The p73 Tumor Suppressor Is Targeted by Pirh2 RING Finger E3 Ubiquitin Ligase for the Proteasome-dependent Degradation*

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Background: The status of p73 expression is linked to the sensitivity of tumor cells to therapy, but how p73 expression is regulated remains uncertain.

Results: Pirh2 E3 ubiquitin ligase promotes the proteasomal turnover of TAp73.

Conclusion: Pirh2 is a novel E3 ligase of p73.

Significance: Targeting Pirh2 to restore TAp73-mediated growth suppression in p53-deficient tumors may be developed as a novel anti-cancer strategy.

The p73 gene, a homologue of the p53 tumor suppressor, is expressed as TA and ΔN isoforms. TAp73 has similar activity as p53 and functions as a tumor suppressor whereas ΔNp73 has both pro- and anti-survival functions. While p73 is rarely mutated in spontaneous tumors, the expression status of p73 is linked to the sensitivity of tumor cells to chemotherapy and prognosis for many types of human cancer. Thus, uncovering its regulators in tumors is of great interest. Here, we found that Pirh2, a RING finger E3 ubiquitin ligase, promotes the proteasome-dependent degradation of p73. Specifically, we showed that knockdown of Pirh2 up-regulates, whereas ectopic expression of Pirh2 down-regulates, expression of endogenous and exogenous p73. In addition, Pirh2 physically associates with and promotes TAp73 polyubiquitination both in vivo and in vitro. Moreover, we found that p73 can be degraded by both 20 S and 26 S proteasomes. Finally, we showed that Pirh2 knockdown leads to growth suppression in a TAp73-dependent manner. Taken together, our findings indicate that Pirh2 promotes the proteasomal turnover of TAp73, and thus targeting Pirh2 to restore TAp73-mediated growth suppression in p53-deficient tumors may be developed as a novel anti-cancer strategy.

p73, a homologue of the p53 tumor suppressor (1–3), is expressed as transcriptional active (TA)2 and ΔN isoforms due to utilization of two separate promoters, the upstream P1 promoter and the P2 promoter in intron 3 (4, 5). The P1 promoter produces TA isoforms, whereas the P2 promoter produces N-terminally deleted (ΔN) isoforms. Both TA and ΔN p73 transcripts undergo C-terminal alternative splicing, resulting in at least seven p73 isoforms (α–γ). Due to the highly conserved amino acid sequence in DNA binding domain and tetramerization domain with p53, p73 is postulated to be a tumor suppressor. TA isoforms are similar to p53 and able to activate an array of target genes promoting cell cycle arrest and cell death (6–8). However, ΔN isoforms can act as dominant negative inhibitors of the full-length proteins and thus inhibits chemotherapy-induced cell death and promotes tumorigenesis in cells containing wild-type p53 and/or TAp73 (9–12). Unlike frequent mutations of p53, somatic mutations of p73 are rare in spontaneous tumors (13–15) and up-regulation of both TAp73 and ΔNp73 is commonly observed in multiple human cancers, including breast, lung, and colorectal cancers (16–18). Mice deficient in p73 are prone to neurological defects, chronic infection, and inflammation (19, 20). However, other studies showed that p73 is involved in the DNA damage response and necessary for the proper function of p53 (6, 7, 21–23). p73 status is linked to increased survival rates and loss of p73 expression is linked to increased metastasis potentials in many types of human cancer (24–26). Likewise, mice heterozygous of p73 are prone to increased tumor burdens and metastasis rates, which are compounded in mouse harboring heterozygous alleles of p53 and/or p63 (27). In addition, mice deficient in TAp73 are prone to spontaneous tumor formation whereas mice deficient in ΔNp73 are hypersensitive to DNA damage (28, 29). This suggests that p73 is involved in tumor suppression and functions as a modulator and effector of the p53 pathway. Because p73 can induce growth suppression in a p53-independent manner, it becomes a potential target for cancer therapeutics.

Pirh2, a RING finger E3 ubiquitin ligase, is transcriptionally regulated by p53 and in turn targets p53 for degradation independent of MDM2 (30). However, unlike MDM2, which dissociates from phosphorylated p53 in response to DNA damage, Pirh2 is capable of inhibiting p53 activation by degrading serine-15-phosphorylated p53 under stress conditions (31). Interestingly, Pirh2 is found to be highly expressed in multiple cancer cell lines regardless of p53 status (32, 33). In addition, Pirh2 transgenic mice are tumor prone (34). These suggest that Pirh2 may promote tumorigenesis in both p53-dependent and -independent manner. Indeed, Pirh2 is found to target the cyclin-dependent kinase inhibitory protein p27 for degradation, which is correlated with pathogenesis of human cancers (35, 36).

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2 The abbreviations used are: TA, transcriptional active; CPT, camptothecin; Dox, doxorubicin; NQO1, NAD(P)H quinone oxidoreductase.

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In this study, we found that Pirh2 promotes the proteasome-dependent degradation of p73. We found that knockdown of Pirh2 up-regulates, whereas ectopic expression of Pirh2 down-regulates, TAp73 expression. In addition, Pirh2 physically associates with TAp73 and promotes TAp73 polyubiquitination and proteasomal degradation. Finally, we showed that TAp73 is required for growth suppression induced by Pirh2 knockdown in both p53-proficient and -deficient cells. Taken together, our findings demonstrate that Pirh2 is a novel E3 ubiquitin ligase of TAp73, and thus depletion of Pirh2 to restore TAp73-mediated growth inhibition in p53-deficient tumors may be developed as a novel anti-cancer strategy.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Constructs**—RKO, H1299 and MCF7 cells were maintained in DMEM ( Gibco ) supplemented with 10% fetal bovine serum and antibiotics. pcDNA3 vectors expressing FLAG-Pirh2, FLAG-Pirh2-DN, FLAG-Pirh2-ΔRING, and FLAG-Ubiquitin were described (37). pcDNA3 vector expressing HA-p73α was generated previously (38). To generate 2×FLAG-tagged ITCH, the cDNA fragment was amplified with ITCH-BF (5′-TATTGCCATCATGCTACATGGATCA-CAACTTG-3′) and ITCH-SR (5′-TTCTGTGCAGCATTTCACTTGTTCTCATCTT-3′). This product was cloned into pcDNA3-2×FLAG vector via BamHI/Sall restriction sites.

**Antibodies**—Rabbit polyclonal anti-p21, anti-FDXR, mouse monoclonal anti-ITCH, and anti-ubiquitin were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-p73 was obtained from Bethyl Laboratories. Other antibodies were anti-HA (HA11, Covance), anti-FLAG (Sigma), anti-p53 (DO-1, PAb1801, PAb240, and PAb421), and anti-actin (sc-1748, Santa Cruz Biotechnology). Mouse monoclonal anti-ITCH, and anti-ubiquitin were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-p21, anti-FDXR, mouse monoclonal anti-ITCH, and anti-ubiquitin were purchased from Santa Cruz Biotechnology. Rabbit polyclonal anti-p73 was obtained from Bethyl Laboratories. Other antibodies were anti-HA (HA11, Covance), anti-FLAG (Sigma), anti-p53 (DO-1, PAb1801, PAb240, and PAb421), and anti-actin (Sigma).

**Small Interference RNA (siRNA)**—Scramble, Pirh2 siRNA #1 (sense 5′-CCACAGCUUGUGAAGAG dTdT-3′ and antisense 5′-UUCCACACAGUCUGUUG dTdT-T3′), Pirh2 siRNA #2 (sense 5′-CAUGCCAACGACACUGUG dTdT-T3′ and antisense 5′-CACAAGUCUGUUGGCAUG dTdT-T3′), TAp73 siRNA #1 (sense 5′-GCAAACUAGCAUGUCAGCA dTdT-T3′ and antisense 5′-UGACAGAUGUGUACUA dTdT-T3′), TAp73 siRNA #2 (sense 5′-GCACAGCUUGCACCU dTdT-T3′ and antisense 5′-AGGGCUACAGUGCUG dTdT-T3′), and p21 siRNA (sense 5′-GCTCUCCCAUCGAGGGUCU dTdT-T3′ and antisense 5′-GAAACGCGGAGAGGAC dTdT-T3′) were purchased from Dharmacon. The siRNA duplex was introduced into cells using Silencerfect (Bio-Rad) according to the manufacturer’s protocol. Cells were harvested 72 h post-transfection.

**Immunoprecipitation-Western blot Analysis**—The assay was carried out as described (37). Briefly, RKO cells were transfected with indicated plasmids and harvested 24 h post-transfection. Cleared cell lysates (300 μg of total proteins) were immunoprecipitated with 1 μg of indicated antibodies and then subject to Western blot analysis.

**In Vitro Proteasome Degradation Assay**—In vitro translated 35S-labeled p73α was incubated in a buffer (20 mM Tris-Cl (pH 7.2), 50 mM NaCl, 10 mM MgCl2 and 1 mM DTT) supplemented with 1 μg of 20 S or 26 S proteasome (Affiniti) along with 80 μM MG132 at 37 °C for indicated times.

**Ubiquitination Assay**—In vivo ubiquitination assay was performed as described (30). Briefly, RKO cells were transfected with indicated plasmids and treated with 5 μM MG132 for 6 h prior to harvest. Total cell lysates were immunoprecipitated with anti-HA antibody followed by Western blot with anti-HA or anti-ubiquitin antibodies to detect p73 ubiquitination.

**In vitro ubiquitination assay** was performed as described (30). Briefly, 35S-labeled p73 protein was mixed with immunopurified FLAG-Pirh2 or FLAG-Pirh2-DN and incubated on ice for 1 h to form Pirh2-p73 complexes. The complexes were then added to ubiquitination buffer containing E1, E2, and ubiquitin, and incubated at 30 °C for 2 h. Finally, 35S-labeled p73 protein was separated on a SDS-PAGE gel and analyzed by autoradiography. E1, E2, and ubiquitin were purchased from Boston Biochem. 35S-labeled p73 was produced by the TNT T7-coupled reticulocyte lysates system (Promega). To purify Pirh2 from RKO cells, whole cell lysates from RKO cells transfected with FLAG-Pirh2 or FLAG-Pirh2-DN expression vector were immunoprecipitated by anti-FLAG antibody (Sigma). To purify recombinant GST-tagged Pirh2 and Pirh2-DN, GST fusion proteins from pGEX-4T-3 expression vectors were expressed in E. coli BL21 (DE3) (Novagen) upon induction with 0.5 mM IPTG for 4 h at 37 °C. Bacterial cells were harvested, sonicated, and clarified by centrifugation. Recombinant GST-tagged proteins were purified by glutathione-Sepharose beads (Amerham Biosciences) as described (39).

**Luciferase Assay**—The dual luciferase assay was performed in triplicate as previously described (38). Briefly, 300 ng of a luciferase reporter (pGL2-p21A promoter), 300 ng of empty vector (pcDNA3) or pcDNA3 that expresses Pirh2 or ITCH, and 3 ng of an internal control Renilla luciferase assay vector pRL-CMV were transfected into H1299 cells by Metfectene pro reagent according to the manufacturer’s instructions (Biontex). Cells were seeded at 4 × 104 per well in 24-well plates. 24 h after transfection, luciferase activity was measured with the dual luciferase kit (Promega) and Turner Designs luminometer. The luciferase activity of each sample was normalized by its Renilla luciferase activity.

**DNA Histogram Analysis**—DNA histogram analysis was performed as previously described (41). Briefly, H1299 cells were transfected with scramble or Pirh2 siRNA for 2 days followed by treatment with doxorubicin (Dox, 500 ng/ml) for 48 h. Both floating cells in the medium and live cells on the plates were fixed in precooled (−20 °C) ethanol (70%) overnight and followed by PI staining. Samples were analyzed by fluorescence-activated cell sorting (BD Biosciences).

**Cell Proliferation and Colony Formation Assay**—For cell proliferation assay, 24 h after transfection, an appropriate number of cells was seeded in 6-well plates in triplicate and cultured over an 8-day period. The medium was replaced every 3 days and cells were harvested and counted at the indicated times. For colony formation assay, 24 h after transfection, cells were seeded at 500 per well in 6-well plates in triplicate and cultured over a 13-day period. Colonies were fixed with methanol/glacial acetic acid (7:1), washed with H2O, and stained with 0.02% crystal violet. The number of total colonies was quantified and pre-
Pirh2-mediated Degradation of p73

FIGURE 1. Pirh2 inhibits p73 expression. A, Western blots were prepared with extracts from RKO (left panel) and H1299 (right panel) cells transfected with scramble or Pirh2 siRNA for 72 h. p73, Pirh2, p53, p21, FDXR, and actin were measured with their respective antibodies. B, Western blots were prepared with extracts from RKO cells transfected with Pirh2 siRNA for 0, 24, 48, and 72 h, p73, Pirh2, p21, and actin were measured with their respective antibodies. C, Western blots were prepared with extracts from RKO (left panel) and MCF7 (right panel) cells transfected with FLAG-tagged Pirh2 for 48 h. The blots were analyzed as in A. D, Western blots were prepared with extracts from RKO cells transfected with HA-p73α along with an increasing dose of FLAG-Pirh2 for 36 h. The blots were analyzed as in A except anti-HA and anti-FLAG antibodies were used to detect HA-p73 and FLAG-Pirh2, respectively.

RESULTS

Pirh2 Inhibits p73 Expression and Physically Associates with p73—To determine whether Pirh2 regulates p73 expression, Pirh2 was transiently knocked down by Pirh2 siRNA in RKO cells. We found that upon knockdown of Pirh2, the level of TAp73α protein was increased (Fig. 1A, left panel). Similarly, p53, which can be degraded following Pirh2-mediated ubiquitination (30), was increased upon knockdown of Pirh2 (Fig. 1A, left panel). The levels of p21 and FDXR, common targets of p53 and p73 (42–44), were increased in Pirh2-KD cells (Fig. 1A, left panel). The level of actin was determined as a loading control.

To rule out a potential cell type-specific effect, Pirh2 was transiently knocked down by Pirh2 siRNA in RKO cells over a 3-day period. We showed that as the level of Pirh2 was progressively decreased (Fig. 1B, Pirh2 panel), the level of endogenous TAp73α protein was gradually increased with concomitant induction of p21 (Fig. 1B, p73 and p21 panels).

Next, we examined whether increased expression of Pirh2 leads to decreased expression of p73. To test this, FLAG-tagged Pirh2 was transiently overexpressed in MCF7 and RKO cells. We found that upon ectopic expression of Pirh2, the level of TAp73α protein was decreased along with a concomitant decrease of p53, p21, and FDXR in both MCF7 and RKO cells (Fig. 1C). To further test this, HA-p73α was transiently expressed in RKO cells along with an increased dose of FLAG-Pirh2 (Fig. 1D, Pirh2 panel). We showed that the levels of ectopic expressed HA-p73α were decreased by FLAG-Pirh2 in a dose-dependent manner (Fig. 1D, p73 panel). As controls, the levels of endogenous p53 and p21 were also gradually decreased by FLAG-Pirh2 (Fig. 1D, p53 and p21 panels).

To determine the mechanism by which Pirh2 regulates p73 expression, we wanted to examine whether Pirh2 physically associates with p73. To test this, endogenous p73 and Pirh2 were immunoprecipitated by anti-Tap73 and anti-Pirh2, respectively. Western blot analysis showed that endogenous Pirh2 was detected in the TAp73α-immunocomplex (Fig. 2A, lane 5). We also showed that TAp73α expression was increased upon treatment with 125 nM camptothecin (CPT), a topoisomerase I inhibitor, for 12 h (Fig. 2A, p73 panel, compare lanes 1–2). This is consistent with the fact that p73 is a DNA damage-responsive gene (45). Likewise, endogenous Pirh2 was also detected in DNA damage-induced TAp73α-immunocomplex (Fig. 2A, lane 6). In addition, ITCH, a well-defined binding partner of p73 (46), was detected in these complexes (Fig. 2A, lanes 5 and 6). Conversely, endogenous TAp73α along with ITCH was detected in the Pirh2-immunocomplex regardless of treatment with CPT (Fig. 2B, lanes 5–6). To confirm the association between Pirh2 and TAp73α, FLAG-tagged Pirh2...
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To validate whether p73 is a direct substrate of Pirh2, in vitro ubiquitination assay was performed with 35S-labeled TAp73α in the presence of recombinant GST-Pirh2 or GST-Pirh2-DN. We showed that TAp73α was polyubiquitinated by Pirh2 but not by Pirh2-DN (Fig. 4D, compare lanes 3–4). To confirm this, in vitro ubiquitination assay was performed with 35S-labeled TAp73α in the presence of immunopurified FLAG-Pirh2 or FLAG-Pirh2-DN. Similarly, we showed that TAp73α was polyubiquitinated by Pirh2 but not by Pirh2-DN (Fig. 4E, compare lanes 3–4). Together, this suggests that Pirh2 promotes polyubiquitination of p73.

p73 is a Downstream Effector of Pirh2 to Modulate Cell Proliferation and Cell Death—To examine the biological significance of TAp73 polyubiquitination by Pirh2, we analyzed the effect of Pirh2 on long-term cell proliferation in MCF7 cells. We found that cell proliferation was inhibited by knockdown of Pirh2 in MCF7 cells (Fig. 5A, top panel, compare the first with the third column). We also found that cell proliferation was increased by knockdown of TAp73 (Fig. 5A, top panel, compare the first two columns). MCF7 is a breast cancer cell line containing wild-type p53, which is regulated by Pirh2 as shown above (Fig. 1). To rule out the effect of p53, colony formation was performed in H1299 cells. Similarly, we showed that the number of colonies was remarkably reduced in Pirh2-KD H1299 cells (Fig. 5B, top panel, compare the first with the third column). Interestingly, upon knockdown of TAp73 in Pirh2-deficient MCF7 cells, growth inhibition induced by Pirh2-KD was markedly diminished (Fig. 5A, top panel, compare the third with the fourth column). Similar result was observed in H1299 cells (Fig. 5B, compare the third with the fourth column). We would like to mention that the effect of Pirh2 on p73-dependent growth suppression was also observed with another set of siRNA (Fig. 5, A and B, compare the columns at the top and bottom panels, respectively). Furthermore, we showed that upon knockdown of Pirh2, TAp73 is accumulated regardless of the set of siRNAs used against Pirh2 (Fig. 5C).

To confirm the effect of Pirh2 on cell proliferation in a p73-dependent manner, short-term cell proliferation assay was performed in H1299 cells. We showed that knockdown of Pirh2 inhibited cell proliferation over a 8-day period (Fig. 5D). In addition, knockdown of TAp73 restored the ability of Pirh2-deficient cells to proliferate (Fig. 5D). To further confirm this, we examined whether Pirh2 regulates p73-mediated transcriptional activity by luciferase assay in H1299 cells. We showed that the transcriptional activity of TAp73 to regulate the p21 promoter was down-regulated by Pirh2 in a dose-dependent manner (Fig. 5E). As a control, the transcriptional activity of TAp73 was also decreased by ITCH (Fig. 5E).

p21, a target of p73 and a key regulator of cell proliferation and cell survival (48), is up-regulated by Pirh2-KD (Fig. 1B). Therefore, it is possible that p21 plays a role in the Pirh2-p73 regulatory loop. To test this, p21 was further knocked down in Pirh2-TAp73 double knockdown MCF7 and H1299 cells. We showed that Pirh2-TAp73-p21 triple knockdown cells exhibited a slightly, but statistically insignificant, higher growth potential than Pirh2-TAp73 double knockdown cells by both long-term and short-term cell proliferation assays (Fig. 5, A, B, and D).
In response to DNA damage, TAp73 induces cell death (6, 7, 21). Thus, we wanted to examine whether Pirh2 plays a role in DNA damage-induced apoptosis via regulating p73. To test this, DNA histogram analysis was performed to measure sub-G1 population of cells, which is believed to represent apoptotic cells. We showed that in the absence of DNA damage, there is little if any apoptotic response in H1299 cells transfected with scramble siRNA or siRNA targeting Pirh2, p73 and/or p21 (Fig. 5F, top panel). This suggests that the increased expression of p73 induced by Pirh2 knockdown is sufficient to inhibit cell proliferation but not sufficient to induce an apoptotic response. However, upon treatment with Dox, a topoisomerase II inhibitor, 17.48% of H1299 cells transfected with scramble siRNA underwent apoptosis (Fig. 5F, bottom panel, left column). The percentage of apoptotic cells was further increased from 17.48 to 21.68% by knockdown of Pirh2 (Fig. 5F, bottom panel, compare left two columns). Most importantly, the increased DNA damage-induced apoptotic response by Pirh2 knockdown was markedly inhibited by knockdown of TAp73 alone and in combination with p21 knockdown (Fig. 5F, bottom panel, right two columns). To validate the effect of Pirh2 on DNA damage-induced cell death in a p73-dependent manner, we measured the extent of cell death in H1299 cells mock-transfected or transfected with Pirh2, TAp73 or both (Fig. 5G). We found that DNA damage-induced apoptosis was decreased by ectopic expression of Pirh2 (27.36%...
versus 13.67%), but increased by ectopic expression of TAp73 (27.36% versus 36.11%). Furthermore, the extent of apoptosis increased by TAp73/H9251 was markedly attenuated by co-expression of Pirh2 (36.11% versus 17.06%). Together, these data indicate that p73 is a downstream effector of Pirh2 to modulate cell proliferation and cell death.

**DISCUSSION**

To maintain tissue homeostasis, the level of p53 protein is tightly regulated by the ubiquitin-proteasomal degradation mechanism. In unstressed cells, MDM2, a RING finger E3 ligase, is a major negative regulator of p53 to promote p53 polyubiquitination and degradation (49). In contrast, in stressed cells, Pirh2 but not Mdm2 is capable of degrading DNA damage-activated p53, such as Ser-15-phosphorylated p53 (30, 47). p73, a p53 homologue, shares structural and functional similarities with the p53 tumor suppressor. Interestingly, MDM2 associates with p73 but does not promote p73 degradation (50, 51). However, the link between p73 and Pirh2 is unclear. Here, we found that knockdown of Pirh2 up-regulates, whereas overexpression of Pirh2 down-regulates, TAp73 expression (Fig. 1). In addition, Pirh2 physically associates with TAp73 and promotes p73 polyubiquitination in vivo and in vitro (Figs. 2 and 4).
Several regulatory factors are known to regulate p73 protein turnover. For examples, HECT-type E3 ligase ITCH and the F-box protein FBXO45 control p73 ubiquitination and the proteasome-dependent degradation of p73 to maintain a low level of p73 expression in unstressed conditions (46, 52). Upon DNA damage, both ITCH and FBXO45 are downregulated to allow for p73 accumulation, leading to p53-independent cell death (46, 52). p73 is also found to undergo ubiquitin-independent 20 S proteasome degradation since NAD(P)H quinone oxidoreductase 1 (NQO1) blocks p73 degradation via association with 20 S proteasome (53). In addition, cyclin G and UFD2a, a U-box type ubiquitin ligase, target p73 for proteolysis without promoting p73 ubiquitination (54, 55). Moreover, p73 ubiquitination by NEDD-4-like ubiquitin protein ligase 2 (NEDL2) enhances p73 transcriptional and proapoptotic activities instead of degradation (56). Together, this indicates that the steady state level of p73 protein is regulated by complex molecular signaling pathways. A few reports showed that Pirh2 exerts its E3 ligase activities and promotes ubiquitin-dependent 26 S proteasome-degradation of several substrates, including p53, p27, and e-COP (30, 35, 57). Previously we showed that Pirh2 promotes degradation of DNA polymerase eta via the ubiquitin-independent 20 S proteasome pathway (37). Here, we showed that p73 can be degraded by both 20 S and 26 S-proteasomes (Fig. 3). This suggests that regulation of p73 by Pirh2 is different from that by other known E3 ligases. Nevertheless, the underlying mechanism needs to be further defined.

Pirh2 is an oncoprotein highly expressed in human cancer and explored as a potential anti-tumor target (32, 33, 40). Here, we found that Pirh2 knockdown leads to growth suppression in both p53-proficient and -deficient cells, which is markedly diminished by TAp73 knockdown (Fig. 5, A–D). Consistent with this, we showed that Pirh2 knockdown enhances DNA damage-induced cell death in p53-deficient H1299 cells in a TAp73-dependent manner (Fig. 5f). Moreover, Pirh2-TAp73-p21 triple knockdown cells exhibit a slightly higher proliferative and survival potential than Pirh2-TAp73 double knockdown cells (Fig. 5, B, D, and F). Conversely, ectopic expression of Pirh2 decreases DNA damage-induced apoptosis in a TAp73-dependent manner (Fig. 5G). These indicate that although Pirh2 degrades p53, TAp73 is a major downstream effector of Pirh2 to regulate cell proliferation and cell survival in both p53-proficient and -deficient cells. p21, as a p73 target, may also play a role in Pirh2 activity. Nevertheless, future studies are needed to determine whether other targets of TAp73 are involved and whether Pirh2 regulates ΔNp73 expression. In summary, our findings suggest that Pirh2 is a negative modulator of TAp73 and depletion of Pirh2 to restore TAp73-mediated growth suppression in p53-deficient tumors may be developed as a novel anti-cancer strategy.
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