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Common Conformational Effects in the P53 Protein of Vinyl Chloride-Induced Mutations

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The tumor suppressor gene p53 has been identified as the most frequent site of genetic alterations in human cancers. Vinyl chloride, a known human carcinogen, has been associated with specific A → T transversions at codons 179, 249, and 255 of the p53 gene. The mutations result in amino acid substitutions of His → Leu at residue 179, Arg → Trp at residue 249, and Ile → Phe at residue 255 in highly conserved regions of the DNA-binding core domain of the p53 protein. We previously used molecular dynamics calculations to demonstrate that the latter two mutants contain certain common regions that differ substantially in conformation from the wild-type structure. In order to determine whether these conformational changes are consistent for other p53 mutants, we have now used molecular dynamics to determine the structure of the DNA-binding core domain of the Leu 179 p53 mutant. The results indicate that the Leu 179 mutant differs substantially from the wild-type structure in certain discrete regions that are similar to those noted previously in the other p53 mutants. One of these regions (residues 204–217) contains the epitope for the monoclonal antibody PAb240, which is concealed in the wild-type structure, but accessible in the mutant structure, and another region (residues 94–110) contains the epitope for the monoclonal antibody PAb1620, which is accessible in the wild-type structure, but concealed in the mutant structure. Immunologic analyses of tumor tissue known to contain this mutation confirmed these predicted conformational shifts in the mutant p53 protein.

KEY WORDS: p53; mutation; vinyl chloride; molecular dynamics; immunohistochemistry.

1. INTRODUCTION

The p53 tumor suppressor gene is the most frequently identified site for mutations in human cancers (Lane, 1994). The encoded wild-type p53 protein is a sequence-specific transcriptional factor that *trans*-activates a number of genes whose products are involved in cell growth regulation (Ko and Prives, 1996). The majority of cancer-related mutations in p53 cluster in several "hot-spot" regions of the protein that have been highly conserved throughout evolution and occur in the sequence-specific DNA-binding core domain of the protein between amino acid residues 102 and 292 (Fromentel and Soussi, 1992). The mutations in p53 found in malignancies could thus result in substitutions of amino acid residues in these regions that are critical for the determination of the structure, and hence cell-cycle regulatory function, of the protein, contributing to the development of cancer.

Vinyl chloride is a known animal and human carcinogen whose electrophilic metabolites react with DNA to form adducts that are known to be mutagenic in bacterial and mammalian cells (Barbin and Bartsch, 1986). The ethenoadenine adducts formed are believed to account for the occurrence of adenine → thymine transversions identified in the p53 tumor suppressor gene in vinyl chloride-

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[6] Abbreviations: AMBER, Assisted Model Building with Energy Refinements; ECEPP, Empirical Conformational Energy Program for Peptides; rms, root-mean-square.

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induced angiosarcomas of the liver in exposed workers. These mutations occur at codons 179, 249, and 255 in different, highly conserved "hot-spot" regions of the p53 protein (Hollstein *et al.,* 1994; Trivers *et al.,* 1995), resulting in the substitution of leucine for the normal histidine at amino acid residue 179, the substitution of tryptophan for the normal arginine at amino acid residue 249, and the substitution of phenylalanine for the normal isoleucine at amino acid residue 255.

Using molecular dynamics calculations and immunologic analyses, we have previously examined the conformational effects of two of these vinyl chloride-induced mutations (Arg → Trp at 249 and Ile → Phe at 255) in the DNA-binding core domain of p53 (Brandt-Rauf *et al.,* 1996). Comparisons of the computed average structures from molecular dynamics for the wild-type and mutant proteins showed that both mutants differed substantially from the wild-type structure in certain common discrete regions, and the movement of one of these regions was confirmed immunologically by the change in detectability of its epitope (Brandt-Rauf *et al.,* 1996). In order to further correlate changes in p53 conformation with cancer-related amino acid substitutions, we have now used similar approaches to examine the effects of the third vinyl chloride-induced mutation (His → Leu at 179) on the structure of the DNA-binding core domain of p53.

2. METHODS

Molecular dynamics calculations were performed as described previously (Brandt-Rauf *et al.,* 1996). The starting structure was the wild-type p53 protein from amino acid residues 94–312 generated from the X-ray crystal coordinates (Cho *et al.,* 1994) and energy-minimized as described (Brandt-Rauf *et al.,* 1996). The conformation of the mutant Leu 179-p53 protein was constructed from the minimized wild-type structure by substituting leucine for the normally occurring histidine at position 179 in the wild-type structure. The backbone dihedral angles for the substituted residue were initially the same as for the corresponding amino acid in the wild-type protein. Starting side-chain conformation for the substituted residue was the lowest energy conformation one for the given backbone conformation as determined by ECEPP[6] (Vasquez *et al.,* 1983). This structure was then subjected to nested molecular dynamics and energy minimizations using ECEPP and AMBER potential functions, as described previously (Brandt-Rauf *et al.,* 1996). Dynamics runs were performed for a total of 700 psec, and the total conformational energy converged to a minimum value. The last 50 isoenergetic structures on the trajectory were employed in computing the average structure and the coordinate fluctuations. The average structure for the mutant form was superimposed on that for the wild-type protein such that the rms deviation of the coordinates of the backbone atoms of one structure from the other was a minimum. The average rms deviation between the mutant protein and the wild-type protein was determined for each amino acid residue.

This approach has been shown to give results that are consistent with experimental data. For example, computed conformational changes in p53 mutants have been confirmed by movement of antibody epitopes (Brandt-Rauf *et al.,* 1996) and phosphorylation sites (Adler *et al.,* 1997) in the predicted regions of the protein. In addition, the X-ray crystal coordinates for the DNA-binding core domain of the Ser 245 mutant p53 have recently been determined (N. Pavletich, personal communication). When this molecular dynamics approach is applied to the determination of the structure of the Ser 245-p53 as constructed from the wild-type starting structure, it yields results in excellent agreement with the Ser 245-p53 X-ray crystal structure, with the changes in overall structure of the computed mutant being very similar to the changes observed in the X-ray-determined mutant (data not shown).

In order to correlate the computational findings for the Leu 179 mutant with conformational changes in the p53 protein *in vivo,* immunohistochemical analyses of sections of tumor tissue from vinyl chloride-induced angiosarcoma of the liver known to contain a His → Leu mutation at codon 179 in the p53 gene were performed, as described previously (Brandt-Rauf-*et al.,* 1996). Sections were stained with primary mouse monoclonal antibody PAb240, which is specific for an epitope between amino acid residues 212 and 217 of p53 (Stephen and Lane, 1992) that was considered on the basis of the computations to be accessible for identification in the mutant protein, but inaccessible in the wild-type, and with primary mouse monoclonal antibody PAb1620, which is specific for an epitope between amino acid residues 106 and 113 of p53 (Ravera *et al.,* 1998) that was considered on the basis of the computations to be inaccessible for identification in the mutant protein, but accessible in the wild-type.

3. RESULTS

Coordinate fluctuations for each residue in the Leu 179-p53 mutant are shown in Fig. 1. Coordinate fluctuations for all residues are less than 1.5 Å, confirming that the protein converges on a specific structure that can be represented by the average structure in this case for the sake of comparison to the wild-type structure.

The overall rms deviation for the average structure of the Leu 179 mutant from the average wild-type struc-

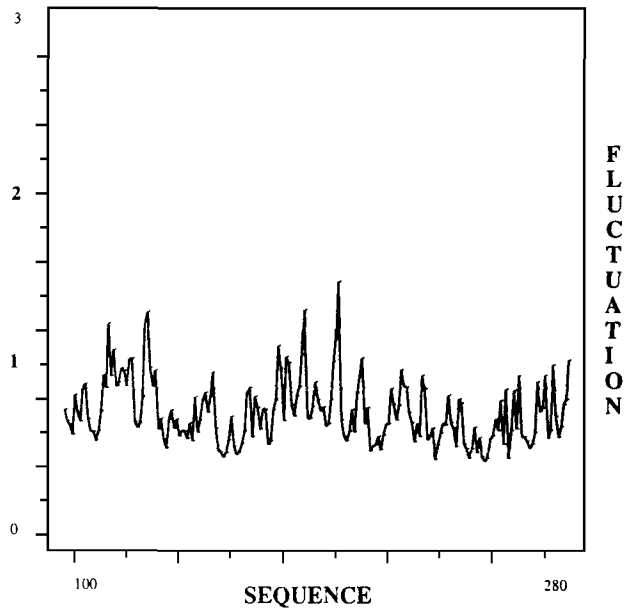

Fig. 1. Coordinate fluctuations for the last 50 low-energy structures on the dynamics trajectory of the Leu 179 p53 protein. Scale is in angstroms.

ture was 3.4 Å. The individual residue rms deviations for the average structure of the mutant compared to the wild-type protein are shown in Fig. 2. A large (9 Å) deviation can be seen around residue 179, the site of the amino acid substitution in the mutant, which is understandable since the normally occurring His 179 is one of the zinc-binding residues in the wild-type protein (Cho *et al.*, 1994); similarly, removal of the zinc atom from the wild-type structure results in major structural changes (data not shown), suggesting that zinc coordination is important for the maintenance of the normal structure.

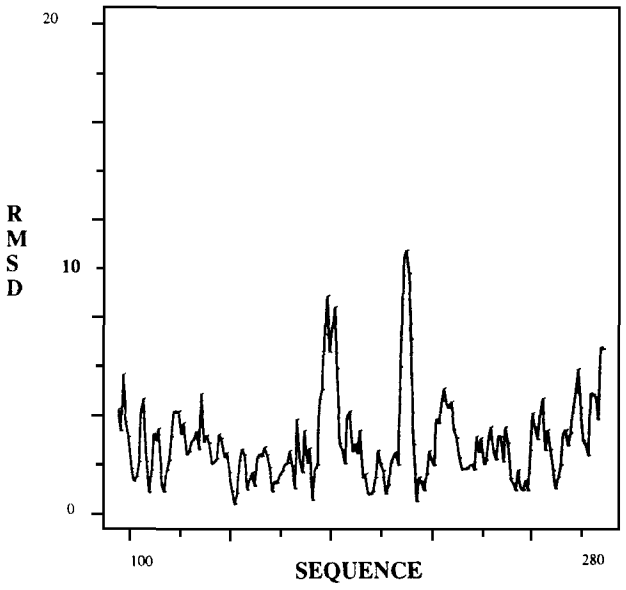

Fig. 2. Individual residue backbone deviations for the coordinates of corresponding amino acid residues of the Leu 179-p53 protein from those of the wild-type average structure. Scale is in angstroms

Additional conformational changes were noted distant from the site of the amino acid substitution. In fact, the largest conformational shift (11 Å) occurred in the region of amino acid residues 204–217. This deviation can be seen in Fig. 3, which presents stereo views of the average structure for the Leu 179 mutant superimposed on the average wild-type structure. In the wild-type structure, the 204–217 region is folded against the central structure of the protein packing into the hydrophobic core of the β-sandwich structure and thus is not exposed. In the mutant, this region is bent out and away from the β-sandwich core and is exposed. The 204–217 region is not just moved in the mutant structure, but can be seen to have adopted a distinctively different conformation (Fig. 4) with an rms deviation of 2.5 Å from the wild-type structure in this region. The conformational shift of this region was confirmed immunologically by detection of the PAb240 epitope in tumor cells containing the mutant protein due to the movement of the epitope to an exposed position on the surface of the protein (Fig. 5), as opposed to its normal unexposed position in the wild-type protein, where it is inaccessible to the antibody and hence nondetectable.

Another region of lesser, but still significant conformational shift (5.8 Å) in the mutant protein can be seen from residues 94 to 110 (Fig. 2). In this case, this region in the wild-type structure is exposed on the surface of the protein, whereas in the mutant structure it is folded into the protein structure packing against the hydrophobic core and thus is not exposed. Once again, the conformational shift of this region was confirmed immunologically by failure of detection of the PAb1620 epitope in tumor cells containing the mutant protein due to the movement of the epitope to an unexposed position in the protein (Fig. 5), as opposed to its normal exposed position in the wild-type protein. Similar regions of minor conformational change (4–6 Å) included residues 223–228, 260–270, and 279–300.

4. DISCUSSION

These results suggest that the Leu 179 substitution in the core DNA-binding domain of p53 produces specific, discrete conformational changes in the protein. Certain of these conformational shifts are similar to those seen in four other p53 mutants, and include amino acid residues 97–117, 172–190, 204–217, 260–281, and 290–300 (Brandt-Rauf *et al.*, 1996; Adler *et al.*, 1997). As in this

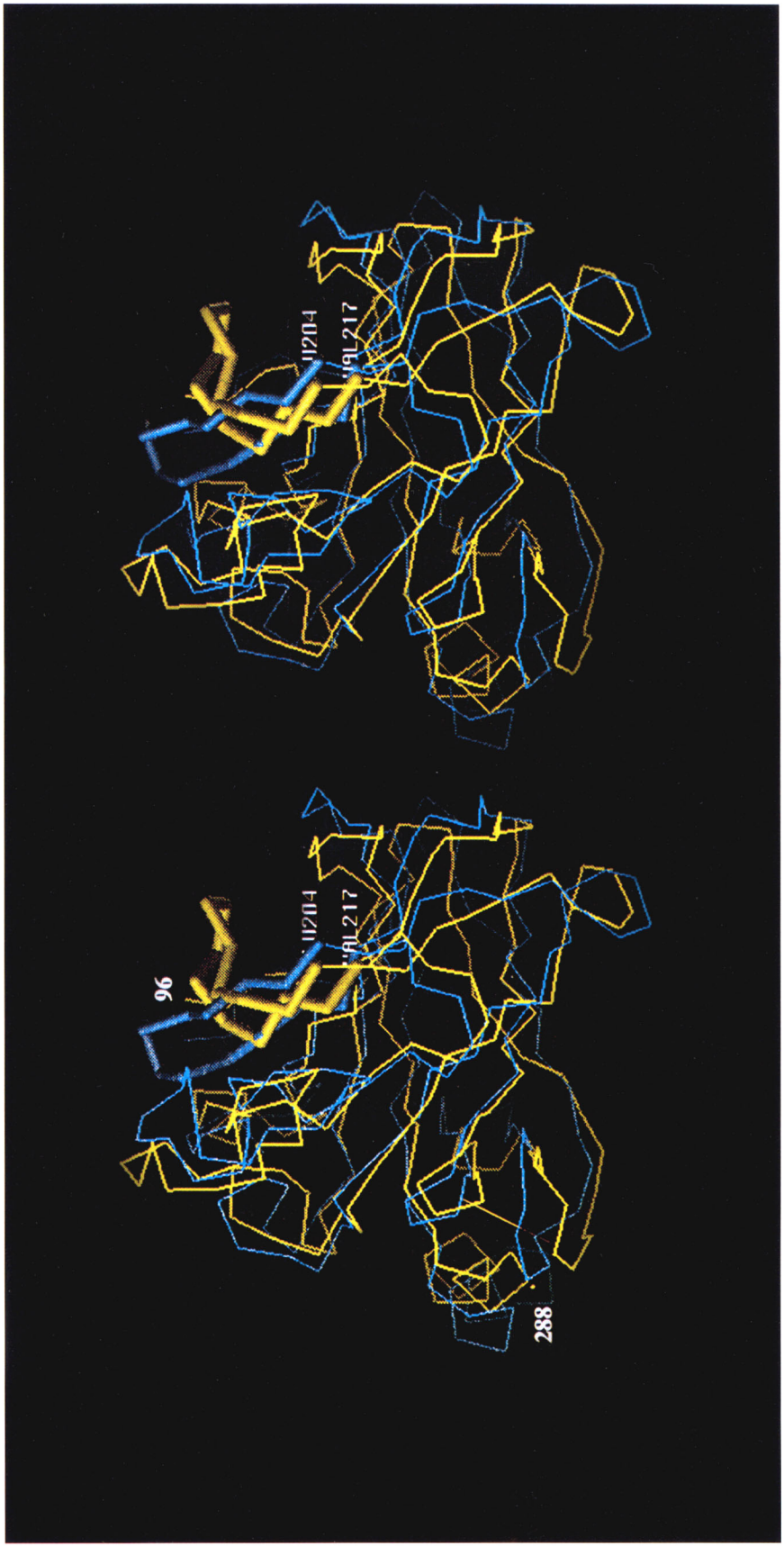

Fig. 3. Stereoview of the superimposed Cα traces of the average structures of the wild-type (blue) and Leu 179 mutant (yellow) p53 proteins obtained from molecular dynamics simulations. The region in bold in both structures is residues 204–217, which is clearly more exposed in the mutant structure.

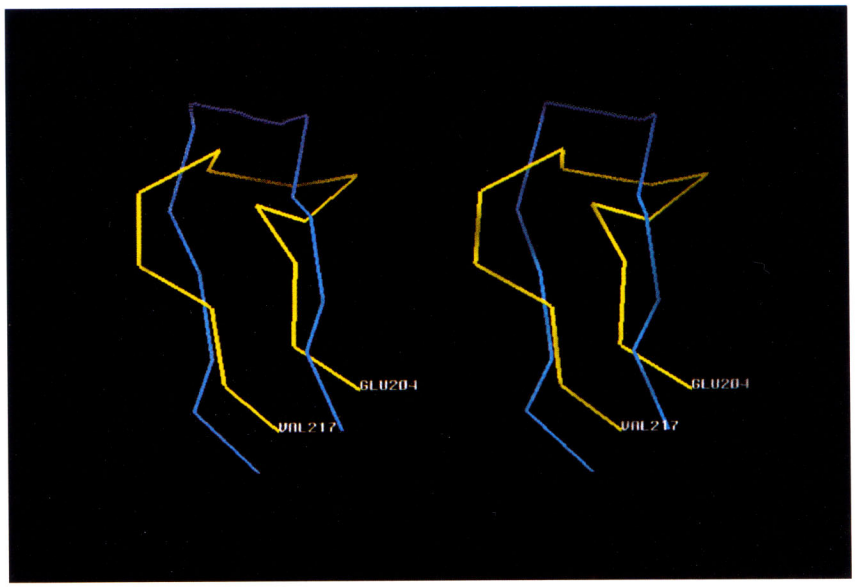

Fig. 4. Stereo view of the superimposed Cα traces of the average structures of the region from residues 204–217 in the wild-type (blue) and Leu 179 mutant (yellow) p53 proteins.

case, experimental support for the computed conformational shifts in the other cases was provided by evidence of epitope shifting (Brandt-Rauf *et al.*, 1996) or altered accessibility for phosphorylation sites (Adler *et al.*, 1997) in these regions. Taken together, the results imply that different amino acid substitutions at different residues in different "hot-spot" regions of p53 can produce common, discrete conformational changes in the protein. These regions are not only similar because they undergo major shifts in all these mutants, but also because they apparently adopt similar conformations that are different from the wild-type conformation. For example, the aforementioned 204–217 region in all five of these mutant cases is distinctly different from the wild-type conformation (rms deviations of at least 2.13 Å), but they are very similar to each other (rms deviations of 0.95–1.40 Å). Studies are currently underway to confirm these common conformational changes in other p53 mutants.

These results also confirm the importance of the residues in "hot-spot" regions of p53 for maintaining the normal structure, and presumably function, of wild-type p53. In some instances, such as this one (Leu 179), this is not at all surprising, since as noted above, the normal His residue at this position in the wild-type protein is

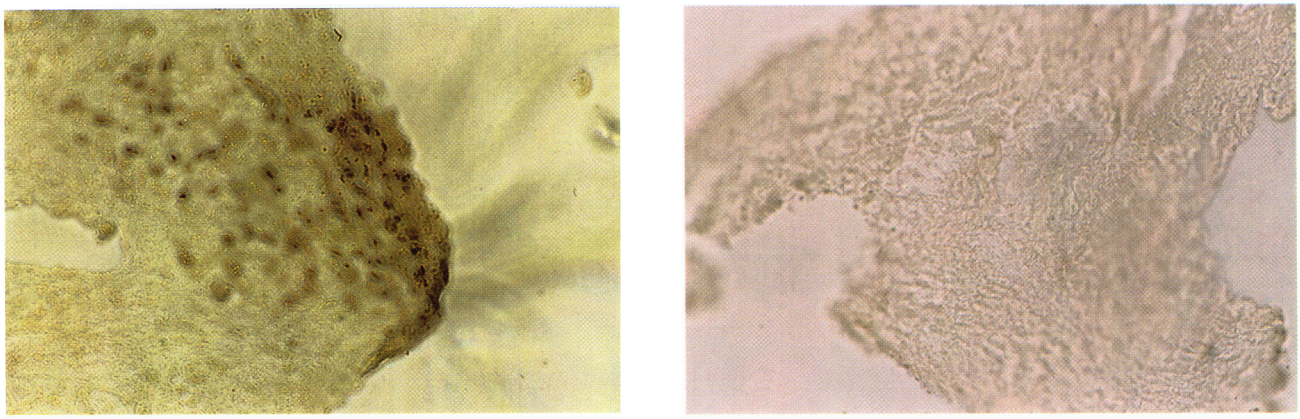

Fig. 5. Representative immunohistochemistry for p53 protein in a tumor known to contain the Leu 179 mutation with immunoperoxidase staining (400×), showing the presence of the PAb240 epitope (left) and the absence of the PAb1620 epitope (right).

involved in coordination of the Zn atom. Thus, this substitution would be analogous to removal of the Zn atom, which also would be expected to produce similar major conformational changes locally and elsewhere in the core DNA-binding domain according to the calculations. This is consistent with recent experimental work in which removal of Zn by chelation of wild-type p53 results in loss of function with adoption of a mutant conformation (PAb240 epitope is exposed and PAb1620 epitope is concealed), while restoration of Zn causes a return to normal structure and function (Verhaegh *et al.,* 1998). On the other hand, in other instances (Brandt-Rauf *et al.,* 1996; Adler *et al.,* 1997), amino acid substitutions in the core DNA-binding domain of p53 produce almost no local conformational change (less than 2 Å), but still produce major conformational changes at common, distant sites.

Knowledge of these sites of major common conformational changes in p53 mutants can lead to potentially useful practical applications. For example, the fact that the PAb240 epitope is exposed and detectable in mutant forms of p53 but not in the wild-type has been used in the case of prior mutations (Brandt-Rauf *et al.,* 1996) as well as the present mutation (Li *et al.,* 1998) to distinguish individuals with vinyl chloride exposure who have developed a p53 mutation from normal, unexposed controls via the presence of the PAb240 epitope in their tissue or serum. Other conformational changes in p53 mutants may similarly help to explain the development of a circulating antibody response to p53 in some vinyl chloride-exposed individuals (Li *et al.,* 1998), based on the notion that the body's immune system recognizes the conformationally altered protein as foreign. Such biomarkers based on conformational alteration of mutant p53 may be of value in identifying individuals who have sustained a cancer-related p53 mutation and thus presumably are at risk for the development of cancer prior to the time of clinical detection of disease (Husgafvel-Pursiainen *et al.,* 1997; Smith *et al.,* 1998).

In addition, regions of common conformational change in diverse p53 mutants are presumably of critical functional importance for p53, for example, potential sites of interaction of p53 with other intracellular elements through which p53 exerts its effects (i.e., they could be "effector" domains of p53). Since these putative "effector" domains are altered in mutant p53s, introduction of peptide sequences corresponding to these domains into cells with p53 mutations may competitively inhibit and thus modulate the effects of these domain interactions. For example, a peptide corresponding to amino acid residues 97–117 can affect the phosphorylation of p53 in this region *in vitro* and in cells in culture (Adler *et al.,* 1997). Furthermore, preliminary evidence suggests that this peptide may be able to induce apoptosis in tumor cells that contain p53 mutations (data not shown). Thus, the identification of regions of common conformational change in p53 mutants may lead to new therapeutic approaches for aborting their malignant effects in cancers that contain p53 mutations.

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