Lack of Correlation between p53-Dependent Transcriptional Activity and the Ability to Induce Apoptosis among 179 Mutant p53s

Yuichi Kakudo, Hiroyuki Shibata, Kazunori Otsuka, Shunsuke Kato, and Chikashi Ishioka

Department of Clinical Oncology, Institute of Development, Aging and Cancer, and Tohoku University Hospital, Tohoku University, Sendai, Japan

Abstract

Tumor suppressor p53-dependent apoptosis is thought to be one of the most important tumor-suppressive functions in human tumorigenesis. However, whether the major mechanism underlying the p53-dependent apoptosis is transactivation-dependent or independent remains unclear. Using 179 mutant p53s with diverse transcriptional activities for distinct p53-binding sequences in yeast, we evaluated both their sequence-specific transcriptional activities on six p53 target genes and their ability to induce apoptosis in Saos-2 cells. These mutant p53s also represented diversity in their ability to both transactivate target genes and induce apoptosis. We identified 17 mutant p53s with superior ability to induce apoptosis than wild-type p53 that tend to cluster at residues 121 or 290 to 292. There was no significant correlation between the two functional properties on any single target gene examined. Furthermore, the 17 mutant p53s were not classified in a specific cluster by hierarchical cluster analysis on their diverse transcriptional activities, indicating that these mutant p53s were not similar in the transcriptional activity of downstream genes. These results suggested that transactivation-dependent apoptosis does not always play a major role in p53-dependent apoptosis, indirectly supporting the importance role of the transactivation-independent mechanism. (Cancer Res 2005; 65(6): 2108-14)

Introduction

Tumor suppressor p53 protein is a 393-amino-acid transcription factor. It is activated by a variety of cellular stresses including DNA damage and hypoxia and is phosphorylated and acetylated after translation (reviewed in refs. 1–4). The activated p53 binds to the specific DNA sequences in the regulatory region of downstream genes, resulting in cellular events including cell cycle arrest and apoptosis. Thus far, several downstream genes have been isolated. These contain a series of genes involved in cell cycle regulation, apoptosis, DNA repair, angiogenesis, and the regulation of p53 stability. Loss of p53 function, therefore, fails to activate these genes after cellular stress and is thought to be a critical cause of tumorigenesis and/or tumor progression.

Approximately 50% of tumors contained the TP53 mutation (5, 6) and the published mutations have been summarized in the TP53 mutation databases (7, 8), which contain more than 15,000 mutations. According to the databases, 74% were missense mutations. Thus far, 1,200 distinct missense mutations have been reported and there are mutation hotspots at residues R175, G245, R248, R249, R273, and R282. Because the common mutant p53s abrogate both the ability to transactivate downstream genes and induce apoptosis, it was concluded at the early stage of p53 studies that sequence-specific transactivation is essential for p53-dependent apoptosis. In other words, sequence-specific transactivation by activated p53 is thought to be one of the most important tumor-suppressive mechanisms in human tumorigenesis. Although many previous studies have supported the hypothesis that p53-dependent cell cycle control depends on the transactivation of specific genes (e.g., p21WAF1[CDKN1A]), the precise mechanism underlying the control of p53-dependent apoptosis is still unclear.

Transactivation-independent tumor suppression and apoptosis induction have been reported from several laboratories (9–13). Since then, accumulating experimental evidence has suggested that there is at least a cytoplasmic p53 function (14) other than the known nuclear p53 function for p53-dependent apoptosis, and the cytoplasmic p53 modulates the localization of death receptors (15) or is localized in mitochondria (16), modulating BH3 family proapoptotic proteins including Bax, Bcl-2, and Bcl-xl (17–21). Because at least some of the common mutant p53s are also inactive in transactivation-independent apoptosis, it remains unclear whether the transactivation-independent pathway can solely induce apoptosis without the transactivation pathway, or vice versa (18).

Recently, we constructed a yeast-based functional assay to construct, express, and evaluate 2,314 mutant p53s representing all possible amino acid substitutions caused by a point mutation throughout the protein (5.9 substitutions per residue; ref. 22). We showed the interrelation among the p53 structure, function, and tumor-derived mutations. The library was a precious resource for the isolation of several temperature-sensitive mutant p53s (23) and second-site suppressor mutations.1 We also showed that there are 635 mutant p53s with either increased activity for specific target gene(s) or decreased activity for a limited number of target gene(s), whereas the remaining ~1,700 mutant p53s showed either wild-type activity or no activity for all the examined target genes. We defined the former subclass as “diverse” mutant p53s because of the diverse transcriptional activity for distinct p53 target genes. Those mutant p53s contained the R175P mutant, a rare and weak-tumorigenic mutant p53 that retained cell cycle arrest but abrogated apoptosis induction probably depending on its diverse transcriptional activity of target genes (24–26). From the results of the R175P studies, we hypothesized that if the cellular response (cell cycle arrest and apoptosis) depends on the group of p53 target genes transactivated, the ability of p53 to induce apoptosis differs among mutant p53s and should correlate with a

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Chikashi Ishioka, Department of Clinical Oncology, Institute of Development, Aging and Cancer, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan. Phone: 81-22-717-8542; Fax: 81-22-717-8548; E-mail: chikashi@idac.tohoku.ac.jp.

©2005 American Association for Cancer Research.

1 K. Otsuka, S. Kato, and C. Ishioka, unpublished data.
transactivation profile of the specific p53 target genes. To examine this hypothesis, we randomly selected 179 (~30%) of such mutant p53s, expressed them in Saos-2 cells, and evaluated their sequence-specific transcriptional activities on six p53 target genes (p21WAF1, BAX, MDM2, 14-3-3σ, p53AIP1, and PUMA) and their ability to induce apoptosis. As expected, there was diversity in their ability to induce apoptosis. We identified 17 mutant p53s with more potent apoptotic activity than that of wild-type p53, and some had comparable ability to S121F mutant p53, previously reported as "super" p53 mutant (27). There was no significant correlation between the ability to induce apoptosis and the transcriptional activity on any single target gene examined and there was no common target gene responsible for their superior ability to induce apoptosis. Combining these results with previous reports, we find at least three possible explanations: (a) the induction of apoptosis by p53 does not always depend on any single p53-downstream gene and requires harmonized transcriptional up- and/or down-regulation of p53 target genes, (b) in some cases, interaction of other proteins with p53 may affect p53-dependent transactivation and apoptosis in the series of mutant p53s, or (c) transactivation-independent apoptosis is more important than transactivation-dependent apoptosis in p53-dependent apoptosis. Additional experiments are necessary to elucidate the molecular mechanisms underlying p53-dependent apoptosis.

**Materials and Methods**

**Cell Culture.** Human osteosarcoma Saos-2 cells were cultured in RPMI 1640 with 10% FCS at 37°C. For cell cycle analysis, immunoblot analysis, and cell proliferation assay, 1.5 × 10^5^ cells were seeded in a 35-mm plate (Corning, Corning, NY) and were incubated for 24 hours until 500 ng of each p53 expression vector was introduced by lipofection using an Effectene transfection reagent (Qiagen, Hercules, CA). The efficiency of the transient transfection was evaluated by counting at least 300 cells for 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) staining in each of six independent cultures using a β-galactosidase expression vector, pCAGGS-lacZ (28). The average fraction of the X-gal staining cells (mean ± SD) was 51.16 ± 1.42%, confirming high efficiency and small SD of the assay. In the cell proliferation assay, the viable cells were counted by the 0.5% trypan blue dye exclusion method at the first, second, or third day after lipofection.

**Plasmid Construction.** The wild-type p53 expression vector, pCR295-p53WT, and some of the mutant p53 expression vectors, pCR295-p53MTs, were constructed previously (22, 23). Most of the pCR295-p53MTs were newly constructed in this study, and the TP53 cDNA of the p53s were sequenced using a dye terminator cycle DNA sequencing kit (Beckman Coulter, Fullerton, CA) and an automated CEQ2000XL DNA analysis system (Beckman Coulter). The quantity and quality of the plasmids were confirmed by spectrometry and 1% agarose gel electrophoresis before lipofection.

**Fluorescence-Activated Cell-Sorting Analysis.** Five hundred nanograms of pCR295-p53WT or each of the pCR295-p53MTs was introduced into the Saos-2 cells (1.5 × 10^5^ cells) by lipofection. After 72 hours incubation, the cells were treated with trypsin and fixed with 70% ethanol in PBS overnight. The cells were then washed once with PBS, incubated in the presence of RNase A (0.25 mg/mL) for 30 minutes at 37°C, collected by centrifugation at 200 × g for 5 minutes, and stained with propidium iodide (50 μL/mL). The cells were filtered through a 50-μm pore size nylon membrane, and the p53 proteins were detected using a polyclonal anti-p53 antibody conjugated with horseradish peroxidase (FL193s sc-6243 HRP, Santa Cruz Biotechnology Inc., Santa Cruz, CA).

**Luciferase Assay.** Reporter plasmids, p21s-luc, pMDM2s-luc, pBAXs-luc, and pSIGMAs-luc were described previously (29). Reporter plasmid pAIPI-1uc was constructed by inserting the EcoRI fragment containing the p53AIP1 promoter (GenBank accession no. AP000920, nucleotides 104403-105010) into the EcoRI site of pGL-Basic in this study. Reporter plasmid Frag1 contains the PUMA promoter (30). When the Saos-2 cells reached about 60% to 80% confluence in 96-well plates (Corning), pCR295-p53WT or pCR295-p53MT (12.5 ng) and one of the six luciferase reporter plasmids (87.5 ng) were cotransfected into the cells by lipofection. After 24 hours, luciferase activity was measured using a Steady-Glo luciferase assay system (Promega, Madison, WI). The mean value of three independent experiments was calculated and was evaluated by the relative value for wild-type p53.

**Definition of Diverse Mutant p53.** The transcriptional activity of each mutant p53 in yeast cells for eight target genes was quantitatively evaluated by their fluorescence intensity for p53-dependent fluorescent protein expression (22). In this study, we defined diverse mutant p53 when the transcriptional activity of a mutant p53 on ≥1 but <8 distinct p53 binding sequences was 1.5-fold higher than that of wild-type p53, or 0.67-fold lower than that of the wild-type; more than 600 mutant p53s were eligible and 179 mutant p53s were randomly selected among these mutant p53s.

**Hierarchical Clustering.** The relative transcriptional activity of each mutant p53 in human cells was depicted as a color gradation from red (high) to green (low) depending on the level of transcriptional activity using the TIGR MeV2.2 program. The transcriptional activities of 185 mutant p53s, wild-type p53, and null p53 on the distinct p53 target genes were analyzed by hierarchical clustering method using the Cluster and Tree View programs and contrasted with the ability to induce apoptosis.

**Results**

**Selection of Diverse Mutant p53s.** In our yeast assay, most of the 2,314 mutant p53s represented either wild-type transcriptional activity or no activity in the examination of p53-binding sequences derived from the p53 target genes. In the remaining mutant p53s (635 mutations), the transcriptional activity was increased in p53 binding sequence(s), decreased in p53 binding sequence(s) or both increased and decreased in distinct p53 binding sequences (22). These mutant p53s with diverse transcriptional activity for distinct p53 binding sequences were defined as diverse mutant p53 (see Materials and Methods). We randomly selected 179 diverse mutant p53s (~30%) to analyze for their ability to induce apoptosis. As shown in Supplementary Table 1, most of the mutant p53s have been reported only a few times, if at all, in human cancers. The diverse mutant p53s were further classified into three subgroups. The first subclass (106) contained mutant p53 with decreased transcriptional activities for one or more but not all of the eight p53 binding sequences; the second subclass (18) contained mutant p53 with increased transcriptional activities for one or more of the eight p53 binding sequences; and the third subclass (55) contained mutant p53s with both decreased and increased transcriptional activities for some of the eight p53 binding sequences. The proportions were nearly identical in the whole diverse mutant p53s (data not shown).
The Ability to Induce Apoptosis among the Selected Diverse Mutant p53s. In addition to the 179 diverse mutant p53s, we used six tumor-derived common mutant p53s (R175H, G245S, R248W, R249S, R273H, and R273C) as known controls with no or less ability to induce apoptosis. The mutant p53-expression vectors with one of the 185 mutations, a null p53 vector, and a wild-type p53 expression vector were introduced into Saos-2 cells. To confirm the expression level of p53 protein, either wild-type p53 (pCR259-p53WT) or each of the eight representative mutant p53s (pCR259-p53MTs) was introduced into Saos-2 cells and the expression of the expressed p53 was detected by immunoblot analysis as described in Materials and Methods. As shown in Supplementary Fig. 1, the amount of expressed p53 protein was comparable and nearly identical among the distinct p53 expression vectors. To evaluate the ability to induce apoptosis in these 185 mutant p53s and wild-type p53, the subG1 was calculated after repeated FACS analyses (see Materials and Methods). The subG1 ranged from 0.2 to 17.9, and there was diversity in the ability to induce apoptosis among 185 mutant p53s (Fig. 1). The subG1 of wild-type p53 was 8.4 ± 3.3 (mean ± SD) and the subG1 of 94 mutant p53s (50.8%) were within this range (5.1-11.7), indicating that these were mutant p53s with equivalent or comparable ability to the wild-type p53. The subG1 of S121F, a known super p53, was 15.7 ± 2.1, significantly larger than that of wild-type p53. The subG1 of 17 mutant p53s (S121C, S121A, S121Y, Q144R, P153H, R156C, I162M, F212Y, H214Q, S215C, E221Q, R290G, K291E, K291Q, K291T, K292T, and K292I) fell at or above this range (>13.6). These mutants were considered to be more potent than wild-type p53 and to be equally or more potent than S121F. The subG1 of R175H, a loss-of-function mutation, was 2.7 ± 1.9. The subG1 of 37 mutant p53s (20.0%) fell at or below this range (<4.6), indicating that these were also loss-of-function mutant p53s, similar to R175H. These results indicated that mutant p53s with diverse transcriptional activities for distinct p53 binding sequences also had diversity in their ability to induce apoptosis. Interestingly, we found that there were several mutant p53s with more potent activity than wild-type p53 in the induction of apoptosis when overexpressed in Saos-2 cells. To confirm this observation, FACS analysis was done thrice on the 17 mutant p53s and S121F as well as wild-type p53, and were shown as the mean ± SD. *, P < 0.1; **, P < 0.05; ***, P < 0.01 (unpaired t test between mutant p53 and wild-type p53).

**K291T, S121A, and S121C** were reconfirmed to have significantly more potent ability to induce apoptosis than wild-type p53 (unpaired t test, P < 0.05; Fig. 2). We defined the nine mutant p53s as novel super p53s. A representative DNA histogram is shown in Fig. 3. The remaining eight mutant p53s (F212Y, E221Q, K291Q, S121Y, R156C, S215C, K292I and P153H) also had more potent ability than wild-type p53 but were not statistically significant (P = 0.053-0.095). The ability to induce apoptosis in the 17 mutant p53s was not statistically different from that of S121F. We noted that the subG1 in the six common mutant p53s, R175H, G245S, R248W, R249S, R273H, and R273C, were 2.7, 3.4, 4.6, 0.7, 1.1, and 0.6, respectively, significantly smaller than wild-type p53.

**Inhibition of Saos-2 Cell Proliferation by Novel Super p53s.** To examine whether novel super p53s inhibit cell proliferation, we selected six of nine super p53s. The viable cells were counted at 2 or 3 days after the introduction of these mutant p53s by lipofection. All the six mutant p53s inhibited Saos-2 cell proliferation more strongly than wild-type p53 (Supplementary Fig. 2). In particular, the inhibition by H214Q, K291E, and K292T was significantly stronger than wild-type p53 (unpaired t test, P = 0.009, 0.003, and 0.017, respectively). These results indicated that the stronger ability to induce apoptosis of mutant p53s correlated with their ability to inhibit cell proliferation.

**Reevaluation of Transcriptional Activity for Each Mutant p53 in Saos-2 cells.** Although the 179 mutant p53s were selected for their transcriptional activity in yeast, we reevaluated those in human cells because we considered that there might be differences in cellular environments and transcriptional machinery between the two cell systems. To analyze the transcriptional activities of the
179 mutant p53s as well as the six common mutant p53s, wild-type p53, and null p53 on the six distinct p53 target genes in human cells, the transcriptional activity of each mutant p53 was evaluated as the relative transcriptional activity for wild-type p53 by luciferase assay (Supplementary Table 2). The mean transcriptional activity in the p53 target genes (WAF1, BAX, MDM2, 14-3-3-σ, p53AIP1, and PUMA) in 179 diverse mutant p53s was 0.680 (0.024-1.531), 0.663 (0.064-2.262), 0.855 (0.033-1.790), 0.998 (0.071-2.354), 0.664 (0.046-1.910), and 1.531 (0.325-3.213), respectively, with a wide range of values indicating that the transcriptional activity of the diverse mutant p53s in yeast was also diverse in human cells. Conversely, those in the six common mutant p53s were 0.019 (0.008-0.067), 0.075 (0.064-0.096), 0.043 (0.030-0.078), 0.111 (0.082-0.20), 0.236 (0.175-0.331), and 0.271 (0.156-0.351), respectively, indicating that the transcriptional activity of the common mutant p53s was significantly lower than wild-type p53 in all of the six p53 target genes as indicated in yeast. Overall, the transcriptional activity of the examined mutant p53s was roughly similar between the two cell systems, although they were not always identical (refer to Supplementary information and Supplementary Fig. 3).

No Evident Correlation between the Ability to Induce Apoptosis and Transcriptional Activity in the 179 Diverse Mutant p53s. To evaluate the correlation between the transcriptional activity (standardized value) on the six distinct p53 target genes and the subG1 in the 179 diverse mutant p53s, these two values were plotted and shown in Fig. 4. The correlation coefficient (r) between the two values on each p53 target gene, WAF1, BAX, MDM2, 14-3-3-σ, p53AIP1, or PUMA, was 0.167 (P = 0.025), 0.002 (P = 0.983), 0.014 (P = 0.848), 0.053 (P = 0.482), −0.008 (P = 0.914), or 0.013 (P = 0.864), respectively. These results indicated that there was no significant correlation between the ability to induce apoptosis and the transcriptional activity in any single, p53 target gene in 179 mutant p53s. The mean transcriptional activities of the S121F and the 17 mutant p53s with potent ability to induce apoptosis (see above) in the six p53 target genes, WAF1, BAX, MDM2, 14-3-3-σ, p53AIP1, and PUMA, were 0.875 (0.141-1.373), 0.730 (0.128-1.169), 0.851 (0.120-1.534), 1.110 (0.291-1.951), 0.612 (0.273-0.870), and 1.538 (0.805-2.443), respectively. Again, there was little relationship between the transcriptional activity and the ability to induce apoptosis in the 18 mutant p53s (Fig. 4).

Hierarchical Clustering of Mutant p53s in Their Transcriptional Activity. The transcriptional activity of 185 mutant p53s, wild-type p53, and null p53 in the distinct p53 target genes were analyzed by hierarchical clustering (see Materials and Methods). The result is shown in Fig. 5. All common mutant p53s but G245S were classified in a single cluster with no transcriptional activity in any target genes. The 18 mutant p53s with potent ability to induce apoptosis were not clustered at all.

Discussion

Identification of the Novel Super p53s. This study started with 635 diverse mutant p53s that had been selected in a yeast-based functional assay (22). From the initial stage of this study, we believed that novel super p53 should be identified from those mutant p53s and considered that such mutant p53s might provide a clue to induce apoptosis in tumor cells efficiently. The 179 mutant p53s were expressed in human cells under the control of a cytomegalovirus promoter and were evaluated for their ability to induce apoptosis. Consequently, we identified 17 mutant p53s with potent activity for apoptosis including nine super p53s (see above). We now predict that there are more unidentified super p53s in the remaining pool of the diverse mutant p53s. We are also trying to introduce the super p53 into a mouse tumor xenograft model to examine the antitumor effect in vivo. The super p53s may also provide a clue to develop novel cancer therapies to efficiently induce apoptosis in cancer cells by analyzing their transcriptional profiles when expressed in cells.

Relationship between the Ability to Induce Apoptosis and p53 Structure. To examine the correlation between the ability to induce apoptosis and the p53 domain, the ΔsubG1, in 179 diverse mutant p53s were plotted on p53 motif for the primary structure (Supplementary Fig. 4). One hundred fifty-six mutant p53s were mapped on the core domain (residue 96-300) containing sequence-specific DNA binding domain (residues 96-286, 146 mutant p53s) and the carboxyl-terminal flanking region (residues 287-300, 10 mutant p53s). The remaining 23 mutant p53s were plotted on the amino-terminal region (residues 1-95, 13 mutant p53s) or the carboxyl-terminal region (residues 301-393, 10 mutant p53s). Obviously, the diverse mutant p53s were concentrated in the central core domain. Interestingly, among the 18 mutant p53s with
more potent ability to induce apoptosis than that of wild-type p53, four (S121F, S121C, S121A, and S121Y) and six (R290G, K291E, K291Q, K291T, K292T and K292I) mutant p53s were mapped at residue 121 in the L1 loop and residues 290 to 292 in the flanking region to the H2 helix, respectively (Supplementary Fig. 5). Although the intramolecular alteration caused by these substitutions was not clear, we speculate two possibilities. First, these substitutions affect the local or global p53 structure and the resulting structures are beneficial for apoptosis induction. Second, these substitutions interfere p53-interacting protein(s) that modulate the apoptotic function of p53. Obviously, additional experiments are necessary to elucidate their molecular mechanisms.

**Lack of Correlation between Ability to Induce Apoptosis and p53 Transcriptional Activity.** Our previous knowledge of p53-dependent apoptosis was that, after genotoxic stress, activated p53 transactivates its downstream genes in a sequence-specific manner and induces apoptosis in cells through the direct and/or indirect induction of the downstream protein(s). The transactivation-dependent theory has been supported by many genetic and biochemical experiments and is supported because the tumor-derived common mutations have no ability to transactivate downstream genes and to induce apoptosis. However, we also considered that the theory should be reevaluated carefully for the following reasons. First, the transactivation-independent mechanism of p53-dependent apoptosis has been reported by several laboratories. Second, most of the remaining mutations have not been examined for their ability to induce apoptosis. Taking advantage of an opportunity to use the comprehensive mutant p53 library (22), we may examine all of the mutant p53s in the future. Unfortunately, plasmid construction and the functional evaluation of ~2,000 mutant p53s in mammalian cells are currently labor-intensive. Selecting a limited number of mutations from the p53 mutation library would be an alternative approach. In this study, we chose to analyze 179 mutant p53s from 635 diverse mutant p53s and avoided selecting mutant p53s from 905 mutant p53s with intact transcriptional activity or from 361 mutant p53s with complete loss of transcriptional activity. This was because most of the former mutant p53s may retain both transactivation-dependent and -independent apoptosis, whereas the later mutant p53s may not retain both. Therefore,

**Figure 4.** Correlation between the ability to induce apoptosis and transcriptional activity in the 179 diverse mutant p53s. Each scatter diagram shows the correlation between ΔsubG1 by FACS analysis and the standardized value of transcriptional activity by luciferase assay in six p53 binding sequences. The correlation coefficient (r) between the two values on each p53 binding sequence, WAF1, BAX, MDM2, 14-3-3σ, p53AIP1, or PUMA, was 0.167 (P = 0.025), 0.002 (P = 0.983), 0.014 (P = 0.848), 0.053 (P = 0.482), −0.008 (P = 0.914), or 0.013 (P = 0.864), respectively. ○, 18 mutant p53s with potent ability to induce apoptosis (see text) including S121F.
Figure 5. Hierarchical clustering of mutant p53s in their transcriptional activities. One hundred eighty-five mutant p53s, null control, and wild-type p53 were clustered on the transcriptional activity in human cells and was shown by DNA microarray analysis (see Materials and Methods). The 18 mutant p53s with potent ability to induce apoptosis (see text) including S121F are shown. *, \( P < 0.1; **, \ P < 0.05; ***, \ P < 0.01 \) (unpaired \( t \) test between each mutant p53 and wild-type p53). Wild-type p53 (arrow) and the six common mutant p53s (bars) are also marked.

we may not be able to address the relationship between the ability to induce apoptosis and p53 transcriptional activity using the intact and inactive mutant p53s, and therefore, cannot estimate the feasibility of the theory or the transactivation-independent mechanism. Our results from the 179 diverse mutant p53s suggested that there was no relationship between the transcriptional activity of any single specific gene and the ability to induce apoptosis. This was also the case in super p53, and there was no common target gene responsible for strong apoptosis by super p53. These results suggested that transactivation-dependent apoptosis does not play a major role in p53-dependent apoptosis.

Combining these results with previous reports, we propose at least three possibilities. First, the induction of apoptosis by p53 does not depend on any single p53 downstream gene and rather requires the harmonized transcriptional up- and down-regulation of p53 target genes. Considering these results, hierarchical clustering for 179 mutant p53s and their transcriptional activity with the six promoters was done because we considered that there might be subcluster(s) containing a specific class of mutant p53s, such as the super p53s, beyond the single gene analysis. As shown in Fig. 5, no cluster(s) preferentially contained super p53s. Because there are several p53 downstream genes, there may still be insufficient information to classify these specific mutant p53s into a specific subcluster. To elucidate this and the transactivation-independent mechanism underlying super p53, we are planning to perform comprehensive expression analysis of the mutant p33-expressing cells by DNA microarray. Second, interaction of other proteins with p53 may affect p53 stability, localization, and p53-dependent apoptosis in the series of mutant p53s, making it difficult to understand the mechanisms in p53-dependent apoptosis. Finally, transactivation-independent apoptosis is more important than transactivation-dependent apoptosis in p53-dependent apoptosis mechanisms. Additional experiments are necessary to evaluate which mechanism is important for p53-dependent apoptosis.

Acknowledgments

Received 8/17/2004; revised 11/30/2004; accepted 1/4/2005.

Grant support: Ministry of Education, Science, Sports and Culture (C. Ishioka and S. Kato), the Sagawa Foundation for Promotion of Cancer Research (C. Ishioka), and the Goryo Medical Foundation (C. Ishioka).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore hereby be marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Jun-ichi Miyazaki and Bert Vogelstein for a kind gift of plasmids, Yuka Fujimaki and Atsuko Takahashi for technical assistance, and Shuang-Yin Han and Wen Liu for construction of the p53 mutation library.

References

3. Hall P, Meek D, Lane D. p53030 integrating the downstream genes, there may still be insufficient information to


Lack of Correlation between p53-Dependent Transcriptional Activity and the Ability to Induce Apoptosis among 179 Mutant p53s

Yuichi Kakudo, Hiroyuki Shibata, Kazunori Otsuka, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/65/6/2108

Supplementary Material
Access the most recent supplemental material at:
http://cancerres.aacrjournals.org/content/suppl/2005/04/05/65.6.2108.DC1

Cited articles
This article cites 29 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/65/6/2108.full#ref-list-1

Citing articles
This article has been cited by 18 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/65/6/2108.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.