Arsenic trioxide reduces the expression of E2F1, cyclin E, and phosphorylation of PI3K signaling molecules in acute leukemia cells

Sanjay Kumar1,2 | Paul B. Tchounwou1

1Cellomics and Toxicogenomics Research Laboratory, NIH/NIMHD-RCMI Center for Environmental Health, College of Science, Engineering and Technology, Jackson State University, Jackson, Mississippi, USA
2Department of life Sciences, School of Earth, Biological, and Environmental Sciences, Central University, Gaya, South Bihar, India

Abstract
Arsenic trioxide (ATO) has been used for the treatment of acute promyelocytic leukemia (APL). Although ATO modulates cell cycle progression and apoptosis in APL cells, its exact mechanism of action remains elusive. In this research, we investigated its effects on E2F1, cyclin E, p53, pRb, and PI3K signaling molecules by western blotting, immunocytochemistry and/or confocal imaging. We found that ATO inhibited the proliferation of APL cells through down-regulation of E2F1 and cyclin E expression, and stimulation of pRb. It also reduced the interaction of pRb and E2F1 with binding to the E2F1 promoter, by stimulating pRb association. ATO also effected the phosphorylation of pRb at S608 and T373 residues and association of E2F1, pRb, and p53, simultaneously. However, in p53-knockdown NB4 cells, ATO did not significantly reduce E2F1 and cyclin E expression. Our findings demonstrate that ATO inhibits APL cell growth through reduced expression of E2F1, cyclin E, and stimulation of pRb. It also effected both interaction and association of E2F1, pRb, and p53 by phosphorylation of pRb at T373 and S608 residues and reduced phosphorylation of PI3K signaling molecules. This novel mode of action of ATO in APL cells may be useful for designing new APL drugs.

KEYWORDS
APL, arsenic trioxide, E2F1, P53, PI3K, pRb

1 | INTRODUCTION

Acute promyelocytic leukemia (APL) is a M3 subtype of acute myeloid leukemia (AML) that affects nearly 1500 patients yearly in United States and abroad. It is formed inside bone marrow cells as a result of chromosomal mutation between chromosomes 15 and 17 in APL patients. It is characterized cytogenetically by a t(15;17) (q22;q11-12) translocation. There are two fusion genes (oncogenes), promyelocytic leukemia–retinoic acid receptor alpha (PML–RARα) and RARα–PML formed as a result of chromosomal translocation. PML–RARα fusion...
transcript is responsible for pathogenesis of APL while RARα-PML fusion gene is a very good molecular marker for the diagnosis and monitoring of APL. ATO has been used successfully in both induction and consolidation therapy for the treatment of all age group of both de novo and relapsed APL patients, for complete remission with highest survival rate. However, recently few ATO-resistant APL patients have been reported underscoring the need to investigate a new target of action of ATO in APL patients.

E2F1 is a transcriptional factor in E2 promoter-binding factor (E2F) family involved in cell proliferation and differentiation. Its activation can initiate expression of related genes to promote progression of cell cycle and trigger the cells to enter the S phase. E2F1 is the only member of E2F family specifically involved in apoptosis and in the induction of signal for accumulation of p53. It regulates cell cycle progression through targeting genes that encode proteins involved in cell cycle regulation, DNA repair, and apoptosis. E2F1 overexpression results in uncontrolled cell proliferation leading to tumorigenesis and cancer. It regulates expression of genes involved in DNA damage, development, and apoptosis. ATO inhibits cell proliferation along with cyclin E and cyclin A.

Retinoblastoma tumor suppressor protein (pRb or Rb) is actively involved in cell proliferation, and belongs to pocket family proteins that regulate E2F1 transcription. Generally, it remains inactive in most of cancer cells and often changes in expression due to post-translational modification such as acetylation and phosphorylation. E2F1 is actively involved in the regulation of genes that modulate Rb activity during cancer cell proliferation. Cell proliferation is commonly depending on interaction between pRb and E2F1 that also depends on phosphorylation of pRb at specific amino acid residues by cyclin dependent kinases (CDKs).

Hypo-phosphorylation of pRb results in the formation of heterodimer between E2F1 and pRb leading to E2F1 activity being repressed and cell proliferation being inhibited. While hyper-phosphorylation of pRb forms heteromerization between pRb and E2F1 leading to upregulation of E2F1 activity and stimulation of cell proliferation. pRb is phosphorylated at nearly 16 different amino acids residues, but phosphorylation at S608 and T373 residues are highly effective in heteromerization between pRb and E2F1 as well as cell proliferation. Phosphorylation of pRb at S608 residue is structurally made order of pocket domain loop and completely inhibits binding with E2F1 transactivation domain, while phosphorylation of pRb at T373 residue produces allosteric changes in pRb and supports interaction of the N-terminus of pocket domain that disrupts heterodimerization to E2F1.

Changes in E2F1 expression induce apoptosis through p53-dependent or p53-independent pathway. It provides a functional relation between p53 and pRb. E2F1 induces phosphorylation and accumulation of p53 leading to apoptosis. P3K signaling molecules are involved in regulation of E2F1 transcriptional output and apoptosis induction. Activation of P3K enhances E2F1 expression in tamoxifen [TAM] resistant breast cancer cells (MCF-7). P3K signaling cascade controls transcription of E2F1 gene in pancreatic ductal adenocarcinomas (PDACs) cells. ATO reduces E2F1 expression in both mesothelioma and lung adenocarcinoma cell lines.

We study a new mode of action of ATO characterized by a reduced expression of E2F1, cyclin E, and phosphorylation of P3K signaling pathway molecules through stimulation of pRb activity in APL cell lines. ATO also affected the interaction of E2F1 and pRb through phosphorylation at S608 and T373 residues and heteromerization leading to the inhibition of APL cell growth.

2 MATERIALS AND METHODS

2.1 Cell line and culture

Three APL cell lines including NB4, KG-1a and HL-60 cells, were used in this study. The cells were purchased from the American Type Culture Collection (Manassas, VA), and maintained at 37°C in an atmosphere of 5% CO2 and 95% air according to standard procedures.

2.2 Chemicals and reagents

Arsenic trioxide (Cat. No. PLAS2-2Y), Alexa Fluor 647 (Cat. No. A32733), and Alexa Fluor 488 (Cat. No. A11029) were purchased from Fisher scientific/Invitrogen (Waltham, MA). Poly-L-Lysine (Cat. No. LSKMAGAG10), and CHIP kit (Cat. No. 17-10 461) were obtained from Millipore- Sigma- (St. Louis, MO). E2F1(Cat. No. 3742), p53(Cat. No.2524), and P3K antibodies kit (Cat. No. 9655) were purchased by Cell Signaling Technology (Danvers, MA). RB (Cat. No. ab226979), RB [T373] (Cat. No. ab52975), and RB [S608] (Cat. No. ab172975) were obtained from Abcam (Cambridge, MA). Hoechst 33342 was purchased from Life Technologies (Grand Island, NY) and cyclin E (Cat. No. sc247) from Santa Cruz Biotechnology Inc. (Dallas, TX).

2.3 Immunoprecipitation and Western blotting

Both NB4 and KG1a cells were treated with different concentration (0,2,4,6, and 8 μg/ml) of ATO for 24 h at 37°C inside a CO2 incubator. After incubation, the cells were collected and protein lysates were prepared in gel, transferred into a nitrocellulose membrane and analyzed by western blotting by using specific antibody/phospho sensitive antibodies, as described previously. The band intensities were quantified using the Image J software freely provided by the National Institutes of Health, as described in our previous publication.

2.4 Immunocytochemistry and confocal microscopy imaging

APL cells (1 × 10⁵) were cultured in presence or absence of ATO and attached to poly-L-lysine coated slides. Immunocytochemistry of attached cells was performed using E2F1 antibody (dilution, 1:100) or pRb (979) antibody from Cell Signaling Technology and imaged by confocal microscopy (Olympus Company, Center valley, PA) as previously described.
2.5 | Chromatin immunoprecipitation [CHIP]

NB4 cells (1 × 10^7) were treated with different concentrations (0, 2, 4, 6, and 8 µg/ml) of ATO for 24 h at 37°C inside a CO2 incubator. After incubation, the cells were washed with ice cold PBS and performed chromatin immunoprecipitation [CHIP] was performed as previously described using EZ-Magna CHIP HiSens (Cat. No.17-10 461) from Millipore company and following kit protocol. In brief, KG1a cells were washed and DNA-proteins cross-linking was performed in 1% formaldehyde with gently swirl mixing and incubating at room temperature for 10 min. After cross-linking, the cells were washed with 2 ml 10× glycine and 1X PBS by centrifugation (1350g) for 5 min each and collection of cell pellets. Cell pellets were mixed with 0.5 ml nuclei isolation buffer with 1× protease inhibitor cocktail and incubated on ice 15 min with every 10 s vortexing. After incubation, cells suspension was centrifuged (800g, 5 min) and the cell pellets were collected and again resuspended in 0.5 ml SCW buffer with few second sonication on wet ice. After sonication, the cell lysates were centrifuged (10 000 g at 4°C for 10mins) and collected the sheared cross-linked chromatin [proteins/DNA] supernatant (0.5 ml) was collected. Magna CHIP A/G beads (10 µl/reaction) were washed three times with SCW (50 µl/reaction) and suspended in E2F1 along with pRb antibody (20 µg each) together and a total volume of 200 µl was made using with SCW buffer and called immunoprecipitation reaction. Immunoprecipitation reaction (200 µl) was placed on rocker (speed, 75 rpm) for 2 h at 4°C. After incubation, immunoprecipitation reaction was centrifuged and magnetic beads were collected. Beads were washed with cold SCW buffer and resuspended in 5 µl sheared cross linked chromatin [~ 100000cells equivalents] and total volume of 500 µl was made using cold SCW buffer and rocking overnight (speed, 75 rpm) at 4°C. After incubation, the reaction was centrifuged and the magnetic beads were collected and washed two times with SCW buffer and cold low stringency buffer each. Finally, the washed beads were suspended in 50-µl CHIP elution buffer by adding 0.6 mAUC Proteinase K and incubated in thermomixer at 65°C for 2 h and 95°C for 15 min. After incubation, the samples were cooled down at room temperature and real time PCR [Step OnePlus Real-Time PCR system; Applied Biosystems (Fisher scientific), Foster City, CA] was performed using 2 µl each sample by TaqMan probe through selecting comparative Ct value [2^ΔΔCt]. Relative/fold expressions of both E2F1and pRb transcriptional factors were calculated with respect of ATO concentration using the Step One software by subtracting background Ct values.

2.6 | Knockdown of p53 in NB4 cells

We made p53-knockdown NB4 cells using lentivirus shRNA (Dharmacon Inc.; Lafayette, CO) method following the previously described standard protocol. In brief, we seeded 10 000 NB4 cells in 25 µl of transduction medium (RPMI 1640) without serum in each well of 96-well plate along with polybrene to a final concentration of 8 µg/ml. Then, we added 40 MOI SMART choice lentiviral p53 shRNA particles (10^5 TU/µl) to each well containing 10 000 NB4 cells in 25 µl and incubated for 20 h at 37°C. After incubation, we added 75 µl of 20% serum containing culture medium in each well and mixed properly by pipetting and further culturing for 2 days at 37°C. We performed microscopic examination and cell viability test and further incubated with puromycin (8 µg/ml final concentration) for a week. Puromycin-selected NB4 cells were further checked through western blotting and fluorescence imagining. We used for our experiment more than 90% p53 knockdown NB4 cells.

2.7 | Statistical analysis

Experiments were performed in triplicates. Data were presented as means ± SDs. Where appropriate, one-way ANOVA or student paired t-test was in the Biostatistics Core Laboratory at Jackson State University. p-values less than .05 were considered statistically significant.

3 | RESULTS

3.1 | Western blot analysis of ATO effect on E2F1, cyclin E, and pRb expression in APL cells

To study the effect of ATO on expression of transcriptional factors (E2F1and pRb) and cyclin E, we treated APL cell lines (KG1a, NB4, and HL-60 cells) with different concentrations of ATO, and performed western blotting. Our results show that ATO treatment reduced expression of both E2F1 and cyclin E by stimulating the expression of retinoblastoma protein (pRb) in a concentration-dependent manner in all three APL cell lines (Figure 1(A-C)).

3.2 | Immunocytochemistry and confocal imaging of ATO effect on E2F1 and pRb in APL cells

To investigate the role of ATO role on the modulation of both E2F1 and pRb, we performed immunocytochemistry assay and confocal imaging of both untreated and ATO-treated KG1a cells. We found that ATO stimulated the accumulation and localization of pRb, and downregulated the expression level of E2F1 in KG1a cells in a concentration-dependent manner (Figure 1 (D&K, i–v)).

3.3 | ATO effects on interaction between pRb and E2F1

To study the effect of ATO on interaction of transcriptional factor (E2F1) and tumor suppressor protein (pRb) associated with the E2F1 promoter, we performed CHIP assay with both untreated and ATO-treated NB4 cells. Our findings indicated that ATO supported the interaction by increasing the expression of pRb and decreasing the expression of E2F1 in NB4 cells (Figure 2(B,A)). It was concluded
FIGURE 1  ATO reduces expression of E2F and cyclin E, and upregulates the expression and accumulation of pRb in APL cells. APL cell lines were treated with different concentrations (0, 2, 4, 6 and 8 μg/ml) of ATO for 24 h. After incubation, cell lysates were prepared in RIPA buffer and the expression profiles of E2F1, cyclin E, and pRb were analyzed by western blotting. ATO reduced the expression levels of E2F1 and cyclin E and activated pRb expression in NB4 (1A), KG1a (1B), & HL-60 (1C) cells. We also checked both expression and localization of E2F1 and pRb in both ATO-treated and untreated KG1a by confocal imaging. ATO reduced the expression of E2F1 and increased accumulation of pRb significantly in KG1a cells (Figure 1(D,E), i–v)). ImageJ bundled with Java 1.8.0_172; URL—https://imagej.nih.gov/ij/download.html. APL, acute promyelocytic leukemia; ATO, arsenic trioxide; E2F1, E2F Transcriptional factor-1; RIPA, radioimmunoprecipitation assay buffer [Color figure can be viewed at wileyonlinelibrary.com]
that ATO promoted association between pRb and E2F1 with E2F1 promoter leading to heteromerization together and repression of E2F1.

### 3.4 | Functional mechanism of ATO-induced E2F1 regulation

ATO stimulates accumulation of p53 and phosphorylation of pRb at different residues leading to heteromerization between E2F1 and pRb in APL cell lines. We evaluated the role of ATO in expression and association E2F1, pRb, and p53 proteins, as well as its effect on phosphorylation of pRb at T373 and S608 residues in KG1a and NB4 cells. Our findings showed that ATO treatment reduced expression E2F1 and cyclin E expression, and stimulated pRb, however these changes in expression level and stimulation were not significant in p53-knock down NB4 cells (Figure 3(A)). We also found that ATO treatment phosphorylated RB at S608 and T373 residues in KG1a and NB4 cells (Figure 3(B,C)) and produced some effect on association of E2F1, pRb, and p53 in KG1a cells (Figure 3(D)).

### 3.5 | ATO reduces phosphorylation of PI3K

To investigate the effect of ATO on phosphorylation of PI3K signaling cascade molecules in APL cell lines, we assessed the phosphorylation of PI3K signaling molecules in both untreated and ATO-treated NB4 and KG1a cells by western blotting with specific phosphor reactive antibodies. Our findings indicated that ATO treatment reduced phosphorylation of most of PI3K signaling molecules in both NB4 and KG1a cells (Figure 4(A,B)).

### 4 | DISCUSSION

Cell cycle progression is regulated mainly by the tumor suppressor protein (pRb) and transcriptional factor (E2F1) simultaneously. pRb is phosphorylated by nearly 16 residues prominently S608 and T373 by CDKs leading to heteromerization with E2F1 and regulated cancer cells proliferation. ATO has been used successfully for treatment of APL patients by inhibiting APL cells proliferation at
higher concentrations. Inside the cells, ATO exerts its action through various molecular mechanisms. It has been reported that ATO inhibits growth of human pulmonary artery smooth muscle cells by increasing mitochondrial O$_2^-$ and depleting the concentration level of GSH. ATO also inhibits murine WEHI-3 leukemia in BALB/c mice. ATO-induced apoptosis is enhanced by dithiothreitol in cultured oral cancer cells via mitochondrial dysfunction and endoplasmic reticulum stress. It also acts via ROS-mediated PERK-eIF2α-CHOP pathway regulating CHOP-DR5 signaling in L-02 cells. However, recently APL patients resistant to ATO have been reported in many places. Molecular mechanism of ATO-induced APL cells proliferation inhibition is not fully elucidated. Accumulating evidences suggested that ATO inhibits cell proliferation through reduced E2F1 and cyclin E expression in BEAS-2B cells, mesothelioma, lung adeno carcinoma, and renal cell carcinoma cell lines by stimulating pRb expression in cervical cancer cells.

We found that ATO treatment reduced the expression of both E2F1 and cyclin E by stimulating expression of retinoblastoma (pRb) in APL cell lines (Figure 1A–C). It also induced accumulation of pRb by downregulating the expression/content level of E2F1 in KG1a cells (Figure 1D,E, i–v). Previous studies have reported that ATO stimulates interaction/association of E2F1 and pRb at E2F1 promoter leading to heteromerization together and reduced transcription as well as expression of E2F1. Our CHIP assay results have shown that ATO activated interaction between both E2F1 and pRb at the E2F1 promoter site leading to reduced expression (DNA level) of E2F1 by stimulating pRb in NB4 cells (Figure 2A,B)). Existing evidence suggests that ATO promotes accumulation of p53 and E2F1 provides a functional platform between p53 and pRb. Our findings have shown that ATO did not significantly reduced expression of E2F1, cyclin E, and stimulation of pRb in p53-knockdown NB4 cells (Figure 3A). Our IP results show that ATO affected the association of E2F1, p53, and pRb in KG1a cells (Figure 3D).

Several research groups have reported that ATO causes phosphorylation of pRb prominently at S608 and T373 residues leading to reduced E2F1 expression in KG1a and NB4 cells (Figure 3B,C). Earlier studies suggested that ATO signaling controls E2F1 both transcriptional activity and expression in breast and PDACs cancer cells. It has been reported that ATO induces apoptosis in chronic B-lymphocytic leukemia cells by suppression of PI3K signaling pathway. Our findings indicated that ATO treatment reduced phosphorylation of most of PI3K signaling molecules in both KG1a and NB4 cells (Figure 4A,B).

Further studies would be needed for functional characterization and confirmation of the interaction between E2F1 and pRb transcriptional factors in the regulation of APL cell growth and proliferation, and to further understand how this interaction is affected by ATO treatment. With regard to functional studies, further experiments would include the use of advanced molecular biology techniques such as CRISPR–Cas9 and other RNA interference methods for the preparation of knockdown and overexpression of both proteins in APL cell lines. These experiments would further elucidate the biomarkers of sensitivity and effect of ATO on cell cycle proliferation and apoptosis in APL cells. In addition, they would help understand how E2F1 and pRb heteromerize together under the influence of higher concentrations of ATO to inhibit APL cell proliferation, in conjunction with the phosphorylation at different residues of pRb as investigated in this manuscript. Furthermore, both proteins interaction and ATO influence can be analyzed by protein–protein interaction techniques such as mass spectroscopy and advanced bioinformatics tools to further elucidate the functional mechanism and strengthen the scientific evidence of E2F1 as a novel target for anti-leukemic drug designing.

Taken together, it can be concluded that ATO inhibits proliferation of APL cells through reduced expression of E2F1, cyclin E, and stimulation of pRb. It also phosphorylated pRb at S608 and T373 residues, reduced phosphorylation of PI3K molecules in KG1a cells. ATO-induced phosphorylated pRb was heteromerized at the E2F1 promoter site leading to reduced expression of E2F1 and inhibition of APL cell lines proliferation (Figure 5). This novel mechanism of action of ATO may be very useful for designing of new anti-leukemic drugs.
Cell proliferation is mainly regulated through the coordination and expression of E2F1 transcriptional factor and pRb tumor suppressor protein. ATO inhibits APL cell proliferation generally through cell cycle regulation and apoptosis. We investigated role of E2F1 and pRb in ATO-induced inhibition of APL cell proliferation through a possible involvement of p53 activation. Our findings revealed that ATO inhibited APL cell proliferation through reduced expression of E2F1 and cyclin E, and stimulation of pRb expression. ATO also regulated the interaction between E2F1 and pRb as well as reduced phosphorylation of PI3K signaling molecules at different residues in APL cell lines. Taken together, ATO inhibits proliferation of APL cells through phosphorylation of pRb at S608 and T373 residues leading to heteromerization of E2F1 and pRb at the E2F1 promoter site, and down-regulation of the expression of both E2F1 and cyclin E (Figure 5). Hence, our research has identified a new target of ATO action in APL cells that can be effectively used in designing new APL drugs, and, therefore, improving the clinical outcome of APL chemotherapy.

ACKNOWLEDGMENTS
This research was financially supported by grants from the National Institutes of Health including NIMHD-Grant No. G12MD007581 (RCMI-Center for Environmental Health), and NIMHD-Grant No. U54MD015929 (RCMI-Center for Health Disparities Research) at Jackson State University, Jackson, Mississippi, USA.

REFERENCES
1. Powell BL. Arsenic trioxide in acute promyelocytic leukemia: potion not poison. Expert Rev Anticancer Ther. 2011;11:1317-1319.
2. Yedjou C, Tchounwou P, Jenkins J, McMurray R. Basic mechanisms of arsenic trioxide (ATO)-induced apoptosis in human leukemia (HL-60) cells. J Hematol Oncol. 2010;3:28-35.
3. Kumar S, Yedjou CB, Tchounwou PB. Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL-60) cells. J Exp Clin Cancer Res. 2014;33:42.
4. Kumar S, Brown A, Tchounwou PB. Trisenox disrupts MDM2-DAAXH-AUSP complex and activates p53, cell cycle regulation and apoptosis in acute leukemia cells. Oncotarget. 2018;9:33138-33148.
5. Grignani F, Fagioli M, Alcalay M. Acute promyelocytic leukemia: from genetics to treatment. Blood. 1994;83:10-25.
6. Lo-Cocco F, Avvisati G, Vignetti M, et al. Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. N Engl J Med. 2013;369:111-121.
7. Tomita A, Kiyoi H, Naoe T. Mechanisms of action and resistance to all-trans retinoic acid (ATRA) and arsenic trioxide (As2O3) in acute promyelocytic leukemia. Int J Hematol. 2013;97:717-725.
8. Pellicelli M, Picard C, Wang D, Lavigne P, Moreau A. E2F1 and TFDP1 regulate PITX1 expression in Normal and osteoarthritic articular chondrocytes. PLoS One. 2016;11:e0165951.
9. Attwooll C, Lazzarini Denchi E, Helin K. The E2F family: specific functions and overlapping interests. EMBO J. 2004;23:4709-4716.
10. Cowell TF, DeGregori J, Leone G, Jakoi L, Nevins JR. E2F1-specific induction of apoptosis and p53 accumulation, which is blocked by Mdm2. Cell Growth Differ. 1998;9:113-118.
11. Sheldon LA. Inhibition of E2F1 activity and cell cycle progression by arsenic via retinoblastoma protein. Cell Cycle. 2017;16:2058-2072.
12. Kent LN, Bae S, Tsai SY, et al. Dosage-dependent copy number gains in E2F1 and E2F3 drive hepatocellular carcinoma. J Clin Invest. 2017;127:830-942.
13. Muller H, Bracken AP, Vernell R, et al. E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. Genes Dev. 2001;15:267-285.
14. Poppy Roworth A, Chari F, La Thangue NB. To live or let die—complexity within the E2F1 pathway. Mol Cell Oncol. 2015;2:e970480.
15. Ohtani K, DeGregori J, Nevins JR. Regulation of the cyclin E gene by transcription factor E2F1. Proc Natl Acad Sci USA. 1995;92:12146-12150.
16. Yang R, Müller C, Huynh V, et al. Functions of cyclin A1 in the cell cycle and its interactions with transcription factor E2F-1 and the Rb family of proteins. Mol Cell Biol. 1999;19:2400-2407.
17. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995;84:323-330.
18. MacDonald JI, Dick FA. Posttranslational modifications of the retinoblastoma tumor suppressor protein as determinants of function. Genes Cancer. 2012;3:619-633.
19. Giacinti C, Giordano A. Rb and cell cycle progression. Oncogene. 2006;25:5220-5227.
20. Dyson N. The regulation of E2F by pRB-family proteins. Genes Dev. 1998;12:2245-2262.
21. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell. 2002;2:103-112.
22. Burke JR, Liban TJ, Restrepo T, Lee HW, Rubin SM. Multiple mechanisms for E2F binding inhibition by phosphorylation of the retinoblastoma protein C-terminal domain. J Mol Biol. 2014;426:245-255.
23. Burke JR, Hura G, Rubin SM. Structures of inactive retinoblastoma protein reveal multiple mechanisms for cell cycle control. Genes Dev. 2012;26:1156-1166.
24. Zarkowska T, U S, Harlow E, Mittnacht S. Monoclonal antibodies specific for underphosphorylated retinoblastoma protein identify a cell cycle regulated phosphorylation site targeted by CDKs. Oncogene. 1997;14:249-254.
25. Burke JR, Deshong AJ, Pelton JG, Rubin SM. Phosphorylation-induced conformational changes in the retinoblastoma protein inhibit E2F transactivation domain binding. J Biol Chem. 2010;285:16286-16293.
26. Zarkowska T, Mittnacht S. Differential phosphorylation of the retinoblastoma protein by G1/S cyclin-dependent kinases. J Biol Chem. 1997;272:12738-12746.
27. Ginsberg D. E2F1 pathways to apoptosis. FEBS Lett. 2002;529:122-125.
28. Hiebert SW, Packham G, Strom DK, et al. E2F-1: DP-1 induces p53 and overrides survival factors to trigger apoptosis. Mol Cell Biol. 1995;15:6864-6874.
29. Wu X, Levine AJ. p53 and E2F-1 cooperate to mediate apoptosis. Proc Natl Acad Sci USA. 1994;91:3602-3606.
30. Rogoff HA, Pickering MT, Debatis ME, Jones S, Kowalik TF. E2F1 induces phosphorylation of p53 that is coincident with p53 accumulation and apoptosis. Mol Cell Biol. 2002;22:5308-5318.
31. Hallstrom TC, Mori S, Nevins JR. An E2F1dependent gene expression program that determines the balance between proliferation and cell death. Cancer Cell. 2008;13:11-22.
32. Lee KY, Lee JW, Nam HJ, et al. P13-kinase/p38 kinase-dependent E2F1 activation is critical for Pin1 induction in tamoxifen-resistant breast cancer cells. Mol Cars. 2011;32:107-111.
33. Schild C, Wirth M, Reichert M, et al. P53K signaling maintains c-myc expression to regulate transcription of E2F1 in pancreatic cancers. Mol Carcinog. 2009;48:1149-1158.
34. Lam SK, Li YY, Zheng CY, Ho JC. Downregulation of thymidylate synthase and E2F1 by arsenic trioxide in mesothelioma. Int J Oncol. 2015;46:113-122.
35. Lam SK, Li YY, Zheng CY, Leung LL, Ho JC. E2F1 downregulation by arsenic trioxide in lung adenocarcinoma. Int J Oncol. 2014;45:2033-2043.