High throughput RNAi screening identifies ID1 as a synthetic sick/lethal gene interacting with the common TP53 mutation R175H

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Abstract. The TP53 mutation (R175H) is one of the most common mutations in human cancer. It is a highly attractive strategy for cancer therapy to find the genes that lead the R175H-expressing cancer cells. The aim of this study was to identify the synthetic sick/lethal gene interacting with R175H. Using lentiviral bar-coded comprehensive shRNA library and a tetracycline-inducible R175H expressed in the SF126 human glioblastoma cell line (SF126-tet-R175H), we conducted high-throughput screening to identify the candidate genes that induce synthetic sickness/lethality in R175H-expressing cells. We identified 906 candidate gene suppressions that may lead to accelerated cell growth inhibition in the presence of R175H. Inhibitor of differentiation 1 (ID1) was one of the candidate genes, and its suppression by siRNA resulted in the acceleration of growth inhibition in cell lines both transiently and endogenously expressing R175H but not in TP53-null cell lines or other common p53 mutants (such as R273H). Flow cytometry analysis showed that ID1 suppression resulted in G1 arrest, and the arrest was accelerated by the expression of R175H. ID1 is a synthetic sick/lethal gene that interacts with R175H and is considered to be a novel molecular target for cancer therapy in R175H-expressing cells.

Introduction

Synthetic sickness/lethality interaction is a highly attractive strategy for cancer therapy (1-4). For example, in cancer cells with a KRAS gene mutation, the inhibition of polo-like kinase 1 (PLK1) resulted in cell death (5). Similarly, cancer cells with the KRAS mutation were sensitive to the suppression of the serine/threonine kinase STK33 (6). Moreover, dysfunction of DNA double-strand break repair caused by mutations in BRCA1 or BRCA2 gene sensitized cells to the inhibition of poly-ADP ribose polymerase (PARP) enzymatic activity, resulting in chromosomal instability, cell cycle arrest, and subsequent apoptosis (7). This concept had been proved by a phase II trial where olaparib, a PARP inhibitor, provided objective antitumor activity in patients with a BRCA1 or BRCA2 mutation (8).

TP53 is the most commonly mutated tumor suppressor gene in several different types of human cancer (9). TP53 encodes the 393 amino acid p53 protein, which binds to specific DNA sequences in the regulatory region of downstream genes (10). A variety of cellular stressors including ultraviolet rays, ionizing radiation, chemotherapeutic drugs, and hypoxia stabilize the p53 protein, and post-translational modifications activate it; this results in various cellular responses including cell cycle arrest, DNA repair and apoptosis (11,12).

According to the TP53 mutation databases, >75% of the mutations are missense mutations (13,14); to date, >1,200 distinct missense mutations have been reported. Among them, those at residues Arg175(R175), Gly245(G245), Arg249(R249), Arg249(R249), Arg273(R273) and Arg273(R273) have been reported most frequently (15). The most common p53 mutant proteins caused by TP53 hot-spot mutations are R175H, G245S, R248W, R248Q, R249S and R273H; these mutations cause a loss of the trans-activation function of downstream genes (16). However, some p53 mutants gain new functions that are not observed in wild-type p53 (so called gain-of-function mutations). For example, mice with the knock-in mutant p53 R172H and R270H, which correspond to human p53 R175H and R273H mutations, develop a variety of novel tumors such as lung adenocarcinoma, renal cancer, hepatocellular carcinoma, and intestinal carcinoma which are not generally observed in TP53-null mice (17). In addition, embryonic fibroblasts derived from p53 R172H knock-in mice gained activities of cell proliferation, DNA synthesis and retroviral transformation (18). Moreover, human p53 R273H or R248W interacted with Mre11 and suppressed the binding of the Mre11-Ras50-NBS1 (MRN) complex to DNA double-strand breaks, resulting in the chromosomal translocation and abrogation of the G2/M check point (19). According to these results, it has been hypothesized that some p53 mutant proteins, such as the activated K-ras
protein, are oncogenic and contribute to carcinogenesis and cancer progression.

In the present study, we conducted high-throughput RNAi screening by a lentiviral gene suppression system to identify synthetic sick/lethal genes in the presence of p53 R175H, which accounts for ~6% of the missense mutations identified in human cancer (20). As a result, we identified that inhibitor of differentiation 1 \((\text{ID}1)\) is the first gene that causes synthetic sickness when paired with p53 R175H mutant protein.

Materials and methods

Cell lines and culture. The stable SF126 cell line expressing the doxycycline (Dox)-inducible p53 R175H mutant (SF126-tet-R175H) was constructed according to the protocol described previously (21). In addition, SF126-tet-TON, which does not express p53, was used as a control (21). Mutant p53 was induced with 10 ng/ml doxycycline (Sigma-Aldrich, St. Louis, MO, USA). Five human cell lines including SKBr3 and HCC1395 (both derived from breast cancer), VMRC-LCD (derived from lung cancer), Detroit 562 (derived from head and neck cancer), and LS123 (derived from colon cancer) express p53 R175H endogenously. Colon cancer cell lines HT-29 and SW480 express p53 R273H endogenously. HCT116 (derived from colon cancer) expressed wild-type p53 endogenously. Four cell lines including PC3 (derived from prostate cancer), H1299 and Calu-1 (both derived from lung cancer), and SK-N-MC (derived from neuroblastomas) are TP53-null. PC3 was purchased from Cell Research Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). SKBr3, HCC1395, LS123, H1299, Calu-1, SK-N-MC, HCT116 and SW480 were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Detroit 562 and VMRC-LCD were purchased from DS Farma Biomedical (Osaka, Japan) and Human Science Research Resources Bank (Tokyo, Japan), respectively. HT-29 was a gift from Dr John M. Mariadason. SKBr3, HCC1395, HT-29, SW480, H1299 and PC3 were cultured in RPMI-1640, and LS123, Calu-1, SK-N-MC, Detroit 562 and VMRC-LCD were cultured in minimum essential medium with 10% FBS at 37˚C. The identity of SF126, PC3, HCC1395, LS123, H1299, Detroit 562, VMRC-LCD and HT-29 cells was tested using DharmaFECT 1 (Dharmacon, Lafayette, CO, USA). Each purified PCR product was labeled with Cy5 (doxycycline-on group) or Cy3 (doxycycline-off group) using Agilent's Genomic DNA Labeling kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and was hybridized on the barcode microarray in the hybridization oven at 65˚C for 17 h. After hybridization, the arrays were scanned with the Agilent DNA microarray scanner to quantify log2 Cy5/Cy3. The ‘log2 Cy5/Cy3’ indicates increase and decrease of cells in the primary screening and negative value of ‘log2 Cy5/Cy3’ shows that the counts of R175H expressing cells (dyed with Cy5) is smaller than the counts of R175H unexpressed cells (dyed with Cy3). We conducted 2 independent experiments, and obtained 3 independent values of log2 Cy5/Cy3 were analyzed by Student’s t-test. Candidate genes were identified after analyzing raw data for each shRNA using the GeneSpring software (Agilent Technologies). Microarray data were deposited in GEO (accession no. 33362).

Mammalian \(\text{p53} \) expression vectors. To express p53 R175H mutant, the p53 expression vectors pCR259-R175H and pCR259 were used (16,22). Each plasmid was transfected into TP53-null cells using Effectene Transfection Reagent (Qiagen, Hercules, CA, USA), following the manufacturer’s recommendations. Expression of p53 R175H was confirmed by western blot analysis.

RNAi screening. One million SF126-tet-R175H and SF126-tet-TON cells were seeded in 10-cm culture plates for 24 h. The medium was removed from the plates and the Decode RNAi Viral Screening Library (Thermo Scientific Open Biosystems, Huntsville, AL, USA) was added to the plate at the multiplicity of infection (MOI) of 0.3 with serum-free medium. After 6 h, the medium was replaced with virus-free medium. After 48 h, puromycin was added at a final concentration of 2 mg/ml to select the infected cells. Finally, 7x10⁶ of lentivirus-infected SF126-tet-R175H and SF126-tet-TON cells were obtained. These cells theoretically contain 70,000 distinct shRNAs, and each cell should express a single shRNA product. These cells were divided into 2 groups, and each group was cultured with or without doxycycline for 10 days. Genomic DNA was extracted from each group using the Blood & Cell Culture DNA Mini kit (Qiagen), according to the manufacturer’s recommendation. Barcode sequences corresponding to specific shRNAs were amplified by the following primers located outside the barcode sequence: forward, 5’-caagggctactttaggagcaattatcttg-3’ and reverse, 5’-ggtgattgcctcaagcgt-3’.

Amplified PCR products were separated in 1.5% TAE agarose gel and extracted using Wizard SV Gel and PCR Clean-Up system (Promega Corporation, Madison WI, USA). Each purified PCR product was labeled with Cy5 (doxycycline-on group) or Cy3 (doxycycline-off group) using Agilent's Genomic DNA Labeling kit (Agilent Technologies, Inc., Santa Clara, CA, USA) and was hybridized on the barcode microarray in the hybridization oven at 65˚C for 17 h. After hybridization, the arrays were scanned with the Agilent DNA microarray scanner to quantify log2 Cy5/Cy3. The ‘log2 Cy5/Cy3’ indicates increase and decrease of cells in the primary screening and negative value of ‘log2 Cy5/Cy3’ shows that the counts of R175H expressing cells (dyed with Cy5) is smaller than the counts of R175H unexpressed cells (dyed with Cy3). We conducted 2 independent experiments, and obtained 3 independent values of log2 Cy5/Cy3 were analyzed by Student’s t-test. Candidate genes were identified after analyzing raw data for each shRNA using the GeneSpring software (Agilent Technologies). Microarray data were deposited in GEO (accession no. 33362).

Knockdown analysis of candidate genes using siRNA. The siRNAs of 50 candidate genes, identified from primary screening, were synthesized by Hokkaido System Science (Hokkaido, Japan). The sequences of synthesized siRNA for candidate genes are listed in Table I. ID1-2 siRNA was synthesized as described previously (23). TP53 siRNA was purchased from Applied Biosystems (Foster City, CA, USA), and TP53-2 siRNA was purchased from Cell Signaling Technology, Inc. (Boston, MA, USA). A total of 3.5-5.0x10⁵ cells/well were seeded and incubated in a 96-well plate for 24 h. Each candidate siRNA and negative control siRNA was added to each cell. Cell proliferation assays were performed using Cell Counting kit-8 (Dojin Laboratories, Kumamoto, Japan), as previously described (21).

Western blot analysis. Cells were harvested and resuspended in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 5 mM EDTA, and 1% protease inhibitor cocktail (Sigma-Aldrich). The lysate was subjected to western blot analysis, as previously described (24). Anti-p53 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-β-actin (Sigma-Aldrich), anti-Id1, and anti-GAPDH (Applied Biosystems) antibodies were used.
Table I. Sequences of synthesized siRNA for 50 candidate genes.

| Gene symbol | siRNA sense sequence | siRNA antisense sequence | Smallest p-value | Largest fold-change |
|-------------|----------------------|--------------------------|------------------|---------------------|
| UROS        | CCTCTGTGGAGAACGCTTGAA | TTAAGCTGCGCTTCACAGAGG   |                  |                     |
| GYP C       | GCTCAGAAGCAGATGGGAAATA | TTAATTCCAAATCGTTCTGAGC  |                  |                     |
| PRO1596     | CGATGAATATCTCTCTGTGAA | TAAATCCACAGATATTCTGCC  |                  |                     |
| CD69        | GGAGCATTTATTTGCTTTGTA | TTTGTCATTTTAAAAATGCTCC |                  |                     |
| PDXP        | GTACAGTTAGTTAGTTCTCTAA | TTAGGACGCTTAAACTGTTAGC |                  |                     |
| THADA       | CGCTTACAGATATGATCTGAAT | ATTCAGAATACATTTGAGG     |                  |                     |
| KCN110      | GCCAGATATCTTGCGTTGGTA | TAAACCGGCAGAAGATTGGC    |                  |                     |
| ABCA12      | CCACATTCTCTCCACAGCTCAA | TTTCAGTGGAGAGAAATTGG    |                  |                     |
| UBA6        | GCTATTGTTACTGCTTTGAAA | TTTCAAGCAGAACATGACTGC   |                  |                     |
| CDCC7       | GCACATTTCCGCTCTGGTATT | ATAGAAACAGAGCTGAAGTGC   |                  |                     |
| ID1         | GGAAATTGCTTTATGGACTAT | ATAGAATACAAAGAAATTTCC   |                  |                     |
| CTBS        | GCCCTCTGTTTATAATCTATAA | TTAATGTTTAATTAAAGAGCCT  |                  |                     |
| EIF2B3      | CGGATGAACTGATCTGPTTAA | ATACCGAATGAGCCATCTGCC  |                  |                     |
| UFM1        | GTGACGGGAAGTTAGTACAGAAGA | TTTATCTGACACTTGGGATC   |                  |                     |
| PTCD1       | CCTGCAATGTGTTACAGGAAT | ATTCACCTGAAACATCGAGG    |                  |                     |
| TPN2C       | GGAGCTTCTGCTCAGGATAT | ATACCCGACGAGGAGTCCT     |                  |                     |
| NEURL       | GTTGAACAAATCTTCTCAGAT | ATACGTGGAGAGTTGTTACC    |                  |                     |
| STAP4       | GCACATTATTAGTTAGTATT | ATACCAACCTTAATATAGTGC   |                  |                     |
| C19orf40    | GAATATTGCTGGAGGAGAAGA | TTTCATGCTGACCACATATGG   |                  |                     |
| C19orf38    | CCAACCTTGGAGTATCAGTAC | CTGAGTATGTCATCAAGTG     |                  |                     |
| C14orf37    | GGAACATCTTCTACAGCAACT | TTATGCCTGTTAAGAGTCTC    |                  |                     |
| MGC42105    | CCACAGCTGACGCCCTTCAGGAA | TTTCTCAGAGGGCGTCAGCTG  |                  |                     |
| GP6         | GGGTCCAGACGGAATCTCTAA | TTAGAGATCCTGCTGGAGCC    |                  |                     |
| BCL2L14     | GCTCAGTGCCTCAAGTCTCATA | TAGAAGATCAGCCATACAGCC   |                  |                     |
| HILSI       | GCCAAAGTCCGACTGCTATAA | TTAATGTCAGGCTACCTGCC    |                  |                     |
| CLNDND2     | GAAGAAATCTGAGTGGAGGAGC | TTTGTCCTTACCAGCACCTTC   |                  |                     |
| UMOD        | CCACTGACACCTCAGACACAA | TTGGCTCTGAGGTCAGTGG     |                  |                     |
| RHAG        | GCCATATTCTTCTAGTATAA | TATAACCTCAAGATATGCCC    |                  |                     |
| GFPT2       | GCTAGGTTTTAGTGCTCAGAAA | TTTCTGCTGACATGTCAGC     |                  |                     |
| SATL1       | AACATGTGAGATTCTCTTAAAT | ATTGTGAAAGTATCAGTCATT  |                  |                     |
| RTN4IP1     | GCTGCAGTGTAAATCTCTATA | TATAGAATTTACACTGCGAGC   |                  |                     |
| DOK7        | CCAAGGCGATTTCTCTTTGGA | TTCAAGATGGAAATCCTGTTG   |                  |                     |
| NUP998      | GCGACAAGCTTTACAAACAA | TTAGGTGTGAAGCCCTGGCGC   |                  |                     |
| PWPP2A      | GCCATGCGCCTCAGAAATGAT | ATTATTTGAGGCGCATAGCG   |                  |                     |
| MEP1A       | GCCATTAGGCGCATCATAGAG | TTCTGATAGTGCTCTATAGG   |                  |                     |
| CCT6B       | TGCGTGAAGCTTTCTTGTACAT | ATGCAACAGACTTCCAGCAGCA |                  |                     |
| PDXP        | GTTACAGTTTGAAGTTCCTTAA | TTAGGAACTCTAAGTTCAGC    |                  |                     |
| ZNF300      | GCTAATGATTCTTGTCTTCAA | TTAAGGACGCTTACATAGGC    |                  |                     |
| DEFB125     | AGAGGATATAAATCTTTGATTA | TAATCCAATTGTTATATCTCCT |                  |                     |
| GJA5        | CTGTTGCATTAAATCTGATAGA | TTTCCAGTAATGAGCAACG     |                  |                     |
| EFNA4       | CCATGGTCTATTTCTCCAGAGA | TTTCCTGAGGAATGACATGG   |                  |                     |
| Double entry |                      |                          |                  |                     |
| NMNAT1      | TACATCTGGAAGTGTACGCTTTA | TTTACGTAAGCTCTTACAGTGA  |                  |                     |
| KLH110      | GCCTAGTACCTCTGAAACAT  | ATTTGTTCTGAAAGTACTCAGC  |                  |                     |
| LMLN        | CCACAGTGAAACATGAGGTTA | TAAACCTGTAGTTCTCAGG     |                  |                     |
| FBXO22      | TCTCTCAGTGGAAATGAAATAA | TTAATTCCCTACATTTGGAGA   |                  |                     |
| ITGB7       | GGAGACTAAATCCTCTCACAAC | TTGGTACAGGAGATTACTGTC   |                  |                     |
| CPT1        | GAAGTAGCAAGAACTTAATTAGT | ATTAATGAGTCTTACCTGCC   |                  |                     |
| COLQ        | GCTCTACATTTGCTTCTCTT   | AAGAGGAAAGCATTGTAAGCC  |                  |                     |
| AP3B2       | GAGTTGCAACCTTGATCTTAA | TTAAGACATGAGTGCTCAATCC |                  |                     |
| ANXA11      | CGTGGTGAATAATGTCAAGAA | TTCTGAGACATTCACACAGC   |                  |                     |
Cell cycle analysis by FACS. A total of 1.5x10^4 cells/plate were seeded and incubated in 6-cm culture plates for 24 h. The cells were further incubated in the presence of drugs for 48 h. These cells were collected, and FACS analysis was performed, as previously described (24).

Results

Screening of synthetic lethal genes that interact with p53 R175H mutant. A flow chart of the high-throughput screening of synthetic lethal genes interacting with p53 R175H is shown in Fig. 1. By comparative analysis, 1,362 candidate genes were identified for synthetic lethality with p53 R175H expression in the SF126-tet-R175H cell line (p<0.05 according to t-test, n=3). Among these, 43 were excluded as suppression of these genes also resulted in decreasing cell numbers in SF126-tet-TON cells after doxycycline treatment (no R175H expression). In the remaining 1,319 genes, 906 genes have validated gene symbols (p-value <0.05). Among these, we selected 50 genes (21 genes from the group with the smallest p-values, 20 genes from the group with the largest fold-change, and 9 genes reproduced by different siRNA sequences) for further validation testing (Table I).

Suppression of candidate genes by siRNA in p53 R175H expressing cell lines and TP53-null cell lines. To investigate whether the suppression of candidate genes by siRNA resulted in p53 R175H-dependent inhibition of cell growth, candidate gene siRNAs were transfected into cell lines expressing endogenous p53 R175H (SKBr3, LS123, HCC1395, Detroit 562 and VMRC-LCD) and TP53-null cells (PC3, H1299, SK-N-MC and Calu-1). We obtained the ratio of cell growth inhibition of candidate gene siRNA transfected cells for negative control siRNA transfected cells on day 4. In 50 candidate genes, suppression of GYPC, NUP98, GP6, EFNA4 and ID1 by siRNA significantly decreased the number of p53 R175H expressing cells compared with TP53-null cells (t-test) (Table II).

To examine whether the cell growth inhibition resulting from suppression of the candidate genes depends on
p53 R175H expression, GYPC, NUP98, GP6, EFNA4 and ID1 were suppressed by specific siRNAs in PC3 cells transiently expressing p53 R175H. Suppression of these genes inhibited cell growth; however, among the candidate genes, ID1 suppression significantly accelerated the cell growth inhibition under transient p53 R175H expression (Fig. 2A and B). ID1 suppression and p53 R175H overexpression did not influence the other protein expression level (Fig. 2C). These results suggest that p53 R175H expression and ID1 suppression cooperate to cause cell growth inhibition.

**Cell growth inhibition by ID1 and/or TP53 suppression in endogenously expressing p53 R175H, wt p53 cells and in TP53-null cells.** To determine whether cell growth inhibition is rescued by the suppression of both candidate genes and p53 R175H, siRNAs of the targeting candidate genes and TP53 were transfected into SKBr3, a p53 R175H expressing cell line. Downregulation of p53 R175H rescued cell growth inhibition caused by ID1 suppression (Fig. 3A), but not by GYPC, NUP98, GP6 and EFNA4 suppression (data not shown). To exclude the off-target effect of siRNA, other siRNA for ID1 and TP53 targeting different sites (ID1-2 and TP53-2) were transfected into SKBr3, and we observed reproducible results to the original siRNAs (Fig. 3B and C). Moreover, similar results were observed only in cell lines expressing p53 R175H (LS123, Fig. 3D and HCC1395, Fig. 3E), but not in wt p53 (HCT116, Fig. 3F), and TP53-null (PC3, Fig. 3G). The quantity of the Id1 protein in SKBr3 was not altered by p53 R175H expression.

Figure 3. Comparison of cell growth inhibition by ID1 suppression alone and ID1/TP53 double suppression in cells expressing p53 R175H, wild-type p53, and TP53-null cells. (A) ID1 and TP53 siRNA were co-transfected into SKBr3 cells to make a final concentration of 100 nM, and cell numbers were measured by performing cell proliferation assays on days 2 and 4. The vertical axis corresponds to the absorbance of the cell proliferation assay. Top of each figure represents expression inhibition of Id1 or p53 by each siRNA. Furthermore, other siRNAs of ID1 (ID1-2) (B) and TP53 (TP53-2) (C) targeting different sites were transfected into SKBr3 cells. The same experiments were conducted in LS123 and HCC1395 cells, p53 R175H expressing cell lines (D and E), HCT116 cells expressing wild-type p53 (F), and the TP53-null cell line PC3 (G). Values shown are means ± SD (n=3) in (A and E). *p<0.05, **p<0.01, between ID1 suppression alone and ID1/TP53 double suppression. (H) Western blot analysis of Id1 and p53 R175H. Knockdown level of Id1 was not rescued by ID1/TP53 double suppression in SKBr3 cells.
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suppression (Fig. 3H), same as p53 R175H transient expression. These results support the finding that cell growth inhibition by ID1 suppression is accelerated by p53 R175H. **Suppression of ID1 in cell lines expressing another common mutant p53 (R273H).** To examine whether cell growth inhibition caused by ID1 suppression is accelerated specifically by
p53 R175H expression, another common p53 mutant (R273H) was expressed in a PC3 cell line (TP53-null). Unlike p53 R175H expression, p53 R273H expression did not accelerate the cell growth inhibition caused by ID1 suppression (Fig. 4A). Furthermore, the cell growth inhibition caused by ID1 suppression was not restored by simultaneous suppression of TP53 in HT-29 cells expressing endogenous p53 R273H (Fig. 4B). Similar results were observed in SW480 cells expressing endogenous p53 R273H/P309S double mutants (Fig. 4C). These results indicated that the growth inhibition induced by ID1 suppression may be accelerated by p53 R175H expression in a specific manner.

Cell cycle analysis under ID1 suppression and ID1/TP53 double suppression. To examine whether ID1 and/or TP53 suppression change the proportion of cell cycle phases, FACS analysis was performed in SKBr3 cells. ID1 suppression did not change the sub-G1 fraction, but significantly decreased the S phase fraction and increased the G1 phase fraction (Fig. 5A). ID1/TP53 double suppression significantly restored the proportion of S phase and G1 phase fractions. These results suggest that p53 R175H potentiates G1 arrest by ID1 suppression. In HCT116 (wild-type p53) and PC3 (TP53-null) cells, ID1 suppression increased the G1 phase fraction and decreased the S phase fraction. However, unlike in SKBr3 cells, ID1/TP53 double suppression did not restore the proportion of S phase and G1 phase fractions (Fig. 5B and C). These results suggest that ID1 suppression induces G1 arrest and the arrest is specifically accelerated by p53 R175H expression.

Discussion

We identified ID1 as a synthetic sick/lethal gene that caused cell growth inhibition in the presence of p53 R175H. Id1 is a member of the helix-loop-helix protein family expressed in actively proliferating cells and regulates gene transcription by hetero-dimerization with the basic helix-loop-helix (bHLH) transcription factor (25). The homodimer of the bHLH transcription factor activates the differentiation, whereas the heterodimer, composed of Id1 and the bHLH transcription factor, attenuates their ability to bind DNA and consequently inhibits cell differentiation (26). Supporting this finding, stable Id1 expression was found to block B cell maturation (27). Moreover, Id1 can inhibit differentiation of muscle and myeloid cells by associating in vivo with E2A proteins (28,29). It has also been reported that Id1 was immunohistochemically expressed in majority of non-small cell lung cancer (NSCLC) samples (30). Furthermore, Id1 protein expression in prostate cancer cells mediated resistance to apoptosis induced by TNFα (31). These lines of evidence also indicate that ID1 may play an essential role in carcinogenesis.

In the present study, we demonstrated that ID1 suppression resulted in cell growth inhibition that was independent of TP53 status. However, cell growth inhibition caused by ID1 suppression was accelerated specifically by the p53 R175H mutant protein. If the accelerated cell growth inhibition is attributable only to loss-of-function in p53 R175H, this phenomenon should also be observed in TP53-null cells and other cells expressing loss-of-function mutations other than p53 R175H. Some p53 mutant proteins acquire additional functions called gain-of-function (32). For example, ectopic expression of p53 R175H resulted in transactivation of genes that are not usually activated by wild-type p53 (33-35). On the basis of these observations, we concluded that the acceleration of cell growth inhibition was likely attributable to gain-of-function of p53 R175H.

To date, synthetic sickness/lethality has been classified into 2 types based on the initial genetic event. The first type is attributable to a loss-of-function mutation in a target gene and the second type is attributable to a gain-of-function or an activated mutation in a target gene. For example, the synthetic lethal interaction between loss-of-function mutations in BRCA1 and BRCA2 genes and PARP inhibition (8) are a former type, and gain-of-function mutations in the KRAS gene and STK33 inhibition (6) are a latter type. Based on our results, it is clear that the synthetic sick/lethal interaction between p53 R175H expression and ID1 suppression is of the latter type. However, there is a clear difference between the activated KRAS-STK33 interaction and the p53 R175H-Id1 interaction. Gain-of-function in activated K-ras depends on STK33 and is therefore blocked by STK33 suppression. By contrast, the accelerated cell growth inhibition observed here cannot be explained only by blockade of gain-of-function in p53 R175H by ID1 suppression. By contrast, it is necessary for accelerated growth inhibition by ID1 suppression. Taken together, these results suggest that the synthetic sickness/lethality of p53 R175H with ID1 suppression may be through a gain-of-function mechanism that is distinct from the previously identified gain-of-function mechanisms. Since both expression and suppression of p53 R175H had no effect on the amount of Id1 protein, p53 R175H may cooperate with downstream factor(s) that are altered by ID1 suppression and may promote synthetic sickness/lethality in cooperation with protein(s) downstream of ID1.

The precise molecular mechanisms of the synthetic sickness/lethality of ID1 suppression and p53 R175H expression remain to be elucidated. In conclusion, Id1 and its associated signaling pathway is one of the molecular targets of cancer cells expressing the common p53 mutant R175H.

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References

1. Molenaar JJ, Ebus ME, Geerts D, et al: Inactivation of CDK2 is synthetically lethal to MYCN over-expressing cancer cells. Proc Natl Acad Sci USA 106: 12968-12973, 2009.
2. Canaani D: Methodological approaches in application of synthetic lethality screening towards anticancer therapy. Br J Cancer 100: 1213-1218, 2009.
3. Kaelin WG Jr: The concept of synthetic lethality in the context of anticancer therapy, Nat Rev Cancer 5: 689-698, 2005.
4. Bandypadhyay N, Ranka S and Kahveci T: Sslpred: predicting synthetic sickness lethality. Pac Symp Biocomput, pp7-18, 2012.
5. Luo J, Emanuele MJ, Li D, et al: A genome-wide RNAi screen identifies multiple synthetic lethal interactions with the Ras oncogene. Cell 137: 835-848, 2009.
6. Scholl C, Fröhling S, Dunn IF, et al: Synthetic lethal interaction between oncogenic KRAS dependency and STK33 suppression in human cancer cells. Cell 137: 821-834, 2009.

7. Farmer H, McCabe N, Lord CJ, et al: Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 434: 917-921, 2005.

8. Kaye SB, Lubinski J, Matulonis U, et al: Phase II, open-label, randomized, multicenter study comparing the efficacy and safety of olaparib, a poly (ADP-ribose) polymerase inhibitor, and pegylated liposomal doxorubicin in patients with BRCA1 or BRCA2 mutations and recurrent ovarian cancer. J Clin Oncol 30: 372-379, 2012.

9. Hollstein M, Sidranksy D, Vogelstein B and Harris CC: p53 mutations in human cancers. Science 253: 49-53, 1991.

10. Levine AJ: p53, the cellular gatekeeper for growth and division. Cell 88: 323-331, 1997.

11. Lill NL, Grossman SR, Ginsberg D, DeCaprio J and Livingston DM: Binding and modulation of p53 by p300/CBP coactivators. Nature 387: 823-827, 1997.

12. Sakaguchi K, Herrera JE, Saito S, et al: DNA damage activates p53 through a phosphorylation-acetylation cascade. Genes Dev 12: 2831-2841, 1998.

13. Soussi T, Dehouche K and Béroud C: p53 website and analysis of p53 gene mutations in human cancer: forging a link between epidemiology and carcinogenesis. Hum Mutat 15: 105-113, 2000.

14. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC and Hainaut P: The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 19: 607-614, 2002.

15. Soussi T, Iishioka C, Claustres M and Béroud C: Locus-specific mutation databases: pitfalls and good practice based on the p53 experience. Nat Rev Cancer 6: 83-90, 2006.

16. Kato S, Han SY, Liu W, et al: Understanding the function-structure and function-relationship of p53 tumor suppressor protein by high-resolution missense mutation analysis. Proc Natl Acad Sci USA 100: 8424-8429, 2003.

17. Olive KP, Tuveson DA, Ruhe ZC, et al: Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell 119: 847-860, 2004.

18. Lang GA, Iwakuma T, Suh YA, et al: Gain of function of a p53 hot spot mutation in a mouse model of Li-Fraumeni syndrome. Cell 119: 861-872, 2004.

19. Song H, Hollstein M and Xu Y: p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol 9: 573-580, 2007.

20. Liu G, McDonnell TJ, Montes de Oca Luna R, et al: High metastatic potential in mice inheriting a targeted p53 missense mutation. Proc Natl Acad Sci USA 97: 4174-4179, 2000.

21. Watanabe G, Kato S, Nakata H, Ishida T, Ohuchi N and Iishioka C: αB-crystallin: a novel p53-target gene required for p53-dependent apoptosis. Cancer Sci 100: 2368-2375, 2009.

22. Shirashi K, Kato S, Han SY, et al: Isolation of temperature-sensitive p53 mutations from a comprehensive missense mutation library. J Biol Chem 279: 348-355, 2004.

23. Dong Z, Wei F, Zhou C, et al: Silencing Id-1 inhibits lymphangiogenesis through down-regulation of VEGF-C in oral squamous cell carcinoma. Oral Oncol 47: 27-32, 2011.

24. Nakakadowa, Y, Shibata H, Otsuka K, Kato S and Iishioka C: Lack of correlation between p53-dependent transcriptional activity and the ability to induce apoptosis among 179 mutant p53s. Cancer Res 65: 2108-2114, 2005.

25. Ling MT, Wang X, Zhang X and Wong YC: The multiple roles of Id-1 in cancer progression. Differentiation 74: 481-487, 2006.

26. Benezra R, Davis RL, Lockshon D, Turner DL and Weintraub H: The protein Id-1: a negative regulator of helix-loop-helix DNA binding proteins. Cell 61: 49-59, 1990.

27. Sun XH: Constitutive expression of the Id1 gene impairs mouse B cell development. Cell 79: 893-900, 1994.

28. Jen Y, Weintraub H and Benezra R: Overexpression of Id protein inhibits the muscle differentiation program: in vivo association of Id with E2A proteins. Genes Dev 6: 1466-1479, 1992.

29. Kreider BL, Benezra R, Rovera G and Kadesch T: Inhibition of myeloid differentiation by the helix-loop-helix protein Id. Science 255: 1700-1702, 1992.

30. Rothschild SI, Kappeler A, Rutschiller D, et al: The stem cell gene 'inhibitor of differentiation 1' (IDI) is frequently expressed in non-small cell lung cancer. Lung Cancer 71: 306-311, 2011.

31. Ling MT, Wang X, Ouyang XS, Xu K, Tsao SW and Wong YC: Id-1 expression promotes cell survival through activation of NF-kB signalling pathway in prostate cancer cells. Oncogene 22: 4498-4508, 2003.

32. Brosh R and Rotter V: When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer 9: 701-713, 2009.

33. Roger L, Jullien L, Gire V and Roux P: Gain of oncogenic function MUTATION R175H

34. Yan W and Chen X: Identification of GRO1 as a critical gene 'inhibitor of differentiation 1' (ID1) is frequently expressed in non-small cell lung cancer. Lung Cancer 71: 306-311, 2011.

35. Liu DP, Song H and Xu Y: A common gain of function of p53 cancer mutants in inducing genetic instability. Oncogene 29: 949-956, 2010.