dependent apoptosis in AML: implications for leukemia therapy. *Blood* 2005;**106**(9):3150–9.
216 Tovar C, Rosinski J, Filipovic Z, et al. Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. *Proc Natl Acad Sci USA* 2006;**103**(6):1888–93.
217 Issaeva N, Bozko P, Enge M, et al. Small molecule RITA binds to p53, blocks p53–HDM-2 interaction and activates p53 function in tumors. *Nat Med* 2004;**10**(12):1321–8.
218 Espinoza-Fonseca LM. Targeting MDM2 by the small molecule RITA: towards the development of new multi-target drugs against cancer. *Theor Biol Med Model* 2005;**2**:38.
219 Krajewski M, Ozdowy P, D'Silva L, Rothweiler U, Holak TA. NMR indicates that the small molecule RITA does not block p53–MDM2 binding in vitro. *Nat Med* 2005;**11**(11):1135–6; author reply 1136–7.
220 Grasberger BL, Lu T, Schubert C, et al. Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. *J Med Chem* 2005;**48**(4):909–12.
221 Koblish HK, Zhao S, Franks CF, et al. Benzodiazepinedione inhibitors of the Hdm2:p53 complex suppress human tumor cell proliferation in vitro and sensitize tumors to doxorubicin in vivo. *Mol Cancer Ther* 2006;**5**(1):160–9.

222 Parks DJ, Lafrance LV, Calvo RR, et al. 1,4-Benzodiazepine-2,5-diones as small molecule antagonists of the HDM2–p53 interaction: discovery and SAR. *Bioorg Med Chem Lett* 2005;**15**(3):765–70.
223 Ding K, Lu Y, Nikolovska-Coleska Z, et al. Structure-based design of spiro-oxindoles as potent, specific small-molecule inhibitors of the MDM2–p53 interaction. *J Med Chem* 2006; **49**(12):3432–5.
224 Lu Y, Nikolovska-Coleska Z, Fang X, et al. Discovery of a nanomolar inhibitor of the human murine double minute 2 (MDM2)-p53 interaction through an integrated, virtual database screening strategy. *J Med Chem* 2006;**49**(13):3759–62.
225 Wilson JM, Henderson G, Black F, et al. Synthesis of 5-deazaflavin derivatives and their activation of p53 in cells. *Bioorg Med Chem* 2007;**15**(1):77–86.
226 Lain S, Hollick JJ, Campbell J, et al. Discovery, in vivo activity, and mechanism of action of a small-molecule p53 activator. *Cancer Cell* 2008;**13**(5):454–63.
227 Guan YS, Liu Y, Zhou XP, Li X, He Q, Sun L. p53 gene (Gendicine) and embolisation overcame recurrent hepatocellular carcinoma. *Gut* 2005;**54**(9):1318–9.
228 INGN 201: Ad-p53, Ad5CMV-p53, Adenoviral p53, INGN 101, p53 gene therapy–Introgen, RPR/INGN 201. *BioDrugs* 2003;**17**(3):216–22.
229 Barnard DL. Technology evaluation: Sch-58500, Canji. *Curr Opin Mol Ther* 2000;**2**(5):586–92.
230 Guan YS, Liu Y, Sun L, Li X, He Q. Successful management of postoperative recurrence of hepatocellular carcinoma with p53 gene therapy combining transcatheter arterial chemoembolization. *World J Gastroenterol* 2005; **11**(24):3803–5.
231 Hansen S, Midgley CA, Lane DP, Freeman BC, Morimoto RI, Hupp TR. Modification of two distinct COOH-terminal domains is required for murine p53 activation by bacterial Hsp70. *J Biol Chem* 1996;**271**(48):30922–8.
232 Orgad S, Goldfinger N, Cohen G, Rotter V, Solomon B. Single chain antibody against the common epitope of mutant p53 restores wild-type activity to mutant p53 protein. *FEBS Lett* 2005;**579**(25):5609–15.
233 Joerger AC, Ang HC, Veprintsev DB, Blair CM, Fersht AR. Structures of p53 cancer mutants and mechanism of rescue by second-site suppressor mutations. *J Biol Chem* 2005;**280**(16): 16030–7.
234 Otsuka K, Kato S, Kakudo Y, Mashiko S, Shibata H, Ishioka C. The screening of the second-site suppressor mutations of the common p53 mutants. *Int J Cancer* 2007;**121**(3):559–66.
235 Suad O, Rozenberg H, Brosh R, et al. Structural basis of restoring sequence-specific DNA binding and transactivation to mutant p53 by suppressor mutations. *J Mol Biol* 2009;**385**(1): 249–65.
236 Foster BA, Coffey HA, Morin MJ, Rastinejad F. Pharmacological rescue of mutant p53 conformation and function. *Science* 1999;**286**(5449):2507–10.
237 Luu Y, Bush J, Cheung KJ, Jr., Li G. The p53 stabilizing compound CP-31398 induces apoptosis by activating the intrinsic Bax/mitochondrial/caspase-9 pathway. *Exp Cell Res* 2002;**276**(2):214–22.
238 Rehman A, Chahal MS, Tang X, Bruce JE, Pommier Y, Daoud SS. Proteomic identification of heat shock protein 90 as a candidate target for p53 mutation reactivation by PRIMA-1 in breast cancer cells. *Breast Cancer Res* 2005;**7**(5):R765–74.
239 Bykov VJ, Issaeva N, Shilov A, et al. Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. *Nat Med* 2002;**8**(3):282–8.
240 Bykov VJ, Issaeva N, Selivanova G, Wiman KG. Mutant p53-dependent growth suppression distinguishes PRIMA-1 from known anticancer drugs: a statistical analysis of information in the National Cancer Institute database. *Carcinogenesis* 2002; **23**(12):2011–8.
241 Weinmann L, Wischhusen J, Demma MJ, et al. A novel p53 rescue compound induces p53-dependent growth arrest and sensitises glioma cells to Apo2L/TRAIL-induced apoptosis. *Cell Death Differ* 2008;**15**(4):718–29.
242 Weinstein JN, Myers TG, O'Connor PM, et al. An information-intensive approach to the molecular pharmacology of cancer. *Science* 1997;**275**(5298):343–9.
243 North S, Pluquet O, Maurici D, El-Ghissassi F, Hainaut P. Restoration of wild-type conformation and activity of a temperature-sensitive mutant of p53 (p53(V272M)) by the cytoprotective aminothiol WR1065 in the esophageal cancer cell line TE-1. *Mol Carcinog* 2002;**33**(3):181–8.
244 Bykov VJ, Issaeva N, Zache N, et al. Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs. *J Biol Chem* 2005;**280**(34):30384–91.
245 Friedler A, Hansson LO, Veprintsev DB, et al. A peptide that binds and stabilizes p53 core domain: chaperone strategy for rescue of oncogenic mutants. *Proc Natl Acad Sci USA* 2002; **99**(2):937–42.
246 Bischoff JR, Kirn DH, Williams A, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996;**274**(5286):373–6.
247 Rothmann T, Hengstermann A, Whitaker NJ, Scheffner M, zur Hausen H. Replication of ONYX-015, a potential anticancer adenovirus, is independent of p53 status in tumor cells. *J Virol* 1998;**72**(12):9470–8.
248 Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff DD, Kirn DH. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nat Med* 1997;**3**(6):639–45.
249 Nemunaitis J, Ganly I, Khuri F, et al. Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial. *Cancer Res* 2000;**60**(22): 6359–66.