Nutlin-3 Affects Expression and Function of Retinoblastoma Protein

ROLE OF RETINOBLASTOMA PROTEIN IN CELLULAR RESPONSE TO NUTLIN-3†

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Wei Du†, Junfeng Wu†, Erica M. Walsh‡, Yujun Zhang§, Chang Yan Chen‡, and Zhi-Xiong Jim Xiao†‡§

From the †Graduate Program in Cellular and Molecular Biology and Department of Biochemistry and the ‡Department of Medicine, Boston University School of Medicine, Boston, Massachusetts 02118 and the §Department of Radiation Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215

The retinoblastoma protein (Rb) plays a pivotal role in regulating cell proliferation and apoptosis. Nutlin-3, a small molecule MDM2 antagonist blocking interaction between MDM2 and p53, activates p53 resulting in cell growth arrest or apoptosis in various cancer cells. However, the molecular basis for the different cellular responses upon nutlin-3 treatment is not fully understood. In this study, we show that nutlin-3 activates p53 resulting in a dramatic increase in MDM2 expression and a marked reduction in total Rb protein levels. Interestingly, nutlin-3 reduces the levels of hypophosphorylated Rb and induces massive apoptosis in SJSA-1 cells, which can be largely rescued by knockdown of MDM2 or by expression of constitutively active Rb. By contrast, nutlin-3 treatment of several human cancer cells, including A549, U2-OS, and HCT116, results in an accumulation of hypophosphorylated Rb and cell cycle arrest but not apoptosis. Furthermore, we show that down-regulation of Rb by nutlin-3 does not lead to E2F1 activation nor does E2F1 play a critical role for nutlin-3-induced apoptosis in SJSA-1 cells. Taken together, these results suggest that Rb plays a critical role in influencing cellular response to activation of p53 pathway by nutlin-3.

The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1–S3.

The retinoblastoma protein (Rb) has been shown to play a pivotal role in regulating cell proliferation, DNA damage response, apoptosis, and differentiation. One major function of Rb is to interact with E2F transcription factors in assembly of transcription repressor complexes to repress expression of E2F downstream targets involved in cell cycle progression and apoptosis (1, 2).

The Rb tumor suppressor function is critically regulated by cyclin/CDK-dependent phosphorylation (3, 4). Notably, there are multiple cyclin/CDK phosphorylation sites throughout the sequence of Rb protein (5) and mutation of those sites, especially the seven Ser/Thr-Pro sites in Rb C terminus (Rb C-pocket) confers Rb with constitutively active growth suppression function to block G1/S transition as well as the S-phase progression (6, 7). Hypophosphorylated Rb has been shown to possess growth suppression function through interaction with a set of cellular proteins including the E2F transcription factors. Notably, MDM2 preferentially binds to hypophosphorylated Rb and facilitates proteasome-mediated Rb protein degradation (8, 9).

Recently, several potent small molecule MDM2 antagonists, the nutlins, have been identified (10). Nutlin-3 specifically binds to MDM2 in the p53-binding pocket and blocks MDM2-p53 interaction, resulting in a dramatic stabilization of p53 and activation of the p53 pathway. In response to nutlin-3 treatment, p53+ cancer cells undergo either cell cycle arrest or apoptosis (11–13). In addition, nutlin-3 can induce differentiation (14) and cellular senescence (15). It has been shown that an array of factors affects the outcome of nutlin-3 treatment, including the single nucleotide polymorphism of MDM2 (16), MDM4 (17, 18), p73 (19), ATM (20), and E2F1 (21, 22).

Because activation of p53 up-regulates p21 and MDM2, both of which are important regulators for Rb, we investigated whether Rb is affected upon nutlin-3 treatment and whether Rb plays a role in cellular response to nutlin-3. In this study, we show that nutlin-3 affects both Rb protein levels and Rb phosphorylation, which significantly impact the cellular responses to nutlin-3.

MATERIALS AND METHODS

Cell Culture, Drug Treatment, and Retroviral Infection—Human IMR90, WI-38, A549, MCF-7, SJSA-1, U2-OS, H1299, and HCT116 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 2 mM l-glutamine, and 1% penicillin/streptomycin in a humidified incubator at 37 °C and 5% CO2. HCT116, HCT116-p53−/−, and HCT116-p21−/− cell lines were kindly provided by Dr. Vogelstein (John Hopkins University). Stock solutions were prepared as follows: Nutlin-3 (Cayman chemical), 10 mM in DMSO; camptothecin (Sigma), 10 μM in DMSO; MG132 (Peptide Institute), 20 μM in DMSO. Exponentially growing cells were treated with either DMSO or nutlin-3 as indicated.

Retrovirus infection was performed as described previously (23). Briefly, 293FT cells were transfected using retroviral vector or plasmid encoding p53shRNA (kindly provided by Dr. Scott Lowe, Cold Spring Harbor Laboratory) and accessory plasmids by Lipofectamine2000. At 48 h after transfection, the media were collected and filtered through a 0.45-μm filter to...
remove debris. The retroviral particles were then concentrated by ultra-centrifugation (27,000 rpm, 1 h 45 min at 4°C), resuspended in fresh medium supplemented with polybrene (10 μg/ml) and used to infect cells. 48 h after infection, cells were selected in growth medium supplemented with puromycin (4 μg/ml).

Western Blot Analysis—Cells were collected, washed with phosphate-buffered saline, and resuspended in EBC250 lysis buffer (250 mM NaCl, 50 mM Tris, pH 8.0, 0.5% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin, and 2 μg/ml leupeptin). Protein concentration was determined using the Bio-Rad protein assay reagent (Bio-Rad). An equal amount of protein was loaded, separated on a 10% SDS-PAGE, transferred to polyvinylidene difluoride membrane (Millipore), and hybridized to an appropriate primary antibody and horseradish peroxidase-conjugated secondary antibody for subsequent detection by ECL. Monoclonal antibody DO-1 specific for p53 (Santa Cruz Biotechnology) was used at a dilution of 1:250. Monoclonal antibody SXM30 specific for p21 (PharMingen) was used at 1:200 dilutions. Cleaved PARP-specific monoclonal antibodies (Cell Signaling Technology) were used at 1:1000. Rb antibodies: monoclonal antibodies G3–245 (BD Pharmingen) were used at 1:200; monoclonal antibodies for hypophosphorylated Rb (BD Pharmingen) were used at 1:200. Polyclonal antibodies specific for hyperphosphorylated Rb were a mixture (1:1000) of antibodies specific for phospho-Rb Ser-780, Ser-795, or Ser-807/811 (Cell Signaling).

Transfection with siRNA Oligos—SJSA-1 cells were transiently transfected with siRNA oligos (Qiagen Inc) using Lipofectamine2000 (Invitrogen) for 16 h. Cells were then treated with 10 μM nutlin-3 for 48 h. Cells were subjected to FACS analysis or Western blot analysis. The siRNA oligonucleotide sequence for human MDM2: 5’-r(GCCUGGCUCUGUGUGUAAU)dTdT-3’ control (lamin) siRNA oligonucleo-

![Western Blot Analysis](image1)

![Transfection with siRNA Oligos](image2)
 tide sequence; 5'-r(CUGGACUUCCAGAAGAACA)dTdT-3'. AllStars Neg. control siRNA were purchased from Qiagen.

FACS Analysis—Cells were trypsinized, washed with cold phosphate-buffered saline, and fixed in 70% ethanol at 4 °C overnight. 1 × 10^6 cells were stained with propidium iodide (50 μg/ml) supplemented with RNase A (100 μg/ml) at room temperature in dark for 1 h. Cells were then subjected to FACS analysis by FACScan Flow Cytometer (Becton Dickson). Data were analyzed using the Cell Quest program.

RESULTS

Nutlin-3 Activates p53 Leading to Accumulation of MDM2 and Reduction of Rb Protein Levels—It is well established that nutlin-3 activates p53 leading to accumulation of MDM2. On the other hand, MDM2 has been shown to play a direct role in inhibition of Rb (8, 9, 24–26). We wanted to examine whether nutlin-3 has an impact on Rb. To do that, we randomly selected p53 positive cancer cells: U2OS, MCF7, HCT116, A549, and SJSA-1 or p53-deficient H1299 cells. These cells were treated with nutlin-3 and the expression levels of p53, MDM2, p21, and Rb protein were examined. Consistent with previous reports (11), treatment of nutlin-3 led to stabilized and activated p53, concomitant with a dramatic increase in p21 and MDM2 expression. Nutlin-3 had no effects on p53, MDM2, and p21 in p53-null H1299 cells, as expected. Strikingly, nutlin-3 also significantly down-regulated Rb protein levels in the p53+ cancer cells (Fig. 1). The nutlin-3-induced down-regulation of Rb is dependent on p53 because nutlin-3 had no effect on Rb protein levels in H1299 cells (Fig. 1B).

Nutlin-3 Affects Both Rb Phosphorylation and Rb Protein Levels—Because Rb function is critically regulated by phosphorylation, and the hypophosphorylated Rb is biologically active in binding to E2F transcription factors, we investigated more vigorously whether nutlin-3 affects Rb phosphorylation. Various human cancer cells (HCT116, U2-OS, A549, MCF-7, SJSA-1, and H1299) or untransformed cells (IMR90 and WI38) were treated with nutlin-3 and subjected to Western blot analysis using antibodies specific for either hypophosphorylated, hyperphosphorylated, or total Rb. In these p53-positive cancer cells, the protein levels of total Rb as well as hyperphosphorylated Rb were dramatically decreased upon treatment with nutlin-3 (Fig. 2).
exception of SJSA-1, which harbors about 25 fold of MDM2 gene amplification (11). In sharp contrast, nutlin-3 led to a clear down-regulation of hypophosphorylated Rb in SJSA-1 cells (Fig. 2B). Again, nutlin-3 did not alter Rb protein levels or Rb phosphorylation status in p53-null H1299 cells (Fig. 2B). Those data indicate that nutlin-3 affects both Rb phosphorylation and Rb protein levels.

**Nutlin-3 Has Profound Impacts on Activation of p53, Induction of MDM2, and Reduction of Hypophosphorylated Rb in SJSA-1 Cells**—We next examined in more detail as to how nutlin-3 affects Rb phosphorylation in these cancer cells. We carefully compared the effects of nutlin-3 on Rb in A549 cells to that of SJSA-1 cells. As shown in Fig. 3A, nutlin-3 had profound effects on activation of p53 in SJSA-1 cells, as evidenced by a robust induction of MDM2 and p21, even at lower doses (0.5 or 2 μM) of nutlin-3. Again, hypophosphorylated Rb is down-regulated in SJSA-1 cells in sharp contrast to up-regulation of hypophosphorylated Rb in A549 cells.

**Nutlin-3 Affects the Levels of Hypophosphorylated Rb in a p53- and p21-dependent Manner**—The alteration of Rb phosphorylation and protein levels upon nutlin-3 treatment is likely dependent on p53 because Rb was not affected in the p53-null H1299 cells (Figs. 1 and 2). To examine this possibility, we generated stable U2-OS cells expressing short hairpin RNA specific for p53 (U2OS-p53shRNA) and the vector control stable cells (U2OS-V). In parallel, we used HCT116 and the derivative, HCT116-p53−/−, which lack endogenous p53. We treated these two matched pairs (HCT116 and HCT116-p53−/−; U2OS-V and U2OS-p53shRNA) with nutlin-3 and examined Rb protein status. Again, nutlin-3 effectively down regulated total Rb protein levels while inducing hypophosphorylated Rb in both HCT116 and U2OS cells. However, nutlin-3 had either little effect (HCT116-p53−/−) or much less effect (U2OS-p53shRNA) on Rb in cells with depleted p53 (Fig. 3B). In addition, hyperphosphorylated Rb was dramatically accumulated in cells lacking p53 or p21 in the absence of nutlin-3, as expected. Under similar experimental settings, nutlin-3 was markedly less potent in inducing accumulation of hypophosphorylated Rb in HCT116-p21−/− cells, indicating that up-regulation of p21 contributes significantly to the accumulation of hypophosphorylated Rb in response to nutlin-3. However, hypophosphorylated Rb was still increased to some extent, suggesting a p21-independent mechanism (Fig. 3B). Interestingly, total Rb protein levels were elevated in cells lacking p53 or p21. Because hyperphosphorylated Rb is preferably targeted for proteasome-mediated degradation (27), it is possible that hyperphosphorylated Rb is more stable due to its inability to bind to MDM2 (8, 9). Taken together, these data suggest that nutlin-3 affects the levels of hypophosphorylated Rb in a p53-dependent manner, in which p21 plays a major role.

**Nutlin-3 Induces MDM2 and Destabilizes Rb**—Our data suggest that nutlin-3 dramatically down-regulates Rb protein levels. Because MDM2 can promote Rb degradation through a proteasome-dependent pathway (9, 26), we tested whether Rb is down-regulated through the proteasome-dependent pathway. As shown in Fig. 4, nutlin-3 treatment of U2-OS and HCT116 cells reduced total Rb protein levels, which was markedly reversed in the presence of a proteasome inhibitor, MG132.
Nutlin-3 Regulates Rb

Nutlin-3 Induces Either Cell Cycle Arrest or Apoptosis—Because nutlin-3 regulates hypophosphorylated Rb differentially in different cancer cells, we asked whether this differential regulation serves as a causative factor affecting cell fate in response to nutlin-3. A549 and SJSA-1 cells were treated with nutlin-3 for 48 h. Cells were then subjected to FACS analysis or Western blot analysis. Consistent with previous reports, nutlin-3 potently induced cell cycle arrest of A549 cells (Fig. 5A) and of most cancer cell lines tested including U2-OS, HCT116, and MCF7 (data not shown), while nutlin-3 induced massive apoptosis in SJSA-1 cells (Fig. 5A), as shown by FACS analysis.

FIGURE 6. Rb plays a critical role in nutlin-3-induced apoptosis. A, SJSA-1 cells were transiently transfected with MDM2 siRNA oligos or control siRNA oligos prior to nutlin-3 (10 μM) treatment for 48 h. Cells were then subjected to Western blot analysis (A) or FACS analysis (B). C, SJSA-1 cells were transiently transfected with constitutively active RbAΔp34 or vector prior to nutlin-3 (10 μM) treatment for 48 h. Cell lysates were subjected to Western blot analysis. Quantitative analyses on the levels of total Rb protein were performed by densitometry scanning and normalized to actin protein. The ratio of total Rb over actin in the control (no nutlin-3) was set as 1.0. D, stable A549 cells expressing Rb shRNA (shRb) or vector (V) were treated with 5, 10, or 20 μM camptothecin (CPT) for 48 h. Cells were then subjected to Western blot analysis as indicated.

Rb Plays a Critical Role in Nutlin-3-induced Apoptosis—SJSA-1 cells harbor MDM2 gene amplification that results in MDM2 protein overexpression. Because MDM2 negatively regulates Rb, we investigated whether MDM2-Rb pathway is important for nutlin-3 effects, using siRNA oligos to knockdown MDM2 expression prior to nutlin-3 treatment. Cells were then subjected to FACS analysis or Western blot analysis. In SJSA-1 cells transfected with siRNA oligos specific for MDM2, nutlin-3-activated p53 and its targets p21 and MDM2, as expected. However, nutlin-3 was unable to fully down-regulate hypophosphorylated Rb and failed to induce apoptosis (Fig. 6, A and B), suggesting that MDM2 is indeed critical for down-regulation of hypophosphorylated Rb upon nutlin-3 treatment.

To further investigate the role of hypophosphorylated Rb in cellular response to nutlin-3, we used a constitutively active murine Rb mutant, RbAΔp34, that is resistant to phosphorylation (28). SJSA-1 cells were transiently transfected with RbAΔp34 followed by nutlin-3 treatment. Cell lysates were then subjected to Western blot analysis. Nutlin-3-activated p53 similarly in SJSA-1 cells expressing RbAΔp34. However, RbAΔp34 significantly suppressed apoptosis of SJSA-1, as shown by dramatically reduced cleavage of PARP (Fig. 6C). Therefore, these data again suggest that down-regulation of hypophosphorylated Rb is necessary for nutlin-3 to trigger apoptosis in SJSA-1 cells. In addition, ectopic expression of wild-type Rb was able to block nutlin-3-induced apoptosis in SJSA-1 cells (supplemental Fig. S2). We next examined the camptothecin (CPT)-induced apoptosis in Rb-knockdown A549 cells. As shown in Fig. 6D, knockdown of Rb significantly increased CPT-induced apoptosis as evidenced by a dose-dependent increase of PARP cleavage, suggesting that Rb may play an important role in p53-based cancer therapy.

E2F1 Is Not Required for Nutlin-3-induced Apoptosis—It has been well documented that Rb interacts with E2F transcription factors and represses E2F transcription activity. Notably, activation of E2F1 can potently induce expression of genes involved in apoptosis. We therefore investigated whether E2F1 is responsible for nutlin-3-induced apoptosis in our experimental setting. We first examined effects of nutlin-3 on E2F1 protein levels and E2F transactivation activity. As shown in Fig. 7A, several p53+ cancer cells displayed significant down-regulation of E2F1 protein levels upon treatment of nutlin-3, in keeping with a previous report (22). In addition, nutlin-3 dramatically inhibited E2F transcription activity in both SJSA-1 and A549 cells as evidenced by E2F-luciferase reporter activity (DHFR-Luc) assay (Fig. 7B). Furthermore, Apaf-1, an impor-

(FIG. 4, A and B, supplemental Fig. S3). Again, nutlin-3 induced hypophosphorylated Rb in those cells, which was further enhanced by proteasome inhibitors. In SJSA-1 cells, inhibition of the proteasome led to an effective reverse in nutlin-3-mediated reduction of hypophosphorylated Rb (Fig. 4C), Together, these data suggest that proteasome-mediated degradation of Rb is an important mechanism with which nutlin-3a regulates Rb.

Nutlin-3 activates p53 similarly in different cancer cells, we asked whether this differential regulation serves as a causative factor affecting cell fate in response to nutlin-3. A549 and SJSA-1 cells were treated with nutlin-3 for 48 h. Cells were then subjected to FACS analysis or Western blot analysis. Consistent with previous reports, nutlin-3 potently induced cell cycle arrest of A549 cells (Fig. 5A) and of most cancer cell lines tested including U2-OS, HCT116, and MCF7 (data not shown), while nutlin-3 induced massive apoptosis in SJSA-1 cells (Fig. 5A), as shown by FACS analysis.

In keeping with the data from FACS analysis, nutlin-3 induced cleavage of poly ADP-ribose polymerase (PARP), a biochemical marker for apoptosis, in SJSA-1 cells but not in A549 or H1299 cells (Fig. 5B). Notably, the nutlin-3-induced outcomes correlated tightly with nutlin-3-mediated differential regulation of hypophosphorylated Rb status, in which hypophosphorylated Rb was increased in A549 cells while decreased in SJSA-1 cells upon nutlin-3 treatment (Figs. 2 and 3). We next examined the effects of nutlin-3 on prostate cancer LNCaP cells, which have been shown to undergo apoptosis upon nutlin-3 treatment (11). Similar to SJSA-1 cells, nutlin-3 significantly induced apoptosis in LNCaP cells, as evidenced by the cleavage of PARP, with tight correlation to down-regulation of hypophosphorylated Rb.

These data are consistent with a previous report that depletion of Rb by siRNA together with nutlin-3 synergistically increases apoptosis (22). Together, these observations suggest that alteration of hypophosphorylated Rb protein levels might be critical for cell fate in response to nutlin-3.
important downstream target of E2F1 involved in apoptotic pathway, was not activated in these cancer cells (Fig. 7A), although both SJSA-1 and LNCaP cells underwent intensive apoptosis upon treatment of nutlin-3.

Next, we examined the effects of knockdown of E2F1 by specific siRNA oligos on nutlin-3-induced apoptosis. SJSA-1 cells were transiently transfected with E2F1 siRNA oligos or control oligos prior to nutlin-3 treatment. As shown in Fig. 7C, nutlin-3 activated p53 and MDM2, and reduced the expression of hypophosphorylated Rb and E2F1, concomitant with PARP cleavage and apoptosis regardless of the presence or absence of E2F1 knockdown. FACS analysis also indicated that knockdown of E2F1 was unable to block nutlin-3-induced apoptosis in SJSA-1 cells (Fig. 7D). These data indicate that E2F1 is not responsible for nutlin-3-induced apoptosis.

Because the CDK4 gene is co-amplified with MDM2 in cancers, we examined whether nutlin-3 affects the expression levels of CDK4. As shown in Fig. 7A, CDK4 protein expression was clearly elevated in SJSA-1 cells, as expected. However, nutlin-3 did not significantly affect CDK4 protein levels. Knockdown of CDK4 by siRNA did not alter hypophosphorylation of Rb nor apoptosis upon nutlin-3 treatment (Fig. 7, E and F).

**DISCUSSION**

Nutlin-3 is a recently developed small molecule that blocks MDM2-p53 interaction, stabilizes p53, and activates p53 target gene expression (13). Interestingly, the cellular response to nutlin-3 seems to be context-specific. Only in a few cancer cell lines, nutlin-3 triggers a potent apoptotic response. While in most cases, nutlin-3 induces cell cycle arrest. It has been reported that, in addition to p53 and MDM2, MDMX (17, 18), p73 (19), ATM (20), and in particular, E2F1 (21, 22) contribute to determination of cell fate upon nutlin-3 treatment in various tumor cells.

In this study, we show that nutlin-3 significantly affects Rb protein levels and Rb phosphorylation, in which p21 and MDM2 play significant roles. Nutlin-3 induces p21 expression through p53, leading to accumulation of active hypophosphorylated Rb. Although p21 is primarily responsible for accumulation of hypophosphorylated Rb, HCT116 cells lacking p21 still exhibited accumulation of hypophosphorylated Rb (Fig. 3B), suggesting that nutlin-3 can also inhibit Rb kinase independent of p21. Nutlin-3 also induces proteasome-mediated degradation of Rb. In the majority of cancer cell lines tested, such as A549 lung cancer cells, nutlin-3 markedly up-regulated hypophosphorylated Rb while in SJSA-1 cells, nutlin-3 down-regulated hypophosphorylated Rb. It is unlikely due to a compromised p21-mediated inhibition of Rb phosphorylation, since nutlin-3 induces p21 production extremely well in these cells. It is possible that the robust accumulation of MDM2 facilitates protein turnover of hypophosphorylated Rb. Work from
others (11) and data from this study clearly indicate that MDM2 is much more dramatically induced by p53 upon nutlin-3 treatment in SJSA-1 cells, which contain ~25-fold MDM2 gene amplification, compared with cancer cells containing a single MDM2 gene locus. On the other hand, nutlin-3 significantly reduces hypophosphorylated Rb and potently induces apoptosis in LNCaP cells although LNCaP cells do not have MDM2 amplification. Furthermore, nutlin-3 does not reduce the levels of hypophosphorylated Rb and does not significantly induce apoptosis of JAR cells that harbor MDM2 amplification (supplemental Fig. S1) (22). Together, these observations strongly correlate the ability of nutlin-3 in reducing hypophosphorylated Rb to its potency in inducing apoptosis.

It is highly likely that the balance of Rb protein turnover and the inhibition of Rb phosphorylation by p21 determines the final outcome of the biologically active Rb species, which functions to inhibit cell cycle progression and apoptosis. In SJSA-1 cells, massive activation of MDM2 by nutlin-3 likely facilitates degradation of hypophosphorylated Rb that renders cells susceptible for apoptosis. This notion is strongly supported by the observation that knockdown of MDM2 in SJSA-1 cells leads to the retention of hypophosphorylated Rb upon nutlin-3 treatment, which leads to inhibition of apoptotic response. In addition, ectopic expression of Rb significantly inhibits nutlin-3-induced apoptosis in SJSA-1 cells. However, how nutlin-3 regulates the levels of hypophosphorylated Rb in other cancer cells, such as LNCaP and JAR, must wait for further investigation.

It is possible that MDM2 expression or MDM2 activity are abnormal in LNCaP cells via other means, such as single nucleotide polymorphism of MDM2 (16), or factors affecting Rb kinase activities such as cyclin D1 (29), Cdns (30), and p21 (31), all of which have impacts on the outcome of nutlin-3 treatment. In addition, other factors such as MDM4 (17, 18), p73 (19) may also contribute to the sensitivity of nutlin-3-induced apoptosis in the absence of amplification of MDM2.

How does reduction in hypophosphorylated Rb in cells lead to apoptosis? It is conceivable that hypophosphorylated Rb binds E2F1 with high affinity. Thus, inhibition of Rb expression or Rb phosphorylation leads to release of E2F1 from Rb repression and that activated E2F1 triggers apoptotic responses. It is reported that E2F1 transcriptional activity correlates with the apoptotic response to nutlin-3 (22). However, our data indicate that E2F1 is unlikely to be responsible to nutlin-3-induced apoptosis. First, nutlin-3 significantly reduces E2F1 protein levels and E2F transactivation activity. In addition, knockdown of E2F1 does not block nutlin-3-mediated apoptosis. How reduction of hypophosphorylated Rb leads to apoptosis independent of E2F1 upon nutlin-3 treatment is not clear.

In summary, this study suggests that upon activation of the p53 pathway by nutlin-3, Rb status is a critical factor influencing cellular response. In most p53−/+ Rb−/− cancer cells, nutlin-3 induces Rb hypophosphorylation, and cells undergo cell cycle arrest. While in a subset of cancer cells, nutlin-3 down-regulates hypophosphorylated Rb and cells undergo apoptosis. Further understanding how Rb modulates cancer cell sensitivity to chemotherapy will help contribute to a better and more specific anticancer therapy.

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