The novel p53 isoform “delta p53” is a misfolded protein and does not bind the p21 promoter site

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Abstract

The tumor suppressor p53 can be expressed as different isoforms because of promoter selection and mRNA editing. One isoform, “delta p53” (Δ p53), results from what would be an unusual alternative splicing of exons 7/8 of the p53 gene, conserving the reading frame and generating a novel protein with proposed transcriptional activity essential for the intra S-phase checkpoint. Here, we show that the deletion of the 66 residues that correspond to strand β 10 and the C-terminal helix of the core domain and the interconnecting linker to the tetramerization domain occurring in the Δ p53 isoform leads to a misfolded and unstable protein, prone to form soluble aggregates, which does not bind the p21 promoter site. The complex of coexpressed Δ p53 and flp53 is soluble in vitro and binds poorly to DNA. Our results provide a structural explanation for the dominant-negative effect of Δ p53 and its lack of transcriptional activity.

Keywords: protein structure/folding; structure/function studies; DNA-binding domains; dominant-negative mutation

Alteration of tissue homeostasis and formation of a tumor result from deregulation of cell differentiation, death rate, and proliferation. p53 is a node in regulation of these processes, and 50% of the human cancers contain mutations and alterations in its gene (Hainaut and Hollstein 2000). The p53-related genes p63 and p73 express a large number of isoforms derived from alternative splicing of the C terminus or species lacking the N-terminal transactivation domain (Deyoung and Ellisen 2007). These can bind to p53 response elements and exert dominant-negative effects either by competing for DNA-binding

sites or by direct protein–protein interactions (Harms and Chen 2005; Deyoung and Ellisen 2007). p53 isoforms arise either from differential promoter selection and/or alternative splicing (Bourdon et al. 2005; Rohaly et al. 2005). One of the proposed novel isoforms, delta p53 (Δ p53), results from alternative splicing of exons 7/8, conserving the frame and generating a deletion of 66 residues (257–322) in the DNA-binding domain and part of the loop linking to the tetramerization domain of p53 (Rohaly et al. 2005). However, the splicing mechanism that would lead to Δ p53 differs from that found in 99% of the genes transcribed by RNA polymerase II (Prives and Manfredi 2005; Murray-Zmijewski et al. 2006), where a 5' donor splice site starting with GU and a 3' acceptor splice site ending with AG are required (Nilsen 1994).

Full-length p53 (flp53) has intrinsically disordered regions (Wells et al. 2008) and two globular domains: the DNA-binding domain, which is unstable per se (Cho et al. 1994; Canadillas et al. 2006; Ho et al. 2006), and the tetramerization domain (Lee et al. 1994; Clore et al.

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Abbreviations: flp53, full-length p53; Δ p53, delta p53 (deletion of residues 257–322); Δ Cd, delta core domain (residues 94–256); SAXS, small-angle X-ray scattering; CD, circular dichroism; AUC, analytical ultracentrifugation; GFP–flp53, flp53 fusion with green fluorescent protein; HA– Δ p53, flp53 fusion with hemagglutinin.

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1995). The quaternary structure of flp53 has an elongated cross-shaped structure with a pair of loosely coupled core domain dimers at the ends, which are accessible for DNA binding (Tidow et al. 2007). Residues 257–322, spliced out in the Δ p53 isoform, correspond to strand β 10 and the C-terminal helix of the core domain and the interconnecting linker to the tetramerization domain (Fig. 1). Here, we show that deletion of the 66 residues from flp53 yields a misfolded protein, and we characterize its structural features. This novel protein was able to form a complex with flp53, modifying its biophysical properties in solution. Δ p53 did not bind the p21 promoter site, and the hybrid complex Δ p53–flp53 bound the DNA very weakly. Δ p53 was mainly localized in the cytoplasm, but when coexpressed with flp53 both proteins colocalized in the nucleus.

Figure 1. Structural elements deleted in Δ p53 with respect to flp53. (A) SAXS model of p53 in solution (Tidow et al. 2007). Core domains (cyan) and tetramerization domain (blue) are shown as cartoons, and connecting linkers in semitransparent spacefill mode. Residues 257–322 that are deleted in Δ p53 are colored orange. For clarity, the flexible N and C termini are not shown. (B) Close-up of p53 DNA-binding core domain with residues deleted in Δ p53 shown in orange.

Results

Biophysical characterization of Δ p53

Residues 94 to 292 of the p53 protein correspond to a structural domain defined as the core domain. It is a DNA-binding domain that belongs to the immunoglobulin-like fold with a central β -sandwich of two antiparallel β -sheets and contains a structural zinc ion chelated by Cys-176, His-179, Cys-238, and Cys-242 (Fig. 1B; Joerger and Fersht 2007). Deletion of residues 257–292 led to a misfolded core domain (Δ Cd). Figure 2A shows the far-UV circular dichroism (CD) spectra of the native core domain and of Δ Cd, indicating a clear loss in its secondary structure content. The native core domain has an apparent melting temperature of 44°C (Ang et al. 2006), while Δ Cd lost all cooperativity of denaturation and displayed only a linear increase of the CD signal at 222 nm (Fig. 2B). In addition, Δ Cd was unstable in solution and prone to form soluble aggregates. The analytical ultracentrifugation (AUC) sedimentation profile of the native core domain corresponded to a molecular weight of $\sim$ 24 kDa, while the AUC profile of Δ Cd corresponded to an average mass of $\sim$ 204 kDa, indicating a high oligomerization state (Fig. 2C). This truncated core domain did not contain Zn as tested by the PAR/PMPS colorimetric assay (Hunt et al. 1985; data not shown). Zn-binding proteins are to be able to form high molecular soluble oligomers in the absence of the metal (Alonso et al. 2004; García-Alai et al. 2007).

Next we carried out structural studies with the Δ p53 isoform in the context of flp53. We used small-angle X-ray scattering (SAXS) to investigate overall protein dimensions in solution. A comparison of the processed experimental patterns (scattering intensity I vs. momentum transfer s , $= 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and $\lambda = 1.5$ Å is the wavelength) is displayed in Figure 3A and the corresponding Kratky plot in Figure 3C. Δ p53 showed a very different scattering pattern from flp53, with a large slope at small angles indicating the presence of soluble high molecular weight oligomers. This observation is consistent with a nonlinear Guinier plot for Δ p53 (data not shown). The distance distribution function revealed that the average particle size of Δ p53 was significantly larger than that of flp53 ($D_{\max}$ 600 Å vs. 240 Å) (Fig. 3B).

As SAXS data represent an average over all species present in solution, the existence of a minor population of tetrameric Δ p53 could not be excluded although the overall parameters indicated that the majority of protein existed as higher oligomeric forms. The AUC sedimentation profile of Δ p53 was consistent with the SAXS data. The absorbance of Δ p53 (recorded at 280 nm) was lower than that measured for flp53 as a result of aggregation and faster sedimentation of the high molecular weight species

Figure 2. Biophysical characterization of p53 truncated core domain. (A) Far-UV CD spectra for p53 core domain (gray) and $\Delta C\delta$ (black). (B) Thermal denaturation for p53 core domain (○) and $\Delta C\delta$ (●). (C) AUC sedimentation profiles for p53 core domain and $\Delta C\delta$ show apparent molecular masses of 24 ± 0.3 kDa and 204 ± 17 kDa, respectively. Data were fitted to a single exponential model. Color code as in B.

during the experiment. The remaining species had a sedimentation coefficient that could correspond to tetramers (data not shown).

We analyzed the thermal stability for both proteins by far-UV CD. The absolute value for the molar ellipticity at 222 nm at 20°C was smaller for $\Delta p53$ than for the full-

length protein, indicating its lower content of α -helical secondary structure (Fig. 4A). flp53 had a first transition (20°C–65°C) with an apparent $T_m = 50 \pm 0.1^\circ\text{C}$ that corresponds to denaturation of the DNA-binding domain in the context of the full-length protein (Fig. 4B; Bullock et al. 2000; Ang et al. 2006). This behavior was not observed for the $\Delta p53$ isoform, which only shows a small amplitude in its curve (Fig. 4A). Both proteins had an increase in their 222-nm molar ellipticity signal $\sim 70^\circ\text{C}$.

Figure 3. SAXS analysis of $\Delta p53$ and flp53. (A) Experimental intensities for $\Delta p53$ (black) and flp53 (gray). The scattering profiles are displaced along the ordinate for better visualization. (B) The distance distribution plots computed from the experimental data and normalized to the maximum value of unity. (C) Kratky plots of SAXS data for $\Delta p53$ and flp53 normalized to the maximum value of unity. Color code as in A. The maximum at small angles indicates the presence of folded domains.

Figure 4. (A) Thermal denaturation curves for flp53 (gray) and $\Delta p53$ (black), molar ellipticity at 222 nm is plotted as a function of temperature. Curves are fitted to a two-state model showing denaturation of the DNA-binding domain for flp53 (B) and tetramerization domain for $\Delta p53$ (C).

This second transition had an apparent $T_m = 74 \pm 0.2^{\circ}\text{C}$ for $\Delta p53$ (Fig. 4C) corresponding to denaturation of the tetramerization domain (Mateu and Fersht 1998, 1999). The $\Delta p53$ isoform had only the transition corresponding to unfolding of the tetramerization domain, indicating it was still capable of oligomerization but lacked a structured DNA-binding domain.

The decrease in CD signal ~ 220 nm is not due to an increase in the helix content of the protein but to the rearrangement of its β -sheets due to hydrophobic interactions. The core domain of p53 has a central β -sandwich of two antiparallel β -sheets. Many β -sheet-containing proteins show this behavior; they display a decrease in signal ~ 217 nm as temperature is increased. This corresponds to the denaturation-aggregation of the protein.

Characterization of the $\Delta p53$ –flp53 complex

Whereas flp53 was expressed as a soluble protein, $\Delta p53$ was expressed in inclusion bodies. But, when flp53 and $\Delta p53$ were coexpressed, both proteins were detected in the soluble fraction. Coexpression of flp53 and $\Delta p53$ led to the formation of a mixed $\Delta p53$ –flp53 complex as revealed by analytical gel filtration. After coexpression only one peak corresponding to the $\Delta p53$ –flp53 complex could be detected in the void volume (Fig. 5). No peak corresponding to tetrameric p53, which has a significantly smaller hydrodynamic radius than $\Delta p53$, could be detected. This implicated that coexpression of flp53 and $\Delta p53$ leads to the formation of mixed oligomers with a large hydrodynamic radius.

While ectopically expressed $\Delta p53$ was localized mainly in the cytoplasm of H1299 cells (p53 null cells) (Fig. 6B), consistent with the deletion of its nuclear localization signal (NLS, residues 316–325) (Shaulsky et al. 1990; Chan and Poon 2007), coexpression of flp53 and $\Delta p53$ causes the $\Delta p53$ –flp53 complex to localize in the nucleus (Fig. 6E). In this case flp53 provided the NLS and shuttled $\Delta p53$ to the nucleus after forming a complex.

$\Delta p53$ does not bind to the p21 response element and significantly reduces DNA binding when forming a mixed $\Delta p53$ –flp53 complex

It has been proposed that $\Delta p53$ transactivates the endogenous p21 and 14-3-3 σ promoters in damaged S-phase cells (Rohaly et al. 2005). In contrast, this isoform does not transactivate either the promoters of p21 and mdm2 in H1299 cells (Chan and Poon 2007). Using fluorescence

Figure 5. Size exclusion chromatography elution profiles for flp53 and $\Delta p53$ when mixed (gray line), and $\Delta p53$ –flp53 complex when coexpressed (black line). Bars denote the positions of the molecular weight standards: (left to right) thyroglobulin (669 kDa); ferritin (440 kDa); aldolase (158 kDa); conalbumin (75 kDa); ovalbumin (43 kDa).

Figure 6. Confocal sections through transiently transfected H1299 cells. GFP-p53 and HA- Δ p53 were transfected alone (A,B), or cotransfected (C,D, and merged in E). HA- Δ p53 is cytoplasmic but relocalized to the nucleus upon coexpression with GFP-p53.

anisotropy, we have previously measured the affinity of tetrameric p53 to fluorescein-labeled specific DNA (Weinberg et al. 2004; Ang et al. 2006). Here, we found that Δ p53 did not bind the p21 response element (Fig. 7). flp53 bound to it with a $K_d = 47 \pm 0.2$ nM. The complex of flp53 and Δ p53 (1 μ M monomer concentration, same as for flp53) had an almost linear increase in anisotropy signal over a protein concentration range of 0–500 nM, showing that the coexpressed Δ p53–flp53 complex bound only weakly to the p21 promoter site. Binding was observed at μ M concentrations of the complex (Fig. 7B) with a $K_d = 3.7 \pm 0.2$ μ M. The binding was most likely due to the presence of a small portion ($\sim 1\%$) of homotetrameric flp53.

Discussion

Editing of mRNA increases the coding capacity of a gene by expressing several related proteins, which introduces diversity in their functions and even antagonistic functions (Kriventseva et al. 2003). Alternative splicing is a crucial mechanism for regulating cell differentiation and developmental stages, and it has been recently shown that splicing patterns can be significantly altered in tumors (Hu and Fu 2007; Karni et al. 2007). The discovery of p53 isoforms could unveil different mechanisms in the tumor suppressor regulation, modulation of its activity, and also novel functions.

Here, we showed that the proposed Δ p53 isoform (Rohaly et al. 2005) is an unfolded protein. This isoform, lacking a part of the DNA-binding domain and connecting the linker to the tetramerization domain, was unstable in solution and prone to form soluble aggregates. Deletion of residues 257–322 from flp53 to form Δ p53 removes essential elements for the structure and the function of

p53; the end of β -strand β 9 and the entire β -strand β 10, which is the longest strand within the core domain, are involved in a strong hydrogen-bond network and form an essential part of the immunoglobulin-like β -sandwich (Fig. 1). This β -sandwich is responsible for the stability of the DNA-binding core domain, and thus removal of a major strand should cause unfolding. The C-terminal helix is involved in DNA binding and further connects the core domain to the tetramerization domain via a linker, which is also entirely removed in Δ p53 (Fig. 1). This would cause the remaining part of the core domain to be positioned in close proximity to the tetramerization domain, most likely introducing steric clashes, even if it were folded. Our results provide for the first time a structural explanation why Δ p53 on its own is not able to bind DNA (Fig. 7), and its complex with flp53 binds only weakly to the p21 site. This lack in DNA-binding ability also occurs in the so-called p53 “hot-spot mutants,” which can be further classified into “structural mutants” and “contact mutants” (Joerger et al. 2005, 2006), both showing little or no transcriptional activity. Thus, it is unlikely that Δ p53 can act as a transcription factor.

Despite the loss of a folded DNA-binding core domain, Δ p53 retains its tetramerization domain (Fig. 4) and has

Figure 7. (A) Binding of p53, Δ p53, and the coexpressed complex Δ p53–flp53 to the p21 DNA promoter site. One μ M (monomer concentration) of flp53 ($\blacktriangle$), Δ p53 ($\blacksquare$), and Δ p53–flp53 ($\bullet$) was measured at 20°C in 25 mM phosphate buffer (pH 7.2), 225 mM NaCl, and 5 mM DTT. (B) Titration using 100 μ M of the complex Δ p53–flp53.

the capacity to form mixed tetramers with p53. We co-expressed p53 and Δ p53 in *Escherichia coli* and purified the complex. The fact that both proteins were expressed in soluble form suggests the existence of mixed oligomers (see Fig. 5), which is consistent with Δ p53 lacking a NLS being imported to the nucleus when coexpressed with flp53 in H1299 cells (Fig. 6). The mixed oligomer species, however, do not show the same DNA-binding activity as tetrameric flp53 anymore. This emphasizes that Δ p53 has a dominant-negative effect on flp53. In general, a similar dominant-negative effect will most likely occur for every p53 splice variant with intact tetramerization domain but defect in the DNA-binding core domain.

The detection of the mRNA encoding for Δ p53 as well as the existence of the polypeptide and its transcriptional activity is the subject of debate (Murray-Zmijewski et al. 2006; Chan and Poon 2007). While Δ p53 mRNA could be detected in human breast tumor samples (Baumbusch et al. 2006), it could not be detected in a large number of normal human tissues or tumors in another study (Murray-Zmijewski et al. 2006). If Δ p53 is a product of alternative splicing and has been positively selected through evolution, the reason for this is most likely inhibition of transcription rather than selective transcriptional activation. There is a correlation between protein thermodynamic stability and degradation (Canadillas et al. 2006), and Δ p53 could also regulate p53 turnover by making the tetramer more unstable.

Whereas the existence of Δ p53 at the mRNA level is still controversial (Murray-Zmijewski et al. 2006), the structural characterization of this novel p53 isoform provides new insights into the importance of defined structural elements and gives a structural explanation for its lack of transcriptional activity. If this protein is the product of a deletion and not the result of splicing, then it could be considered as a dominant-negative mutation.

Materials and Methods

Plasmids and recombinant proteins

flp53 and core domain were expressed in *E. coli* and purified as described (Veprintsev et al. 2006). Δ Cd was amplified by PCR with the following primers: Fw5'-GGTGGATCCTCATCTTC TGTCCC-3' and Rev5'-TCGAATTCAGGTGTTGTTGGGCA-3'. The Δ p53 was amplified by inverse PCR from the wild-type p53 clone, deleting residues 257 to 322 using the following primers: Fw5'-CTGGATGGAGAATATTCACC-3' and Rev5'-TGTGATGATGGTGAGGATGGG-3'. Both Δ Cd and Δ p53 were expressed as inclusion bodies. These were dissolved in 6 M GdmHCl, diluted in buffer A (20 mM sodium phosphate [pH 7.2], 150 mM NaCl, and 6 M urea), loaded onto a HisTrap column (GE Healthcare), and eluted in buffer A containing 250 mM imidazole. The refolding step was carried out at 4°C by dialysis against 20 mM sodium phosphate (pH 7.2), 40 μ M ZnSO₄, and 1 mM DTT. Further purification of both proteins

was performed as for flp53 (Veprintsev et al. 2006). For the Δ p53–flp53 complex, BL21 (DE3) were cotransformed with pET24– Δ p53 and pRSET–flp53 and grown at 22°C in 2TY media containing 50 and 100 μ g/mL kanamycin and ampicillin, respectively. Cells were harvested 18 h after induction. Both proteins were expressed as soluble proteins and purification of the complex was carried out as described for flp53 (Veprintsev et al. 2006). For cell transfections flp53 and Δ p53 were subcloned into pCDNA 3.1. For immunofluorescence the following vectors were constructed: pEGFP–flp53 and pCHA– Δ p53.

Small-angle X-ray scattering (SAXS)

SAXS data were collected at the X33 beamline at EMBL/DESY, Hamburg (Roessle et al. 2007), following standard procedures. Repetitive data collection on the same sample was performed to monitor possible radiation damage, and no damage was detected. The camera length was 2.7 m at X33 covering the range of scattering vectors $0.012 < s < 0.47 \text{ \AA}^{-1}$ at a wavelength $\lambda = 1.5 \text{ \AA}$. The sample concentration was 3.5 mg mL⁻¹ in 25 mM sodium phosphate (pH 7.2), 150 mM NaCl, 5 mM DTT. The data were processed using PRIMUS (Konarev et al. 2003). The radius of gyration (R_g) was evaluated using the Guinier approximation (Guinier 1939) ($I(s) = I(0)\exp(-s^2 R_g^2/3)$ for $sR_g < 1.3$) and also from the entire scattering curve with the program GNOM (Svergun 1992), the latter also provided distance distribution function $p(r)$ and the maximum dimension $D_{\max}$. The M_r of the solutes were evaluated by comparison of the forward scattering intensity with that from a BSA reference solution ($M_r = 66 \text{ kDa}$).

Circular dichroism

Measurements were performed using a Jasco J-815 spectropolarimeter; 20 μ M of p53 core domain, Δ Cd, flp53, or Δ p53 were incubated for 4 h in 20 mM Tris (pH 7.5), 150 mM NaCl, and 1 mM DTE. Melting curves were monitored following the amplitude of negative band at 222 nm as a function of temperature with an increasing slope of 1°C/min and fitted to a two-state model as described (Neira et al. 2000).

Analytical gel filtration

A 0.5 mL of 30 μ M of flp53, Δ p53, and the complex p53– Δ p53 were injected onto an analytical Superdex 200 HR10/30 column. Buffer conditions were 20 mM sodium phosphate buffer (pH 7.2), 150 mM KCl, 10 mM 2-mercaptoethanol, and 10% glycerol. Molecular weight standards (Pharmacia) were run under the same conditions; V0 corresponds to elution of blue-dextran.

Analytical ultracentrifugation

Equilibrium sedimentation experiments were performed with a Beckman XL-I Ultracentrifuge equipped with a Ti-60 rotor and 6-sector cells at 3000, 10,000 and 30,000 rpm, respectively. Data were collected at 10°C following absorbance at 280 nm, at a protein concentration of 20 mM. Buffer conditions were 25 mM sodium phosphate (pH 7.2), 150 mM NaCl, and 1 mM DTT. Samples were considered to be at equilibrium as judged by a

comparison of the several scans of each. Data were analyzed using the UltraSpin software (<http://ultraspin.mrc-cpe.cam.ac.uk/>).

DNA binding

Fluorescence anisotropy measurements were recorded on a Cary Eclipse fluorescence spectrophotometer equipped with a Hamilton Microlab titrator controlled by laboratory software. The excitation and emission wavelengths were 480 nm and 530 nm, respectively, and the slit widths for excitation and emission were 20 nm with photomultiplier voltage of 600 V. The concentrations of flp53 and Δ p53 were 1 μ M in terms of monomer. The concentration of the flp53- Δ p53 complex titrant, in terms of monomer species, was 1 μ M and 100 μ M, respectively. The initial concentration of DNA was 20 nM. Experiments were performed at 20°C in 25 mM phosphate buffer (pH 7.2), 225 mM NaCl, 5 mM DTT. p53 protein was titrated into a cuvette containing 1.2 mL fluorescein-labeled DNA as described in Weinberg et al. (2004). Fluorescence anisotropy was calculated from the fluorescence intensities (Lakowicz 1999) and analysis of binding was performed using a simple one-site binding isotherm with 4:1 stoichiometry (Weinberg et al. 2004).

Cell culture

H1299 cells were grown and maintained in Roswell Park Memorial Institute (RPMI) medium (Invitrogen) supplemented with 10% fetal calf serum (FCS). Cells were subcultured at a ratio of 1:6 the day before transfection in 35-mm culture wells and transfected with Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were grown on glass coverslips. The coverslips were removed 24 h after transfection and rinsed briefly with PBS. The cells were fixed for 20 min with 4% paraformaldehyde in PBS, then permeabilized by washing three times with PBS + 0.1% Triton X-100 (TX100). For blocking, the coverslips were incubated for 30 min with PBS + 0.1% TX100 + 1% BSA and incubated for 1 h with rat anti-HA (Roche) diluted 1:100 in the same solution. Coverslips were then washed three times with PBS + 0.1% TX100 and incubated for 1 h in the dark with Alexa Fluor 488 goat anti-mouse antibody (Molecular Probes) diluted 1:200 in blocking solution. Coverslips were again washed three times with PBS + 0.1% TX100, then air-dried and mounted in Fluoromount-G (SouthernBiotech). Images were collected on a Bio-Rad Radianc confocal microscope.

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References

- Alonso, L.G., García-Alai, M.M., Smal, C., Centeno, J.M., Iacono, R., Castano, E., Gualfetti, P., and de Prat-Gay, G. 2004. The HPV16 E7 viral oncoprotein self-assembles into defined spherical oligomers. *Biochemistry* **43**: 3310–3317.
- Ang, H.C., Joerger, A.C., Mayer, S., and Fersht, A.R. 2006. Effects of common cancer mutations on stability and DNA binding of full-length p53 compared with isolated core domains. *J. Biol. Chem.* **281**: 21934–21941.
- Baumbusch, L.O., Myhre, S., Langerod, A., Bergamaschi, A., Geisler, S.B., Lonning, P.E., Deppert, W., Dornreiter, I., and Borresen-Dale, A.L. 2006. Expression of full-length p53 and its isoform Δ p53 in breast carcinomas in relation to mutation status and clinical parameters. *Mol. Cancer* **5**: 47. doi: 10.1186/1476-4598-5-47.
- Bourdon, J.C., Fernandes, K., Murray-Zmijewski, F., Liu, G., Diot, A., Xirodimas, D.P., Saville, M.K., and Lane, D.P. 2005. p53 isoforms can regulate p53 transcriptional activity. *Genes & Dev.* **19**: 2122–2137.
- Bullock, A.N., Henckel, J., and Fersht, A.R. 2000. Quantitative analysis of residual folding and DNA binding in mutant p53 core domain: Definition of mutant states for rescue in cancer therapy. *Oncogene* **19**: 1245–1256.
- Canadillas, J.M., Tidow, H., Freund, S.M., Rutherford, T.J., Ang, H.C., and Fersht, A.R. 2006. Solution structure of p53 core domain: Structural basis for its instability. *Proc. Natl. Acad. Sci.* **103**: 2109–2114.
- Chan, W.M. and Poon, R.Y. 2007. The p53 isoform Δ p53 lacks intrinsic transcriptional activity and reveals the critical role of nuclear import in dominant-negative activity. *Cancer Res.* **67**: 1959–1969.
- Cho, Y., Gorina, S., Jeffrey, P.D., and Pavletich, N.P. 1994. Crystal structure of a p53 tumor suppressor-DNA complex: Understanding tumorigenic mutations. *Science* **265**: 346–355.
- Clore, G.M., Ernst, J., Clubb, R., Omichinski, J.G., Kennedy, W.M., Sakaguchi, K., Appella, E., and Gronenborn, A.M. 1995. Refined solution structure of the oligomerization domain of the tumour suppressor p53. *Nat. Struct. Biol.* **2**: 321–333.
- Deyoung, M.P. and Ellisen, L.W. 2007. p63 and p73 in human cancer: Defining the network. *Oncogene* **26**: 5169–5183.
- García-Alai, M.M., Dantur, K.L., Smal, C., Pietrasanta, L., and de Prat-Gay, G. 2007. High-risk HPV E6 oncoproteins assemble into large oligomers that allow localization of endogenous species in prototypic HPV-transformed cell lines. *Biochemistry* **46**: 341–349.
- Guinier, A. 1939. La diffraction des rayons X aux tres petits angles: Application a l'étude de phenomenes ultramicroscopiques. *Ann. Phys.* **12**: 161–237.
- Hainaut, P. and Hollstein, M. 2000. p53 and human cancer: The first ten thousand mutations. *Adv. Cancer Res.* **77**: 81–137.
- Harms, K.L. and Chen, X. 2005. The C terminus of p53 family proteins is a cell fate determinant. *Mol. Cell. Biol.* **25**: 2014–2030.
- Ho, W.C., Fitzgerald, M.X., and Marmorstein, R. 2006. Structure of the p53 core domain dimer bound to DNA. *J. Biol. Chem.* **281**: 20494–20502.
- Hu, A. and Fu, X.D. 2007. Splicing oncogenes. *Nat. Struct. Mol. Biol.* **14**: 174–175.
- Hunt, J.B., Neece, S.H., and Ginsburg, A. 1985. The use of 4-(2-pyridylazo) resorcinol in studies of zinc release from *Escherichia coli* aspartate transcarbamoylase. *Anal. Biochem.* **146**: 150–157.
- Joerger, A.C. and Fersht, A.R. 2007. Structure-function-rescue: The diverse nature of common p53 cancer mutants. *Oncogene* **26**: 2226–2242.
- Joerger, A.C., Ang, H.C., Veprintsev, D.B., Blair, C.M., and Fersht, A.R. 2005. Structures of p53 cancer mutants and mechanism of rescue by second-site suppressor mutations. *J. Biol. Chem.* **280**: 16030–16037.
- Joerger, A.C., Ang, H.C., and Fersht, A.R. 2006. Structural basis for understanding oncogenic p53 mutations and designing rescue drugs. *Proc. Natl. Acad. Sci.* **103**: 15056–15061.
- Karni, R., de Stanchina, E., Lowe, S.W., Sinha, R., Mu, D., and Krainer, A.R. 2007. The gene encoding the splicing factor SF2/ASF is a proto-oncogene. *Nat. Struct. Mol. Biol.* **14**: 185–193.
- Konarev, P.V., Volkov, V.V., Sokolova, A.V., Koch, M.H.J., and Svergun, D.I. 2003. PRIMUS: A Windows PC-based system for small-angle scattering data analysis. *J. Appl. Crystallogr.* **36**: 1277–1282.
- Kriventseva, E.V., Koch, I., Apweiler, R., Vingron, M., Bork, P., Gelfand, M.S., and Sunyaev, S. 2003. Increase of functional diversity by alternative splicing. *Trends Genet.* **19**: 124–128.
- Lakowicz, J.R. 1999. *Principles of fluorescence spectroscopy*, 2d ed. Kluwer Academic/Plenum Publishers, New York.
- Lee, W., Harvey, T.S., Yin, Y., Yau, P., Litchfield, D., and Arrowsmith, C.H. 1994. Solution structure of the tetrameric minimum transforming domain of p53. *Nat. Struct. Biol.* **1**: 877–890.
- Mateu, M.G. and Fersht, A.R. 1998. Nine hydrophobic side chains are key determinants of the thermodynamic stability and oligomerization status of tumour suppressor p53 tetramerization domain. *EMBO J.* **17**: 2748–2758.
- Mateu, M.G. and Fersht, A.R. 1999. Mutually compensatory mutations during evolution of the tetramerization domain of tumor suppressor p53 lead to impaired hetero-oligomerization. *Proc. Natl. Acad. Sci.* **96**: 3595–3599.
- Murray-Zmijewski, F., Lane, D.P., and Bourdon, J.C. 2006. p53/p63/p73 isoforms: An orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ.* **13**: 962–972.
- Neira, J.L., Vazquez, E., and Fersht, A.R. 2000. Stability and folding of the protein complexes of barnase. *Eur. J. Biochem.* **267**: 2859–2870.

- Nilsen, T.W. 1994. RNA-RNA interactions in the spliceosome: Unraveling the ties that bind. *Cell* **78**: 1–4.
- Prives, C. and Manfredi, J.J. 2005. The continuing saga of p53—more sleepless nights ahead. *Mol. Cell* **19**: 719–721.
- Roessle, M.W., Klaering, R., Ristau, U., Robrahn, B., Jahn, D., Gehrmann, T., Konarev, P., Round, A., Fiedler, S., Hermes, C., et al. 2007. Upgrade of the small-angle X-ray scattering beamline X33 at the European Molecular Biology Laboratory, Hamburg. *J. Appl. Crystallogr.* **40**: S190–S194.
- Rohaly, G., Chemnitz, J., Dehde, S., Nunez, A.M., Heukeshoven, J., Deppert, W., and Dornreiter, I. 2005. A novel human p53 isoform is an essential element of the ATR-intra-S phase checkpoint. *Cell* **122**: 21–32.
- Shaulsky, G., Goldfinger, N., Ben-Ze'ev, A., and Rotter, V. 1990. Nuclear accumulation of p53 protein is mediated by several nuclear localization signals and plays a role in tumorigenesis. *Mol. Cell. Biol.* **10**: 6565–6577.
- Svergun, D.I. 1992. Determination of the regularization parameter in indirect-transform methods using perceptual criteria. *J. Appl. Crystallogr.* **25**: 495–503.
- Tidow, H., Melero, R., Mylonas, E., Freund, S.M., Grossmann, J.G., Carazo, J.M., Svergun, D.I., Valle, M., and Fersht, A.R. 2007. Quaternary structures of tumor suppressor p53 and a specific p53 DNA complex. *Proc. Natl. Acad. Sci.* **104**: 12324–12329.
- Veprintsev, D.B., Freund, S.M., Andreeva, A., Rutledge, S.E., Tidow, H., Canadillas, J.M., Blair, C.M., and Fersht, A.R. 2006. Core domain interactions in full-length p53 in solution. *Proc. Natl. Acad. Sci.* **103**: 2115–2119.
- Weinberg, R.L., Veprintsev, D.B., and Fersht, A.R. 2004. Cooperative binding of tetrameric p53 to DNA. *J. Mol. Biol.* **341**: 1145–1159.
- Wells, M., Tidow, H., Rutherford, T.J., Markwick, P., Jensen, M.R., Mylonas, E., Svergun, D.I., Blackledge, M., and Fersht, A.R. 2008. Structure of tumor suppressor p53 and its intrinsically disordered N-terminal transactivation domain. *Proc. Natl. Acad. Sci.* **105**: 5762–5767.