Oncogene (2005) 24, 6976–6981

www.nature.com/onc

SHORT REPORTS

The relationship among p53 oligomer formation, structure and transcriptional activity using a comprehensive missense mutation library

Tomohiro Kawaguchi[1,2,3] Shunsuke Kato[1,3], Kazunori Otsuka[1], Gou Watanabe[1], Toshihiro Kumabe[2], Teiji Tominaga[2], Takashi Yoshimoto[2] and Chikashi Ishioka*[,1]

[1]*Department of Clinical Oncology, Institute of Development, Aging and Cancer (IDAC), and Tohoku University Hospital, Tohoku University, Sendai 980-8575, Japan;* [2]*Department of Neurosurgery, Tohoku University Graduate School of Medicine, Sendai 890-8574, Japan*

Tumor suppressor p53 forms a homo-tetramer through its COOH-terminal oligomerization domain and acts as a sequence-specific transcription factor. We have analysed the interrelation among the transcriptional activities, the structure and the cancer-related mutations in the oligomerization domain by using a comprehensive missense mutation library. Here, we examined the ability of 184 mutant p53s in the domain to form an oligomer by expressing these mutant p53s in yeast, and compared the data with the previous information. We showed that specific residues in the α-helix and the β-strand of the oligomerization domain were critical for both oligomer formation and sequence-specific transactivation, and the activities were closely related. In particular, the α-helix was more sensitive to amino-acid substitutions than the β-strand. We found identity in the interrelation between the two activities, that is, monomer mutants were transcriptionally inactive whereas dimer and tetramer mutants retained their transcriptional activities. In *TP53* mutation databases, a small number of mutations have been reported in this domain. Surprisingly, most do not encode p53s defective in functional properties. These results indicate that, although oligomer formation is essential for p53 transactivation function, the inactivation of oligomer formation and therefore the inactivation of transactivation may not be essential for tumor suppression by p53 because they do not lead to oncogenic proteins.
Oncogene (2005) **24,** 6976–6981. doi:10.1038/sj.onc.1208839; published online 20 June 2005

Keywords: p53; oligomerization; transactivation

p53 plays an important role to maintain genomic integrity from cellular stress by *trans*-activating the downstream genes involved in different cellular functions, such as cell cycle arrest (Slingerland and Benchimol, 1991; el-Deiry *et al*., 1993; Tanaka *et al*., 2000) and apoptosis (Miyashita and Reed, 1995; Owen-Schaub *et al*., 1995; Polyak *et al*., 1997; Oda *et al*., 2000). Cellular stress, including DNA damage and hypoxia, stabilizes p53 protein by post-transcriptional modification, such as phosphorylation (Sakaguchi *et al*., 1998; Ashcroft *et al*., 1999), acetylation (Barlev *et al*., 2001) and prolyl isomerization (Zacchi *et al*., 2002; Zheng *et al*., 2002) in specific residues outside the central DNA-binding domain, converting the latent form of p53 into the active form. p53 acts as a tetramer through the carboxy-terminal oligomerization domain and several studies have shown the importance of this domain for p53 transcriptional activity, tumor suppressive activity and dominant negative effect of p53 (Shaulian *et al*., 1992, 1993; Unger *et al*., 1992; Halazonetis and Kandil, 1993). The structure of the oligomerization domain has been solved by both X-ray crystallography and NMR (Lee *et al*., 1994; Clore *et al*., 1995; Jeffrey *et al*., 1995; Miller *et al*., 1996; Chene *et al*., 1997; Mittl *et al*., 1998) and the structural information of specific residues in the solved 'V-shaped' structures is useful to better understand oligomerization. However, the contribution of each residue in oligomerization has not been validated biochemically.

Previously, we have randomly mutagenized the domain by an error-prone PCR technique and yeast-based functional assay (Ishioka *et al*., 1995). We have isolated two interesting mutant p53s encoded by two missense mutations. The first mutant, L344P, identified as a loss of transactivation mutant, is located in the α-helix and had no ability to form dimers or tetramers (monomer mutant). The second mutant, K351E, identified as a partial-transactivation mutant, has the ability to form dimers but not tetramers (dimer mutant) (Ishioka *et al*., 1997). L344P is not only defective in the *in vitro* experimental setting but also in the pathogenic setting because it has been reported as a causative germline mutation in a family with classic Li–Fraumeni syndrome (Varley *et al*., 1996). This mutant p53 is also shown to be a monomer mutant (Ishioka *et al*., 1997; Davison *et al*., 1998; Lomax *et al*., 1998). These results suggest that oligomer (either dimer or tetramer) formation is essential for the p53 transactivation function and show that mutations defective in oligomer formation are as pathogenic as common

*Correspondence: C Ishioka, Department of Clinical Oncology, IDAC and Tohoku University Hospital, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan; E-mail: chikashi@idac.tohoku.ac.jp
[3]These authors contributed equally to this work
Received 14 February 2005; revised 5 May 2005; accepted 6 May 2005; published online 20 June 2005

mutations in the DNA-binding domain. Although the importance of several specific residues for both oligomer formation and transcriptional activity have been demonstrated by an alanine substitution at nine residues in the domain (Chene *et al*., 1997; Mateu and Fersht, 1998; Chene and Bechter, 1999), more information about the relationships among p53 structure, transactivation activity and oligomer formation is necessary to elucidate the tumor suppressive role of p53 oligomer formation. Recently, we have constructed all possible amino-acid substitutions caused by point mutations throughout the protein (5.9 substitution per residue) and have shown that mutant p53s in the oligomerization domain are sensitive in transcriptional activities in yeast and clarified interrelations between their transcriptional activities and the structure (Kato *et al*., 2003).

To investigate whether the oligomer formation correlates with the transactivation activity and the structural information of the oligomerization domain, we analysed the ability to form oligomers and compared the results with the known information on the transactivation and the structure. Among 193 mutant p53s constructed in 33 residues from 322 to 354, we chose to examine 184 (95.3%) mutant p53s. The mutant p53s or wild-type p53 protein expressed in yeast cells were incubated in the presence or absence of 0.1% glutaraldehyde (a cross-linker) and were separated by SDS/PAGE with a 5–15% polyacrylamide gradient. The p53 protein was visualized by immunoblotting analysis using a polyclonal p53 antibody. The results of representative residue, in which mutant p53s predominantly form tetramer (R336) or dimer (E343), or either tetramer, dimer, or monomer depending on the amino-acid substitution (A347), are shown in Figure 1. The results of all 184 mutant p53s are summarized in Figure 2. In wild-type p53, only the tetramer form was observed and no or a trace monomer and dimer form was visible in the presence of glutaraldehyde, indicating that wild-type p53 predominantly forms tetramers (Figure 1). In contrast, neither dimers nor tetramers were formed in the known monomer mutant L344P as reported previously (Ishioka *et al*., 1997) (Figure 1).

The oligomerization domain consists of two secondary structures, a β-strand (residues 326–333) and an α-helix (residues 335–354), and the two structures form a V-shaped structure connected by a hinge residue at G334 (Clore *et al*., 1995). Of the 33 residues analysed, 24 residues were affected with the oligomer formation by at least one mutant p53. All four residues (P322, L323, D324 and G325) outside of the secondary structures and five (E326, Y327, F328, T329 and Q331) of eight residues in the β-strand were not sensitive to amino-acid substitutions. Like wild-type p53, all mutant p53s in nine residues predominantly formed tetramers (tetramer mutant), although a small amount of the dimer form was also seen in some mutant p53s of these residues (data not shown). Figure 1a indicates representative tetramer mutant p53s in the E336 residue. In contrast, all but four (R335, E336, E339 and Q354) residues in the α-helix (16 of 20) as well as the hinge residue (G334) were sensitive for amino-acid substitutions.

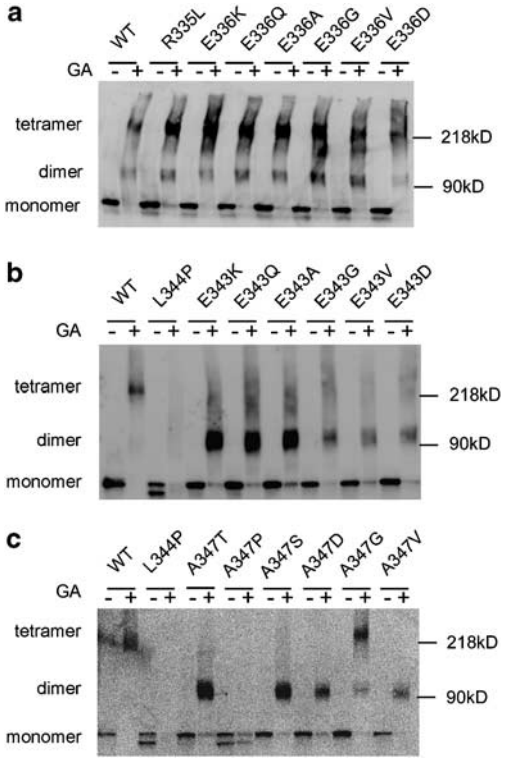

Figure 1 Analysis of oligomer formation of mutant p53s. The p53-expressing yeast cells were cultured in 1.2 ml of synthetic complete (SC) liquid media at 30°C for 16 h in a 96-well plates and 0.4 ml of the culture medium was inoculated in 0.8 ml of yeast extract/peptone/dextrose (YPD) liquid media and further cultured at 37°C for 6 h. The cells were precipitated by centrifugation and resuspended in 200 μl of DOC buffer (Ishioka *et al*., 1997). The cells were then homogenized using a Mixer Mill MM300 (Qiagen) with glass beads for 1 min and chilled for 1 min on ice four times. In total, 25 μl of cell lysates were incubated at room temperature for 20 min in the absence or presence of 0.1% glutaraldehyde (a cross-linking agent) and boiled in a sample loading buffer containing 1% SDS. The reaction mixtures were separated by SDS/PAGE (with a 5–15% polyacrylamide gradient), followed by immunoblotting analysis using a HRP-conjugated polyclonal p53 antibody (FL-393, Santa Cruz). The p53 protein was visualized using an enhanced chemiluminescent technique (ECL kit, Amersham). (**a**) Mutant p53s in Glu336 predominantly form tetramers as wild-type p53 (WT). One (R335L) of other tetramer mutants was also shown. (**b**) Mutant p53s in Glu343 predominantly form dimers. WT and L344P are tetramer and monomer controls, respectively. (**c**) Mutant p53s in Ala347 form monomers (A347P), dimers (A347T, A347S, A347D and A347V) and tetramers (A347G) depending on the specific mutant p53. GA is indicated in the absence (−) or presence (+) of 0.1% glutaraldehyde. We note that monomeric p53 forms of L344P and L347P seem to be unstable in our experimental setting because of unknown reason

A small hydrophobic cluster consisting of the side chains of three amino acids, I332, F338 and F341, causes a V-shaped structure (Clore *et al*., 1995). Among the mutant p53s in three residues, five of six mutants in F341 were unable to form tetramers. There were four monomer mutants (F341V, F341Y, F341S and F341C) and one dimer (F341L) mutant. In residue I332, two (I332N and I332S) of the six mutants were monomer mutants and the remaining four mutants represented

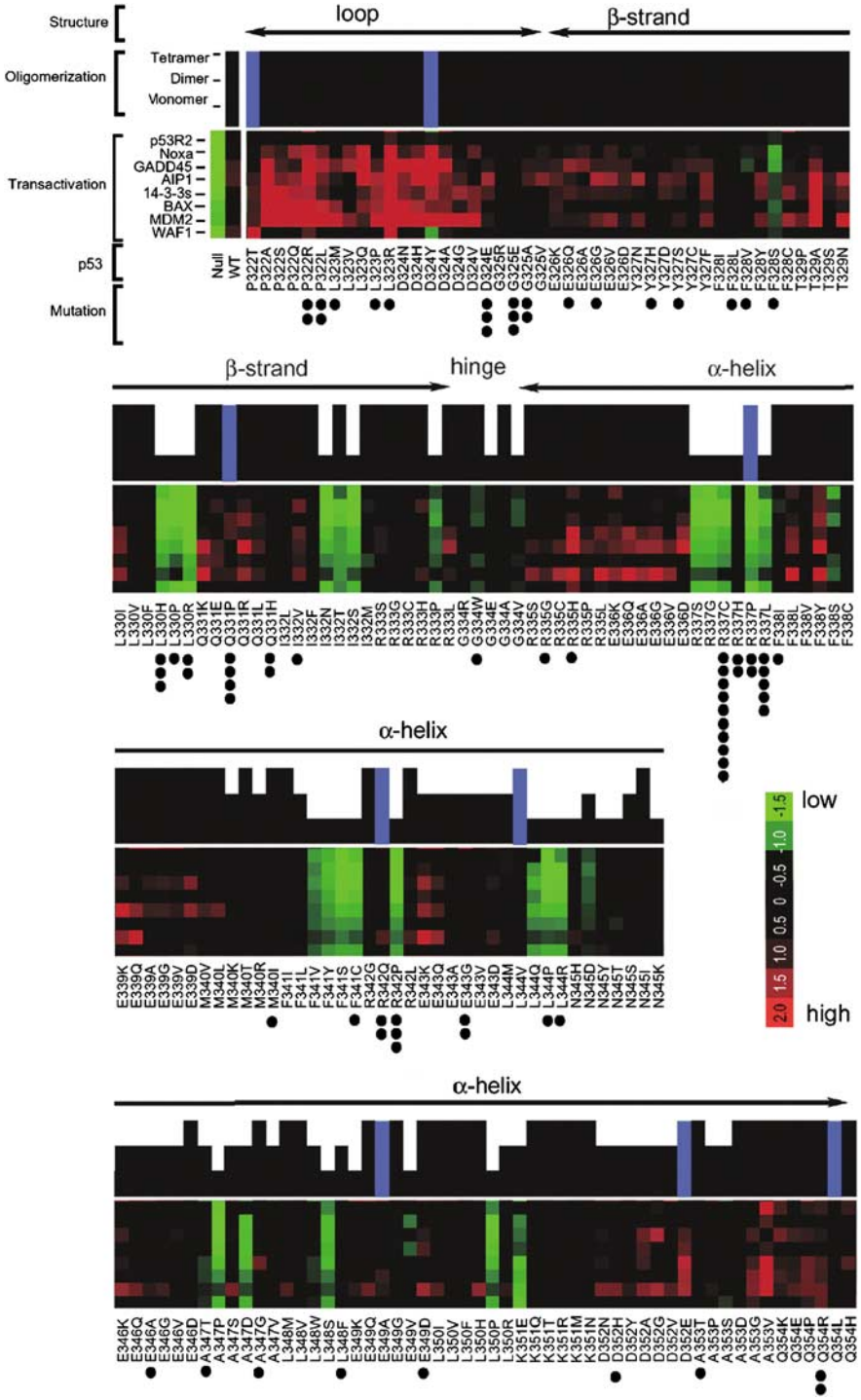

Figure 2 The ability to form oligomers and transactivate p53 target genes on 184 mutant p53s. The ability to form oligomers of each mutant p53 is indicated graphically from the NH_2 terminal portion (top panel) to the COOH terminal portion (bottom panel). A total of 10 mutant p53s were not examined and are indicated as blue bars. The ability to transactivate eight p53 target genes on each mutant p53 originated from our previous study (Kato *et al*., 2003) and showed a color gradation according to the standardized value of transactivity (reference bar). Red, green or black indicates higher, lower or intermediate activity, respectively). Mutant p53s reported as somatic mutation (p53 web site, http://p53.free.fr/) were marked (closed circle) by the number of mutation frequencies

predominant tetramer formation. In contrast, the six mutants in F338 did not affect the tetramer formation.

Two p53 monomers associate via their β-strands to form an antiparallel double-stranded sheet and via the antiparallel association of their α-helix to create a double-helical bundle (Clore *et al*., 1995). Eight residues, F328, L330, I332, R337, F338, F341, N345 and D352, are involved in the monomer–monomer interaction. A hydrophobic core assembles hydrophobic residues including F328, L330 and I332 from the β-strands and

F338, F341 and N345 from the α-helix (Clore *et al*., 1995). All residues but two (F328 and F338, see above) were sensitive to amino-acid substitutions and, as expected, many of the mutant p53s (17 of 48) constructed in these residues were monomer mutants. These were three mutants (L330H, L330P and L330R) in L330, two mutants (I332N and I332S) in I332, all examined mutants (R337S, R337G, R337C, R337P and R337L) in R337, four mutants (F341V, F341Y, F341S and F341C) in F341 and three mutants (N345H, N345Y and N345T) in N345. Two specific residues, R337 from one monomer and D352 from the other monomer, make a salt bridge (Clore *et al*., 1995); however, interestingly, the biochemical effects of mutants in the two residues were different. Whereas all examined mutants in R337 were monomer mutants, all six mutants (D352N, D352H, D352Y, D352A, D352G and D352V) constructed on D352 were dimer mutants, indicating that D352 may be a more important residue for dimmer–dimer interaction rather than for monomer–monomer interaction. Three other dimer mutants (F341L, N345D, N345S) were also found in this category, suggesting the importance of F341 and N345 in dimmer–dimer interaction (see below).

Two dimers associate through double helical bundles, and the dimmer–dimer interaction involves hydrophobic residues in the α-helix, M340, F341, L344, A347, L348 and L350 (Clore *et al*., 1995). As expected, many of the mutant p53s (11 of 33) constructed in these residues were dimer mutants. There were two mutants (M340K and M340R) in M340, one mutant (F341L) in F341, one mutant (L344M) in L344, four mutants (A347 T, A347S, A347D and A347V) in A347 (Figure 1c), two mutants (L348W and L348F) in L348 and one mutant (L350R) in L350. Interestingly, most of the mutant p53s in F341 (four of six, F341V, F341Y, F341S and F341C) and L344 (three of four, L344Q, L344P and L344R) were monomer mutants, and the remaining mutants contained dimer mutants (F341L and L344M). These results indicated that, similar to F341, L344 and N345 (see above) are also important for both monomer–monomer interaction and dimmer–dimer interaction.

Eight residues (R333, R342, E343, E346, E349, K351, A353 and Q354) have not been specified in the previous structural studies. Among the mutants in these residues, six (R333P, R342P, E349K and E349V) were monomer mutants, indicating that R333, R342 and E349 may contribute to monomer–monomer interaction. There were 13 dimer mutants. All mutants (E343K, E343Q, E343A, E343G, E343V and E343D) in E343 (Figure 1b) and all (E346K, E346Q, E346A, E346G and E346V) but one mutant in E346 were dimer mutants, indicating that these two residues are important for dimmer–dimer interaction. There were two other dimer mutants, including K351E, a known dimer mutant (Ishioka *et al*., 1997), and A353P, suggesting that these two residues are also involved in dimmer–dimer interaction. Neither monomer nor dimer mutants were found in Q354.

The monomer mutants tended to cluster in the residues involved in the monomer–monomer interface (Supplementary Figure 1) and the dimer mutants tended to cluster in the residues involved in the dimmer–dimer interface (Supplementary Figure 2). These results indicate that the ability to form oligomers of p53 mutants in the tetramerization domain and the structure are well interrelated. The ability to form oligomers of p53 mutants in the tetramerization domain and their ability to transactivate downstream genes are summarized in Table 1. Obviously, the α-helix was more sensitive to amino-acid substitutions than the β-strand and the loop in the 33 amino-acid peptides, even if the β-strand was conserved as well as the α-helix (Figure 3). All dimer mutants and the majority of monomer mutants are found in the α-helix. There was identity in the relation between oligomer formation and transcriptional activity, that is, monomer mutants were transcriptionally inactive whereas dimer and tetramer mutants retained their transcriptional activity. These data also suggest that only *TP53* mutations encoding monomer mutants may be pathogenic, whereas those of dimer mutants may not. Among the 193 mutant p53s constructed in the fragment, only 78 mutant p53s have been reported as somatic missense mutations in the *TP53* mutation databases. Surprisingly, half of these *TP53* missense mutations did not encode biochemically and/or transcriptionally inactive mutant p53s. Among the reported mutants, all but three inactive mutants (Q331P, R337C and R337H) have been reported only three times or less. According to our reassessment of the *TP53* mutation database, the *TP53* mutations reported fewer than three times are suspicious (accidental mutations or technical errors) for their functional effect on carcinogenesis (Soussi *et al*., 2005, and unpublished data). Real pathogenic mutations, therefore, seem to be very few although 23.9% (44 of 184) encoded transcriptionally inactive mutant p53s such as common mutant p53s that occurred in the DNA-binding domain (Table 1). While the oligomer formation

Table 1 Ability to form oligomer of the mutant p53s in the tetramerization domain

	Loop 322–325	β-*Strand 326–333*	*Hinge 334*	α-*Helix 335–354*	*Conserved residues*[a]	*Reported mutation*[b]	*Full length 322–354*
Tetramer mutant	20 (0)[c]	40 (6)	3 (2)	58 (4)	50 (7)	35 (4)	121 (12)
Dimer mutant	0 (0)	0 (0)	0 (0)	33 (6)	10 (1)	6 (1)	33 (6)
Monomer mutant	0 (0)	6 (6)	2 (2)	22 (18)	10 (10)	29 (29)	30 (26)
Total	20 (0)	46 (12)	5 (4)	113 (28)	70 (18)	70 (34)	184 (44)

[a]A total of 12 residues containing D324, E326, L330, I332, R333, G334, F338, E339, M340, E346, L348 and L350. [b]Among 78 *TP53* mutations, 70 were examined for both oligomer formation and transcriptional activity. [c]Number of the transcriptionally inactive mutants were shown in parenthesis

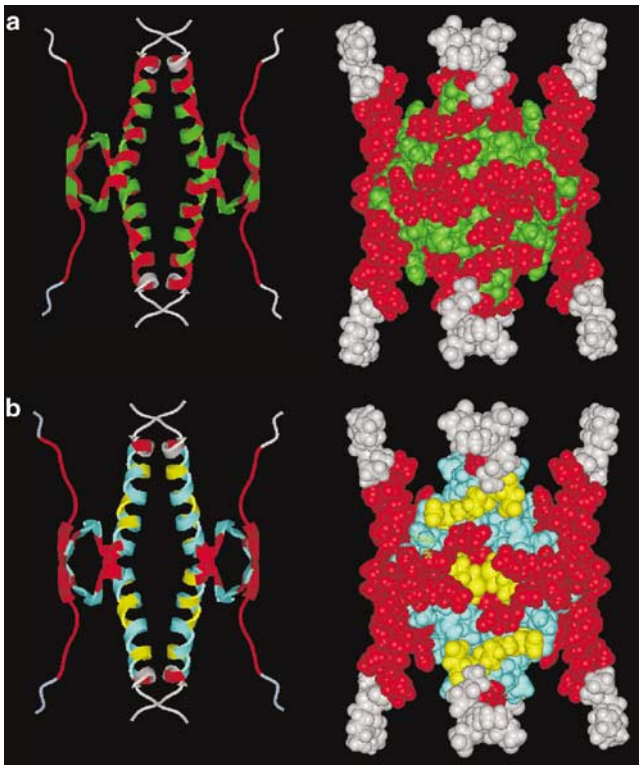

Figure 3 3D-structure and functional properties of p53 tetramerization domain. The 3D-structure p53 tetramerization domain was based on 3SAK (PDB) and was shown as ribbon diagram (left panel) for backbone structure or ball diagram (right panel) containing the side chain structure. (**a**) Residues from 322 to 354 were evaluated based on the transcriptional activity of each mutant p53 (Kato *et al*., 2003) and colored. Red, transactivity of any mutant p53s in a residue was not affected. Green, a residue in which transactivity of at least one mutant p53 was inactivated. (**b**) Residues from 322 to 354 were colored based on the oligomerization ability of mutant p53s. Red, a residue in which any mutant p53s were tetramer mutants. Light blue, a residue in which at least one mutant p53 was monomer mutant. Yellow, a residue in which at least one mutant p53 was dimer mutant and no mutant p53 was monomer mutant. Gray in both (**a**) and (**b**), unexamined residues in the 3SAK

was closely linked to the p53 transactivation function, the inactivation of oligomerization seems insufficient to abrogate the full p53 tumor suppressive function. We propose two possible explanations: first, such mutant p53s defective in oligomerization have no trans-dominant effect as reported in some mutant p53s in the DNA-binding domain (Srivastava *et al*., 1993, Brachmann *et al*., 1996) because the effect requires p53 oligomer formation (Shaulian *et al*., 1993, Unger *et al*., 1993); second, transactivation is not the only function of p53-mediated tumor suppression apoptosis. There are many reports supporting the importance of the transactivation-independent cytoplasmic function of p53 (Marchenko *et al*., 2000; Mihara *et al*., 2003; Kakudo *et al*., 2005) and mutant p53s defective in oligomerization might retain the function. We note that these possibilities may explain, at least in part, why fewer mutations have been reported in the carboxyl terminal domain.

Among the 184 mutant p53s, there were 22 exceptions to the oligomerization–transactivation relationship, including four monomer mutants (N345H, N345Y, N345K, E349K) that retained transcriptional activity, 12 tetramer mutants (F328V, F328S, I332F, I332T, I332M, R333G, G334W, G334A, F338I, F338V, F338S, F338C) and six dimer mutants (N345D, A347T, A347D, A347V, L348W, K351E) that were transcriptionally inactive. We note that half of them (F328V, I332F, I332M, R333G, G334A, F338I, F338V, F338C, A347T, A347V, L348W) retained partial transactivity. Although the molecular mechanism of the discrepancy in each mutant p53 is still unclear, we speculate at least two possibilities explaining the exceptions. First, our two experimental systems (both transactivation assay and oligomer formation analysis) may not always represent a subtle environmental difference and that the oligomerization status in the living cells might differ from that in yeast cell lysates depending on environmental factors such as pH. Second, some of the exceptions may affect the cellular localization of p53 rather than the oligomer formation. The p53 nuclear localization is regulated by its nuclear import and nuclear export in mammalian cells. Especially, the nuclear export is regulated by MDM2 binding to the NH_2 terminal transactivation domain of p53 (Roth *et al*., 1998; Tao *et al*., 1999) and by CRM1 binding to a nuclear export signal (NES) in the α-helix portion of the tetramerization domain (residues 340–351) (Stommel *et al*., 1999). Therefore, not only oligomer formation but also nuclear export of p53 may affect the transactivation although there has never been a study showing the cellular localization of the p53 protein in yeast.

Apart from the exceptions, there were at least two cases with discrepancy between the current study and the previous report. One mutant p53, R337H, showed a different result from the previous report. R337H has been reported in Brazilian childhood adrenocortical carcinoma, is highly sensitive to pH on its tetramerization and retains wild-type activity under the physiological pH condition (DiGiammarino *et al*., 2002). In contrast, R337H was a monomer mutant under our cross-linking conditions and was mostly inactive in transcription assay in our experimental system (Figure 2). We speculate that there might be slight differences in pH, redox control and thiol content among human and yeast cells and this might be a reason explaining the discrepancy. Another mutant p53 is D352A, which has been reported as a tetramer mutant when it is expressed as a small p53 peptide (Mateu and Fersht, 1998) but was a dimer mutant in full-length p53 in this study. We speculate that the outside of the oligomerization domain might affect the oligomer formation.

In the tetramerization domain, 12 residues (D324, E326, L330, I332, R333, G334, F338, E339, M340, E346, L348 and L350) are well conserved among vertebrate p53 and p53 family members (Supplementary Figure 3). However, their sensitivity to mutations in oligomer formation and their transcriptional activity were different. In all, 10 monomer and 10 dimer mutants were observed in this category, but no oligomer defective or transcriptionally inactive mutant was found in the four (D324, E326, F338 and E339) residues.

Therefore, it seems difficult to predict the functional impact of mutations from specific amino-acid substitution and amino-acid conservation in the domain.

In conclusion, we intensively analysed the p53 oligomerization domain by using a comprehensive p53 missense mutation library and showed the interrelation among oligomer formation, transactivation and the structure. We showed the first evidence that p53 oligomerization is closely related to sequence-specific transactivation in each mutant p53 level. The data presented here underline the previous data showing the importance of the p53 oligomerization domain for the sequence-specific transactivation and should be useful to better understand the structure and function relationship of p53 and possibly other oligomer proteins.

Acknowledgements
We thank Shuang-Yin Han and Wen Liu for their contribution to the construction of the p53 mutation library. We also thank Yuka Fujimaki for technical assistance. This study was supported in part by grants-in-aid from the Ministry of Education and Science, Sports and Culture (to CI and SK) and the Gonryo Medical Foundation (CI).

References

Ashcroft M, Kubbutat MH and Vousden KH. (1999). *Mol. Cell. Biol.*, **19,** 1751–1758.
Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD and Berger SL. (2001). *Mol. Cell*, **8,** 1243–1254.
Brachmann RK, Vidal M and Boeke JD. (1996). *Proc. Natl. Acad. Sci. USA*, **93,** 4091–4095.
Chene P and Bechter E. (1999). *J. Mol. Biol.*, **288,** 891–897.
Chene P, Mittl P and Grutter M. (1997). *J. Mol. Biol.*, **273,** 873–881.
Clore GM, Omichinski JG, Sakaguchi K, Zambrano N, Sakamoto H, Appella E and Gronenborn AM. (1995). *Science*, **267,** 1515–1516.
Davison TS, Yin P, Nie E, Kay C and Arrowsmith CH. (1998). *Oncogene*, **17,** 651–656.
DiGiammarino EL, Lee AS, Cadwell C, Zhang W, Bothner B, Ribeiro RC, Zambetti G and Kriwacki RW. (2002). *Nat. Struct. Biol.*, **9,** 12–16.
el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B. (1993). *Cell*, **75,** 817–825.
Halazonetis TD and Kandil AN. (1993). *EMBO J.*, **12,** 5057–5064.
Ishioka C, Englert C, Winge P, Yan YX, Engelstein M and Friend SH. (1995). *Oncogene*, **10,** 1485–1492.
Ishioka C, Shimodaira H, Englert C, Shimada A, Osada M, Jia LQ, Suzuki T, Gamo M and Kanamaru R. (1997). *Biochem. Biophys. Res. Commun.*, **232,** 54–60.
Jeffrey PD, Gorina S and Pavletich NP. (1995). *Science*, **267,** 1498–1502.
Kakudo Y, Shibata H, Otsuka K, Kato S and Ishioka C. (2005). *Cancer Res.*, **65,** 2108–2114.
Kato S, Han SY, Liu W, Otsuka K, Shibata H, Kanamaru R and Ishioka C. (2003). *Proc. Natl. Acad. Sci. USA*, **100,** 8424–8429.
Lee W, Harvey TS, Yin Y, Yau P, Litchfield D and Arrowsmith CH. (1994). *Nat. Struct. Biol.*, **1,** 877–890.
Lomax ME, Barnes DM, Hupp TR, Picksley SM and Camplejohn RS. (1998). *Oncogene*, **17,** 643–649.
Marchenko ND, Zaika A and Moll UM. (2000). *J. Biol. Chem.*, **275,** 16202–16212.
Mateu MG and Fersht AR. (1998). *EMBO J.*, **17,** 2748–2758.
Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P and Moll UM. (2003). *Mol. Cell*, **11,** 577–590.
Miller M, Lubkowski J, Rao JK, Danishefsky AT, Omichinski JG, Sakaguchi K, Sakamoto H, Appella E, Gronenborn AM and Clore GM. (1996). *FEBS Lett.*, **399,** 166–170.
Mittl PR, Chene P and Grutter MG. (1998). *Acta Crystallogr D Biol. Crystallogr.*, **54,** 86–89.
Miyashita T and Reed JC. (1995). *Cell*, **80,** 293–299.
Oda E, Ohki R, Murasawa H, Nemoto J, Shibue T, Yamashita T, Tokino T, Taniguchi T and Tanaka N. (2000). *Science*, **288,** 1053–1058.
Owen-Schaub LB, Zhang W, Cusack JC, Angelo LS, Santee SM, Fujiwara T, Roth JA, Deisseroth AB, Zhang WW, Kruzel E and Radinsky R. (1995). *Mol. Cell. Biol.*, **15,** 3032–3040.
Polyak K, Xia Y, Zweier JL, Kinzler KW and Vogelstein B. (1997). *Nature*, **389,** 300–305.
Roth J, Dobbelstein M, Freedman DA, Shenk T and Levine AJ. (1998). *EMBO J.*, **17,** 554–564.
Sakaguchi K, Herrera JE, Saito S, Miki T, Bustin M, Vassilev A, Anderson CW and Appella E. (1998). *Genes Dev.*, **12,** 2831–2841.
Shaulian E, Zauberman A, Ginsberg D and Oren M. (1992). *Mol. Cell. Biol.*, **12,** 5581–5592.
Shaulian E, Zauberman A, Milner J, Davies EA and Oren M. (1993). *EMBO J.*, **12,** 2789–2797.
Slingerland JM and Benchimol S. (1991). *J. Cell. Physiol.*, **148,** 391–395.
Soussi T, Kato S, Levy PP and Ishioka C. (2005). *Hum. Mutat.*, **25,** 6–17.
Srivastava S, Wang S, Tong YA, Pirollo K and Chang EH. (1993). *Oncogene*, **8,** 2449–2456.
Stommel JM, Marchenko ND, Jimenez GS, Moll UM, Hope TJ and Wahl GM. (1999). *EMBO J.*, **18,** 1660–1672.
Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y and Nakamura Y. (2000). *Nature*, **404,** 42–49.
Tao W and Levine AJ. (1999). *Proc. Natl. Acad. Sci. USA*, **96,** 6937–6941.
Unger T, Mietz JA, Scheffner M, Yee CL and Howley PM. (1993). *Mol. Cell. Biol.*, **13,** 5186–5194.
Unger T, Nau MM, Segal S and Minna JD. (1992). *EMBO J.*, **11,** 1383–1390.
Varley JM, McGown G, Thorncroft M, Cochrane S, Morrison P, Woll P, Kelsey AM, Mitchell EL, Boyle J, Birch JM and Evans DG. (1996). *Oncogene*, **12,** 2437–2442.
Zacchi P, Gostissa M, Uchida T, Salvagno C, Avolio F, Volinia S, Ronai Z, Blandino G, Schneider C and Del Sal G. (2002). *Nature*, **419,** 853–857.
Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP and Xiao ZX. (2002). *Nature*, **419,** 849–853.

Supplemenatry Information accompanies the paper on Oncogene website (http://www.nature.com/onc)