Polyamine depletion induces G₁ and S phase arrest in human retinoblastoma Y79 cells

Akiko Ueda¹, Makoto Araie¹ and Shunichiro Kubota*²

Address: ¹Department of Ophthalmology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan and ²Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

Email: Akiko Ueda - aueda-tky@umin.ac.jp; Makoto Araie - araie-tky@umin.ac.jp; Shunichiro Kubota* - kubota@idaten.c.u-tokyo.ac.jp

* Corresponding author

Abstract

Background: Polyamines and ornithine decarboxylase (ODC) are essential for cell proliferation. DL-α-difluoromethylornithine (DFMO), a synthetic inhibitor of ODC, induces G₁ arrest through dephosphorylation of retinoblastoma protein (pRb). The effect of DFMO on cell growth of pRb deficient cells is not known. We examined the effects of DFMO on pRb deficient human retinoblastoma Y79 cell proliferation and its molecular mechanism.

Methods: Using cultured Y79 cells, the effects of DFMO were studied by using polyamine analysis, western blot, gel shift, FACS and promoter analysis.

Results: DFMO suppressed the proliferation of Y79 cells, which accumulated in the G1 and S phase. DFMO induced p27/Kip1 protein expression, p107 dephosphorylation and accumulation of p107/E2F-4 complex in Y79 cells.

Conclusion: These results indicate that p107 dephosphorylation and accumulation of p107/E2F-4 complex is involved in G₁ and S phase arrest of DFMO treated Y79 cells.

Background

The polyamines, spermidine, spermine, and their precursor putrescine are essential for cell growth and the regulation of the cell cycle [1,2]. Many reports describe increased polyamine and ODC levels in various cancers [3-7]. Intracellular polyamine levels are regulated and primarily depend on the activity of ornithine decarboxylase (ODC), which catalyzes the first rate-limiting step in polyamine biosynthesis [8]. Depletion of polyamines by DL-α-difluoromethylornithine (DFMO), a specific inhibitor of ODC, has been reported to inhibit growth of various kinds of cells [3,4,9-11]. DFMO induces expression of CDK inhibitors such as p21 and p27 [12-14] and G₁ arrest associated with hypophosphorylation of pRb [13].

P16, one of the major p16 CDK inhibitor family, competes with cyclin D to bind with CDK4 and CDK6, and the both p21 and p27 which are p21 CDK inhibitor family associate with cyclin/CDK complexes including CDK2, 4 and 6. It is established that increased expression of p16, p21 or p27 suppresses CDK activities, which leads to cell cycle arrest [15,16].

The pRb protein and E2F are thought to be a critical component in the control of the restriction point of the G₁/S transition of the cell cycle [17]. Free E2F activates E2F-dependent transcription of genes required for S-phase entry. E2F/pRb complex represses transcriptional activity of E2F [18]. When pRb protein is phosphorylated by cyc-
lin CDK complexes, the E2F/pRb complex is disrupted, and the released E2Fs from pRb lead to activation of E2F target genes [17,18]. p107 and p130 are similar to pRb in their structures and functions [18,19]. E2F-1, E2F-2, and E2F-3 bind almost exclusively to pRb; E2F-4 binds to p107 and p130 with high affinity, and also associates with pRb in some cell types; E2F-5 associates with p130 [18].

Overexpression experiments have revealed that each of pRb family proteins can induce G1 cell cycle arrest [20-23]. Recently, it has been reported that p107 blocks cell cycle inside S phase in addition to G1 arrest [24].

Since little is known about effects of DFMO on cell growth of pRb deficient cells, we examined whether DFMO has an anti-proliferative effect and how DFMO affects cell cycle in human retinoblastoma Y79 cells that lack functional pRb protein.

Results
Inhibition of Y79 cell growth and induction of G1 and S phase arrest by DFMO
Preliminary experiment showed that DFMO (1–5 mM) inhibited Y79 cell growth in a dose-dependent manner (not shown). Therefore, we used 5 mM DFMO in the present study. The effects of 5 mM DFMO on Y79 cell growth were studied. After 72 h and 96 h, 5 mM DFMO inhibited Y79 cell growth 34.5 ± 3.97 and 53.5 ± 3.7%, respectively, compared to control without DFMO treatment (p < 0.001) (Fig. 1A). The growth suppression by 5 mM DFMO was completely restored by addition of 20 mM putrescine, indicating the specificity of the effect of DFMO on cell growth and the involvement of polyamines in Y79 cell growth. Viabilities of the cells treated with 5 mM DFMO were 96.6 ± 1.08 %, 96.67 ± 2.65 %, 95.88 ± 1.32 %, and 96.83 ± 1.58 % after 24 h, 48 h, 72 h, and 96 h respectively (not significant, compared to that observed at 0 h) (Fig. 1B). The results suggest that DFMO did not induce Y79 cell death. We next analyzed the effect of DFMO on ODC activities and polyamine levels in cells. DFMO (5 mM) treatment decreased ODC activities 96.7 ± 0.81 % and 99.6 ± 0.01 at 24 h and 48 h, respectively, compared to that observed at 0 h (p < 0.001) (Table 1). The levels of putrescine, spermidine, and spermine were significantly reduced at 24 h and 48 h (Table 2).

Cell cycle analysis was done using a flow cytometer. As shown in Fig. 2A and 2B, the percentages of G1 phase cells were significantly increased at 48 h and 72 h. The percentages of G2 phase cells were significantly reduced at 48 h and 72 h. These data suggest that DFMO treatment blocked cell cycle inside S phase in addition to G1 arrest.

Figure 1
Effects of DFMO on Y79 cell growth. A: Y79 cells were treated with 5 mM DFMO over a 96 h period, and cell number was counted. B: Cell viability was analyzed with trypan blue dye exclusion test. Significantly different compared to 0 h: *p < 0.01

Effects of polyamine depletion on expression of CDK inhibitors and pRb family proteins
To study the mechanism of S and G1 phase arrest, we studied the effect of DFMO on the expression of CDK inhibitors and pRb family proteins. The expression of p27 was markedly induced by DFMO treatment. The magnitude of induction at 24 h and 48 h was 235% and 268%, respectively, compared to that at 0 h (100%). Addition of
putrescine rescued the effect of DFMO. In contrast, p16 and p21 were not significantly changed (Fig. 3A). We confirmed that Y79 cells did not express pRb using western blot analysis (Fig. 3B), and examined the expression of p107 and p130 proteins. Hypophosphorylated forms of p107 accumulated at 24 h and 48 h after DFMO treatment (Fig. 3B). On the contrary, the expression levels of each form of p130 protein did not change until 48 h after DFMO treatment (Fig. 3B).

Polyamine depletion increased p107/E2F-4 complex
Since p107 is known to be associated with E2F-4 [24,25], C-Myc [26,27], and B-Myb [28], and suppress their transcriptional activities, we investigated whether DFMO induced binding of these proteins with p107. Whole cell extracts (100 μg protein) derived from cells treated with DFMO for 0 h, 24 h, and 48 h were immunoprecipitated with antibody against E2F-4 or C-Myc. Immunoprecipitated proteins were analyzed by western blotting using anti p107 antibody. The p107/E2F-4 complex was detected at 0 h, and DFMO treatment induced 1.6 ± 0.2- and 1.8 ± 0.2-fold increase in amount of the complex at 24 h and 48 h, respectively, compared to 0 h (p < 0.01) (Fig. 4A). Putrescine (20 μM) blocked the increase of the amount of p107/E2F-4 complex, indicating the specificity of the effect of DFMO on binding of E2F-4 with p107. Other E2F family proteins (E2F1, 2, 3, and 5) were detected in DFMO treated Y79 cells (Fig. 4B, lower panel), but the complex between p107 and these proteins were not detected (Fig. 4B, upper panel). Although C-Myc was detected in DFMO treated Y79 cells (Fig. 4C, right panel), the complex of p107 and c-Myc was not detected (Fig. 4C, left panel). Whole cell extracts (100 μg protein) derived from cells treated with DFMO for 0 h, 24 h, and 48 h were immunoprecipitated with antibody against p107. Immunoprecipitated proteins were analyzed by western blotting using anti B-Myb antibody and the complex of p107 and B-Myb was not detected (Fig. 4D, left panel). It has been reported that pRb and pRb family protein can directly regulate DNA replication by their association with MCM7/CDC47 [29]. Therefore, we analyzed the MCM7-immunoprecipitated whole cell extracts by western blotting using anti p107 antibody. The complex of p107 and MCM7 was detected, but it was not increased by DFMO treatment (Fig. 4E).

p107/E2F-4 complex binds to E2F binding site
To clarify whether p107 is involved in repression of E2F by DFMO we performed gel shift assay using E2F consensuss oligonucleotides. A fast migrating complex (Fig. 5A, b), and a complex migrating at slower rate (Fig. 5A, a) were apparent in DFMO treated Y79 cells. Complex b did not change until 48 h after DFMO treatment (Fig. 3B).

Table 2: Polyamine levels of Y79 cells treated with DFMO

|        | Putrescine | Spermidine | Spermine |
|--------|------------|------------|----------|
| 0 h    | 1643.7 ± 143.2 | 1014.1 ± 235.1 | 659.3 ± 96.2 |
| 24 h   | 90.5 ± 22.7* | 597.7 ± 118.2** | 405.7 ± 102.9*** |
| 48 h   | 45.8 ± 9.5*  | 221.7 ± 87.6** | 422.8 ± 87.5*** |

Significantly different compared to 0 h
*p < 0.0001, **p < 0.002, ***p < 0.03

Figure 2
Effects of DFMO on Y79 cell cycle. Y79 cells were treated with 5 mM DFMO for 24, 48, and 72 h. Cell cycle was analyzed using a flow cytometer (A), as described under Experimental Procedures, and percentages of G1, S, and G2 phase cells are shown (B). Significantly different compared to 0 h: *p < 0.01
not change significantly by DFMO treatment. The magnitudes of increase of complex a were $2.4 \pm 0.3$- and $2.4 \pm 0.5$-fold at 24 h and 48 h, respectively, compared to 0 h ($p < 0.01$) (Fig. 5A lanes 2 and 3). The binding activity in these complexes was due to E2F, as it was competed out by excess unlabeled wild type E2F consensus binding site.
oligonucleotides, but not by their mutants (Fig. 5A lanes 8 and 9). The addition of specific antibodies against E2F-4 and p107 to the reaction mixtures confirmed that the complex a contains E2F-4 and p107 (Fig. 5A lanes 6 and 7).

**Effect of DFMO on E2F transcriptional activity**

To further clarify whether p107 is involved in transcriptional repression of E2F by DFMO we performed reporter assay using artificially synthesized four tandem repeats (E2WTx4) of the adenovirus E2 enhancer, which has two typical E2F-binding sites [30]. The plasmids, pE2WTx4 and pRL-CMV were cotransfected into Y79 cells. After 24 h culture media were changed to RPMI 1640 media with or without 5 mM DFMO or 5 mM DFMO and 20 μM putrescine. Cell lysates were prepared at indicated times after the addition of DFMO, and examined for luciferase activity to monitor the promoter activity. Luciferase activity was reduced by DFMO treatment to 76.7 ± 3.3%, 66.7 ± 5.5%, 55.7 ± 4.1% at 24 h, 48 h, and 72 h, respectively, compared to control (p < 0.02) (Fig. 5B). The addition of 20 μM putrescine completely restored the luciferase activity to the level of control, indicating the specificity of DFMO effect on E2F promoter activity.

**Discussion**

Our results demonstrate that DFMO has anti-proliferative effects even in pRb deficient cells, and suggest that p107 may play an important role in cell cycle control of Y79 cells.

There are several reports that DFMO induces G1 arrest in various kinds of cells [12-14]. It was reported that pRb is dephosphorylated by DFMO treatment [13]. Induction of CDK inhibitors that regulate pRb phosphorylation has been reported in cells treated with DFMO [12-14]. However, it is unknown about the effect of DFMO on growth and cell cycle of cells that lacks pRb expression. In this study we showed for the first time that p107 is involved in cell cycle control in response to polyamine depletion in Y79 cells.

In the present study we showed that expression of p27, but not p16 and p21, was increased after DFMO treatment. As it is established that p27 suppresses CDK activities and leads to cell cycle arrest [15,16], it is reasonably assumed that increased expression of p27 by DFMO suppressed CDK activities, which led to cell cycle arrest in the present study.

p107 plays an important role in cell cycle control by making complex with E2F-4 [24,25], c-Myc [26,27], B-Myb [28] and MCM7 [29]. When E2F/p107 complex binds to promoters, p107 recruits histone deacetylase complexes, which repress promoter activity [30-34]. Binding of E2F-4...
to p107 was reported to protect E2F-4 from degradation, and this may contribute to the maintenance of active transcriptional repression in quiescent cells [35]. It was reported that p107 act as a tumor suppressor function in pRb deficient mouse [36]. Recently, the unique role of p107 inhibiting S phase progression of cells that have already passed the G_{1} restriction point receives much attention [24,37,38]. Kondo et al. reported the involvement of p107 in the inhibition of S phase progression in response to DNA-damaging agent [37]. Our data suggest that p107 is involved in not only S phase arrest but also G_{1} phase arrest in DFMO-treated Y79 cells by binding with E2F-4.

Although the mechanism of accumulation of hypophosphorylated forms of p107 remains to be elucidated, CDK inhibitors may be the most possible regulators, as p107 contains multiple CDK phosphorylation sites [18]. There may be other factors that control cell cycle. However, since targeted disruption of three Rb-related genes leads to loss of transcriptional repression in quiescent cells [35]. It was already passed the G_{1} restriction point receives much attention [24,37,38].

In conclusion, we report here that DFMO induced G_{1} and S phase arrest of Y79 cells and the cell cycle arrest was mediated through dephosphorylation of p107 and accumulation of p107/E2F-4 complex which repress E2F transcriptional activity.

Methods

Cells and reagents

The human retinoblastoma cell line Y79 and the human T cell lymphoma cell line Jurkat were obtained from the RIKEN cell bank (Tsukuba, Japan). Cells were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) (JRH Biosciences, Lenexa, KS) in humidified air with 5% CO_{2} at 37°C. DFMO was kindly provided by Dr. P. McCann, Merrell Dow Research Center. The antibodies against p16, p27, p107, p130, E2F-1, E2F-2, E2F-4, E2F-5, B-Myb, cytochrome c, and c-Myc were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The antibody against p21 was obtained from BD Transduction Laboratories (Franklin Lakes, NJ). The antibodies against E2F-3 and MCM7 were obtained from Neo Markers (Fremont, CA). Propidium iodide and protein A-sepharose CL-4B are purchased from Amersham Biosciences (Tokyo, Japan). The antibodies against pRb was purchased from Promega (Madison, WI). FuGene™6 Transfection Reagent was purchased from Roche.

General experimental protocols

Cells (10^5 cells/ml) were treated with or without 5 mM DFMO, or 5 mM DFMO and 20 μM putrescine for the indicated times. At 0 h, 24 h, 48 h, 72 h, and 96 h, the cells in each treatment group were collected. Viable cells were counted using trypan blue exclusion method.

ODC enzyme assay and polyamine analysis

ODC activity was determined using DL-[1-^{14}C]-ornithine as a substrate, as described previously [39]. Polyamine analysis was determined using high-performance liquid chromatography (HPLC) as described [40].

Cell cycle analysis

At 0 h, 24 h, 48 h, and 72 h, the cells were collected and washed twice with PBS and fixed with chilled 70% ethanol on ice for 30 minutes, treated with RNase A (0.5 mg/ml) for 20 minutes at 37°C, and stained with 50 mg/ml propidium iodide for 30 minutes at 4°C. Stained cells were analyzed using EPICS XL flow cytometer (Beckman Coulter, Tokyo, Japan) and WinCycle software (Phoenix Flow Systems, Inc, San Diego, CA).

Immunoprecipitation and western blot analysis

For immunoprecipitation, whole cell extracts (100 μg protein) were incubated at 4°C for 16 h with specific antibodies prebound to protein A- sepharose CL-4B prewashed in IP buffer (50 mM HEPES, pH 7.4, containing 150 mM NaCl, 10 mM EDTA, 100 mM NaF, and 2 mM sodium orthovanadate). For western blotting, extracted proteins (100 μg) were separated by SDS polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane, probed with diluted antibody (1:1,000), and visualized by ECL (Amersham Biosciences). Quantification of the bands was performed using NIH image Version 1.62 (Wayne Rasband, National Institutes of Health, U.S.A). The protein concentration was measured using a Bradford assay kit (Bio-Rad, Hercules, CA).

Electrophoretic mobility shift assays

At 0 h, 24 h, and 48 h, the cells were collected and washed twice with PBS. Nuclear extracts were prepared as described [41]. The sequences of the E2F oligonucleotides used were 5’-ATTIAAGTGTCGCCCTTTTCCTCAA-3’ (wild) and 5’-ATTIAAGTGTCGATCCCCCTTCTCAA-3’ (mutant). The wild type oligonucleotides were labeled with [γ-^{32}P] ATP and T4 polynucleotide kinase. Ten μg of nuclear extracts were incubated for 20 min at room temperature in 12.5 mM HEPES buffer (pH 7.9), containing 100 mM KCl, 10% glycerol, 0.1 mM EDTA, 0.75 mM DTT, 0.2 mM phenylmethylsulfonyl fluoride, 3 μg of poly (dl-dC), and 32P-labeled E2F probe (10,000 cpm). For com-
petition, 50 ng of unlabeled competitor was added. For antibody studies, extracts were preincubated with 4 μg of antibody for 20 min at room temperature. The reaction mixtures were separated on 6 % nondenaturing polyacrylamide (37.5:1) gels. The gels were autoradiographed using Fujix Bio Imaging Analyzer BAS2000 (Fujifilm, Tokyo, Japan).

**Transient transfection and promoter assay**

One μg of pE2WTx4-Luc and 10 ng of pRL-CMV was co-transfected into Y79 cells (5 × 10^5) using FuGene™6 Transfection Reagent according to the manufacturer's instructions (Roche). After 24 h culture media were changed to RPMI 1640 media with or without 5 mM DFMO, or 5 mM DFMO and 20 μM putrescine. Promoter activities were analyzed by dual luciferase reporter assay system according to the manufacturer's protocol (Promega).

**Statistical analysis**

Data from two independent experiments performed in triplicate are presented as mean ± SD unless otherwise indicated. Statistical analysis was performed using analysis of variance, with comparison of different groups by Fisher's partial least-squares difference (PLSD) test (Statview 4; Abacus Concepts, Berkeley, CA). The P value less than 0.05 was considered to be significant.

**Authors’ contributions**

AY did all of the experiments. MA and SK showed and helped how to do the experiments side by side. SK organized whole the experiment. Three authors read the final version of the manuscript.

**Acknowledgements**

We thank Dr. K. Ohtani, Tokyo Medical and Dental University, for the generous gift of pE2WTx4-Luc and pE2MTx4-Luc plasmids. This work was supported by a grant under the Ministry of Education, Sports, Sciences, and Culture, Japan.

**References**

1. Pegg AE: Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. Cancer Res 1988, 48:759-774.
2. Tabor CW, Tabor H: Polyamines. Annu Rev Biochem 1984, 53:749-790.
3. Luk GD, Casero RA Jr: Polyamines in normal and cancer cells. Adv Enzyme Regul 1987, 26:91-105.
4. Luk GD, Yang P: Polyamines in intestinal and pancreatic adaptation. Gut 1987, 28:95-101.
5. Luk GD, Mosher JA, Ehringpreis MN: Ornithine decarboxylase as a marker for colorectal polyps and cancer. Prog Clin Biol Res 1988, 279:227-239.
6. Auvinen M, Paasinen A, Andersson LC, Holta E: Ornithine decarboxylase activity is critical for cell transformation. Nature 1993, 360:355-358.
7. Clifford A, Morgan D, Yusha SU, Soler AP, Gilmore S: Role of ornithine decarboxylase in epidermal tumorigenesis. Cancer Res 1993, 53:1680-1686.
8. Russell DH: Ornithine decarboxylase as a biological and pharmacological tool. Pharmacology 1980, 20:117-129.
9. Kubota S, Ohashi N, Takaku F: Effects of DL-alpha-difluoroornithine on the growth and metastasis of B16 melanoma in vivo. Cancer Res 1987, 47:24-27.
10. Mc Cann PP, Pegg AE: Ornithine decarboxylase as an enzyme target for therapy. Pharmacol Ther 1992, 54:195-215.
11. Pegg AE, Shantz LM, Coleman CS: Ornithine decarboxylase as a target for chemoprevention. J Cell Biochem Suppl 1995, 22:132-138.
12. Ray RM, Zimmerman BJ, McCormack SA, Patel TB, Johnson LR: Polyamine depletion arrests cell cycle and induces inhibitors p21(Waf1/Cip1), p27(Kip1), and p53 in IEC-6 cells. Am J Physiol 1999, 276:684-691.
13. Choi SH, Kim SW, Choi DH, Min BH, Chun BG: Polyamine-depletion induces p27Kip1 and enhances dexamethasone-induced G1 arrest and apoptosis in human T lymphoblastic leukemia cells. Leuk Res 2000, 24:119-127.
14. Nemoto T, Kamei S, Seyama Y, Kubota S: p53 independent G (1) arrest is induced by DL-alpha-difluoromethylornithine. Biochem Biophys Res Commun 2001, 280:848-854.
15. Sheer CJ, Roberts JM: CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 1999, 13:1501-1512.
16. Tabor JW, Elledge SJ: CDK inhibitors in development and cancer. Curr Opin Genet Dev 1996, 6:56-66.
17. Weinberg RA: The retinoblastoma protein and cell cycle control. Cell 1995, 81:323-330.
18. Dyson N: The regulation of E2F by pRB-family proteins. Genes Dev 1998, 12:2245-2262.
19. Mulligan G, Jacks T: The retinoblastoma gene family: cousins with overlapping interests. Trends Genet 1998, 14:223-229.
20. Goodrich DW, Wang NP, Qian YW, Lee EE, Lee WH: The retinoblastoma gene product regulates progression through the G1 phase of the cell cycle. Cell 1991, 67:293-302.
21. Hinds PW, Mitnacht S, Dulic V, Arnold A, Reed SI, Weinberg RA: Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. Cell 1992, 69:993-1006.
22. Zhu L, van den Heuvel S, Helin K, Fattaey A, Ewen M, Livingston D, Dyson N, Harlow E: Inhibition of cell proliferation by p107, a relative of the retinoblastoma protein. Genes Dev 1993, 7:1111-1125.
23. Hoshikawa Y, Mori A, Animoto K, Iwabe K, Hatakeyama M: Control of retinoblastoma protein-independent hematopoietic cell cycle by the pRB-related p107. Proc Natl Acad Sci USA 1998, 95:8574-8579.
24. Jiang H, Karnezis AN, Tao M, Guida PM, Zhu L: pRB and p107 have distinct effects when expressed in pRb-deficient tumor cells at physiologically relevant levels. Oncogene 2000, 19:3878-3887.
25. Sage J, Mulligan G, Attardi LD, Miller A, Chen S, Williams B, Theodorou E, Jacks T: Targeted disruption of the three Rb-related genes leads to loss of G (1) control and immortalization. Genes Dev 2000, 14:3037-3050.
26. Beijersbergen RL, Hijmans EM, Zhu L, Bernards R: Interaction of c-Myc with the pRB-related protein p107 results in inhibition of c-Myc-mediated transactivation. EMBO J 1994, 13:4080-4086.
27. Gu W, Bhatia K, Magrath IT, Dang CV, Dalla-Favera R: Inhibition of the retinoblastoma family member p107 binds to B-MYB and suppresses its autoregulatory activity. J Biol Chem 1996, 271:28738-28740.
28. Sterner JM, Dew-Knight S, Musahl C, Kornbluth S, Horowitz JM: Negative regulation of DNA replication by the retinoblastoma protein is mediated by its association with MCM7. Mol Cell Biol 1998, 18:2748-2757.
29. Sterner JM, Dew-Knight S, Musahl C, Kornbluth S, Horowitz JM: Negative regulation of DNA replication by the retinoblastoma protein is mediated by its association with MCM7. Mol Cell Biol 1998, 18:2748-2757.
30. Sterner JM, Dew-Knight S, Musahl C, Kornbluth S, Horowitz JM: Negative regulation of DNA replication by the retinoblastoma protein is mediated by its association with MCM7. Mol Cell Biol 1998, 18:2748-2757.
31. Sterner JM, Dew-Knight S, Musahl C, Kornbluth S, Horowitz JM: Negative regulation of DNA replication by the retinoblastoma protein is mediated by its association with MCM7. Mol Cell Biol 1998, 18:2748-2757.
32. Sterner JM, Dew-Knight S, Musahl C, Kornbluth S, Horowitz JM: Negative regulation of DNA replication by the retinoblastoma protein is mediated by its association with MCM7. Mol Cell Biol 1998, 18:2748-2757.
ability to repress E2F activity through recruitment of a histone deacetylase. Proc Natl Acad Sci USA 1998, 95:10493-10498.

34. Luo RX, Postigo AA, Dean DC: Rb interacts with histone deacetylase to repress transcription. Cell 1998, 92:463-473.

35. Hateboer G, Kerkhoven RM, Shvarts A, Bernards R, Beijersbergen RL: Degradation of E2F by the ubiquitin-proteasome pathway: regulation by retinoblastoma family proteins and adenovirus transforming proteins. Genes Dev 1996, 10:2960-2970.

36. Robanus-Maandag E, Dekker M, van der Valk M, Carrozza ML, Jeanny JC, Dannenberg JH, Berns A, Riele H: p107 is a suppressor of retinoblastoma development in pRb-deficient mice. Genes Dev 1998, 12:1599-1609.

37. Kondo T, Higashi H, Nishizawa H, Ishikawa S, Ashizawa S, Yamada M, Makita Z, Koike T, Hatakeyama M: Involvement of pRB-related p107 protein in the inhibition of S phase progression in response to genotoxic stress. J Biol Chem 2001, 276:17559-17567.

38. Ohtani K, Iwanagi R, Arai M, Huang Y, Matsumura Y, Nakamura M: Cell type-specific E2F activation and cell cycle progression induced by the oncogene product Tax of human T-cell leukemia virus type I. J Biol Chem 2000, 275:11154-11163.

39. Kubota S, Kiyosawa H, Nomura Y, Yamada T, Suyama Y: Ornithine decarboxylase overexpression in mouse 10T1/2 fibroblasts: cellular transformation and invasion. J Natl Cancer Inst 1997, 89:567-571.

40. Morgan DM: Determination of polyamines as their benzoylated derivatives by HPLC. Methods Mol Biol 1998, 79:111-118.

41. Dignam JD, Lebovitz RM, Roeder RG: Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res 1983, 11:1475-1489.