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Largazole Arrests Cell Cycle at G1 Phase and Triggers Proteasomal Degradation of E2F1 in Lung Cancer Cells

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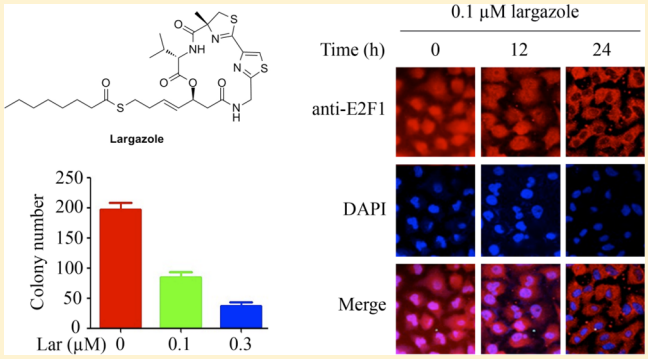
S *Supporting Information*

ABSTRACT: Aberration in cell cycle has been shown to be a common occurrence in lung cancer, and cell cycle inhibitor represents an effective therapeutic strategy. In this study, we test the effects of a natural macrocyclic depsipeptide largazole on lung cancer cells and report that this compound potently inhibits the proliferation and clonogenic activity of lung cancer cells but not normal bronchial epithelial cells. Largazole arrests cell cycle at G1 phase with up-regulation of the expression of cyclin-dependent kinase inhibitor p21. Interestingly, largazole enhances the E2F1-HDAC1 binding affinity and induces a proteasomal degradation of E2F1, leading to suppression of E2F1 function in lung cancer but not normal bronchial epithelial cells. Because E2F1 is overexpressed in lung cancer tumor samples, these data indicate that largazole is an E2F1-targeting cell cycle inhibitor, which bears therapeutic potentials for this malignant neoplasm.



KEYWORDS: *Lung cancer, cell cycle, largazole, E2F1, degradation*

Lung cancer is the leading cause of cancer-related death in both males and females, accounting for 13% (1.6 million) of the total cancer cases and 18% (1.4 million) of the deaths in 2008.[1] On the basis of histological characteristics, lung cancer can be divided into non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC), which comprise 85% and 15% of all cases, respectively. Chemotherapy is currently the mainstay of treatment, which has apparently reached a plateau of effectiveness in improving survival,[2] and new agents such as gefitinib and erlotinib will eventually fail because of the development of drug resistance.[3] Therefore, novel approaches are urgent needs to improve the prognosis of patients with lung cancer.

Aberration in cell cycle has been shown to be one of the major features of human cancers.[4] In eukaryotic cells, the restriction point that controls the entry into S phase comprises the retinoblastoma (Rb) and the E2F family proteins.[5,6] During G1 phase, cyclin D1 titrates CDK inhibitors such as $p27^{Kip1}$ and $p21^{Cip1}$ and inactivates the growth-suppressive function of Rb through its phosphorylation.[7,8] E2F1 is subsequently released from the Rb/E2F1 complex and translocated into the nucleus, where it transactivates the transcription of genes required for entry into S phase.[6,9,10] Loss of p21 expression was frequently observed and is associated with poor prognosis in NSCLC.[11,12] P21 is also required for NSCLC cells' sensitivity to gefitinib treatment.[13] However, recent studies show that E2F1 can promote cancer progression and confer chemoresistance to cancer cells.[9] In NSCLC, E2F1 is overexpressed and correlates with the expression of thymidylate synthase (TS) and survivin and is associated with adverse prognosis.[14] E2F1 is also up-regulated in SCLC.[15] Moreover, antiproliferative effects of gefitinib are associated with suppression of E2F1 expression and telomerase activity.[16] These results suggest that p21-E2F1 cascade could be a drug target for lung cancer.

Natural compounds remain a source for anticancer drug development, because 47% of the 155 anticancer drugs approved during the period from 1940s to 2006 were either natural products or directly derived therefrom.[17] We therefore collected a set of natural compounds and investigate their anticancer activities and mechanisms of action, with the aim to

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help anticancer drug development. Among them, largazole (Figure 1A) is a natural macrocyclic depsipeptide isolated from

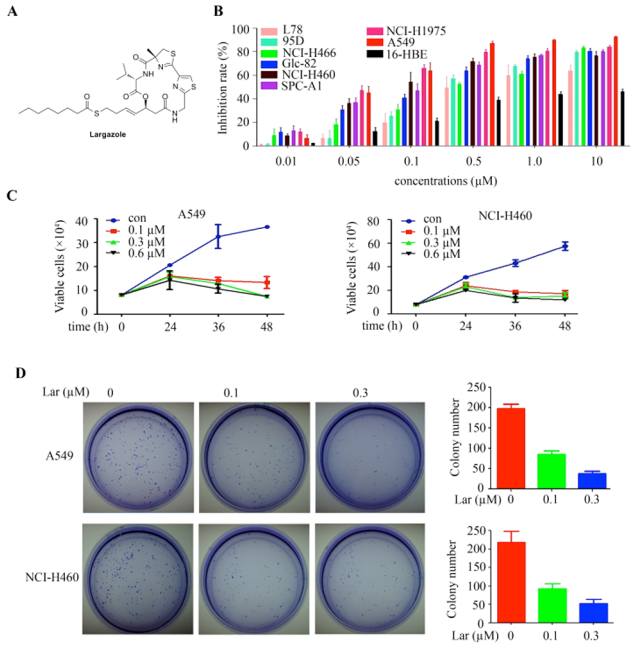

Figure 1. Inhibitory effects of largazole on lung cancer cells. (A) Chemical structure of largazole. (B) The cells were treated with different concentrations of largazole for 48 h, and cell proliferation was measured by the CCK-8 assay. (C) The cells were treated with largazole for indicated time points, and cell viability was evaluated by trypan blue exclusion assay. (D) Clonogenic assay of A549 and H460 cells treated with largazole. The cells were pretreated with largazole or DMSO for 24 h and seeded in medium containing 0.3% soft agar in the upper layer and 0.6% soft agar in the lower layer. After 10 days, the cell clones were counted with a microscope. Data are shown as mean ± SD for three independent experiments.

a cyanobacterium of the genus *Symploca*[18] that can inhibit class I histone deacetylases (HDAC)[19,20] and ubiquitin activating enzyme (E1) mediating ubiquitin conjugation to $p27^{Kip1}$ and TRF1.[21] Largazole exhibits antiproliferative activity against lung, colorectal, melanoma, ovarian, renal, and breast cancer and leukemia cells.[19,22,23] In this study, we tested the cytotoxicity as well as the underlying mechanisms of largazole on lung cancer cells.

We evaluated the effects of largazole on lung cancer lines expressing wide-type (WT) or mutant EGFR and normal human bronchial epithelial 16-HBE cell line. The compound was synthesized as described by Jiang's group.[19] We found that largazole significantly inhibited cell proliferation of A549, NCI-H1975, SPC-A1, GLC-82, L78, H460, 95D, and H466 lung cancer lines but not 16-HBE cells (Figure 1B), with the IC_{50} values ranging from 0.077 to 0.57 μM for cancerous cells but more than 10 μM for 16-HBE cells (Table 1). By using trypan blue dye exclusion analysis, we found that largazole at 0.1 μM significantly inhibited the growth of A549 and H460 cells (Figure 1C). Moreover, we showed that largazole markedly inhibited the colony forming activity of A549 and H460 cells in a dose-dependent fashion (Figure 1D). These results demonstrate that largazole has potent and selective cytotoxicity to lung cancer cells as compared to normal human bronchial epithelial cells.

We tested the effect of largazole on cell cycle and cell death. To do this, the cells were synchronized, stained by propidium iodide (PI), and analyzed by flow cytometry. Our results showed that in A549 and H460 cells, treatment with largazole at 0.06 to 0.1 μM for 24 h led to the arrest of cell cycle at G1 phase (Figure 2A). We further showed that treatment with

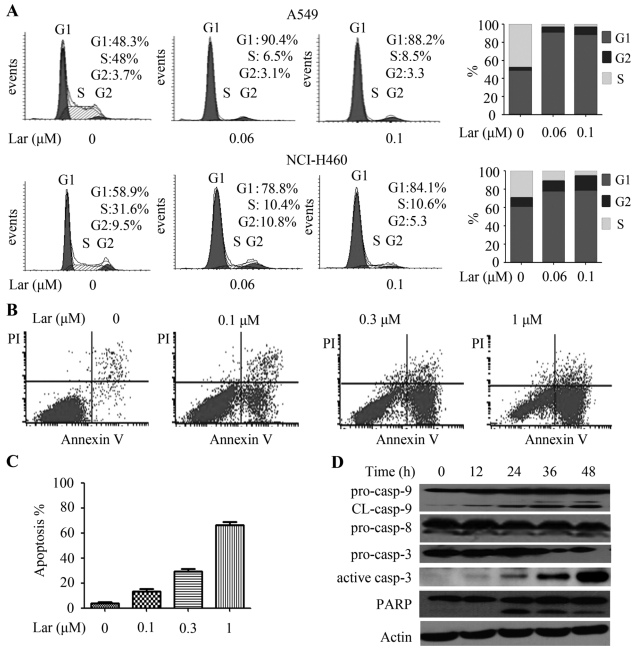

Figure 2. Largazole induces cell cycle arrest and subsequent apoptosis in lung cancer cells. (A) The synchronized cells were treated with largazole for 24 h, stained with PI, and analyzed with flow cytometry for cell cycle distribution. (B,C) A549 cells were treated with largazole for 48 h and assessed by Annexin V/PI staining and flow cytometry. (D) A549 cells were treated with largazole (0.1 μM) for indicated time points, lysed, and subjected to Western blot assay using indicated antibodies. CL-casp-9, cleaved casp-9.

largazole at a relatively high concentration (0.3 to 1 μM) for 48 h induced apoptosis in a significant proportion (up to 70%) of A549 cells, revealed by the Annexin V/PI staining and flow cytometry analysis (Figure 2B,C). In these cells, treatment with largazole for 48 h caused down-regulation of pro-casp-9 with generation of activated casp-9, while the expression of casp-8 was not markedly affected (Figure 2D). The intrinsic apoptotic

Table 1. Concentration of Largazole Required to Inhibits 50% of Cell Growth (IC_{50}) in Lung Cancer and Noncancerous Cell Lines[a]

cell lines	A549	H1975	H460	GLC-82	L78	SPC-A1	95D	H466	16-HBE
cancer type	Ade	Ade	Ade	Ade	SCC	LCC	LCC	SCLC	NBEC
IC_{50} (μM)	0.077	0.083	0.12	0.19	0.57	0.14	0.42	0.52	>10

[a]Ade, adenocarcinoma; ADSC, adenosquamous carcinoma; LCC, large cell carcinoma; SCC, squamous cell carcinoma; SCLC, small cell lung cancer; NBEC, normal bronchial epithelial cells.

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signal led to activation of casp-3, reflected by a decrease of pro-casp-3 and increase of active casp-3 with cleavage of its substrate poly(ADP-ribose) polymerase (PARP) (Figure 2D). These results indicate that largazole causes cell cycle arrest at a lower concentration and induces a mitochondrial-dependent caspase activation to trigger apoptosis of lung cancer cells at a higher concentration with a relatively longer treatment time course.

We investigated the mechanisms underlying largazole-induced G1 phase arrest. By Western blot analysis of cell cycle regulators essential for G1 phase progression in A549 cells upon largazole at 0.1 μM, we found that the expression of p21 was up-regulated drastically at 6 h, while p27 was slightly increased at 12 h of treatment time course (Figure 3A).

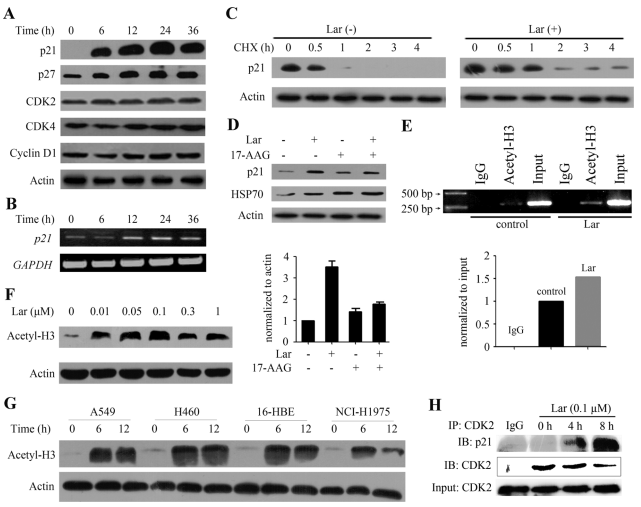

Figure 3. Largazole up-regulates p21 in lung cancer cells. (A) A549 cells were treated with largazole (0.1 μM) at indicated time points, lysed, and subjected to Western blot assay using indicated antibodies. (B) A549 cells were treated with largazole (0.1 μM) at indicated time points and lysed, and RNA was extracted for RT-PCR analysis using indicated primers. (C) A549 cells were treated with CHX (100 μg/mL) and/or largazole (0.1 μM) for indicated time points, lysed, and subjected to Western blot analysis. (D) A549 cells were treated with Hsp90 inhibitor 17-AAG (1 μM) and/or largazole (0.1 μM) for 6 h, lysed, and subjected to Western blot analysis (upper panel). Protein expression of p21 was quantified by the densitometry analysis and normalized against β-actin expression (lower panel). (E) Pharmacologic modulation of acetyl histone H3 on p21 promoter. ChIP assay was done in A549 cells treated with largazole (0.1 μM) using antiacetyl histone H3 antibody (upper panel), and acetyl histone H3-bound p21 promoter was quantified by the densitometry analysis and normalized against input (lower panel). (F,G) A549 or indicated cells were treated with largazole at indicated concentration for 4 h (F) or at 0.1 μM for 6 to 12 h (G), lysed, and subjected to Western blotting. (H) A549 cells were treated with largazole and lysed, and the lysates were analyzed by immunoprecipitation and Western blot using indicated antibodies.

However, the expression of cyclin D1, CDK2, or CDK4 was not dramatically affected (Figure 3A). By RT-PCR assay, we found that treatment of A549 cells with largazole at 0.1 μM for 12 h up-regulated p21 at mRNA level (Figure 3B). These results indicate that largazole may affect the post-translational modification/degradation as well as transcriptional activation to up-regulate the expression of p21. Indeed, in A549 cells upon protein synthesis inhibitor cycloheximide (CHX, 100 μg/mL), p21 was decreased markedly at 1 h and became undetectable at 2 h (Figure 3C, left panel), suggesting that p21 can be modified and degraded rapidly within cells. However, in cells coincubated with CHX and largazole, p21 was slightly down-regulated at 1 h and could be detected in 4 h (Figure 3C, right panel), indicating that largazole can stabilize this protein. Heat shock protein 90 (Hsp90) is shown to have a vital role in p21 stabilization.[24] We observed that at the presence of Hsp90 inhibitor 17-AAG, largazole-induced p21 accumulation was markedly attenuated (Figure 3D), indicating that Hsp90 also has an essential role in largazole-induced up-regulation of p21.

Largazole can inhibit class I HDAC.[19,20] We investigated the effect of this compound on the binding affinity between p21 promoter and acetyl H3 by a chromatin immunoprecipitation (ChIP) assay and found that largazole enhanced the recruitment of p21 promoter to acetyl H3 (Figure 3E). We further showed that in A549 cells treatment with largazole at 0.01 to 1 μM for 4 h led to hyperacetylation of histone H3 (Figure 3F). Moreover, treatment with largazole at 0.1 μM for 6 to 12 h resulted in accumulation of hyperacetylated H3 in A549, H460, and H1975 as well as 16-HBE cells (Figure 3G). We performed immunoprecipitation assay to test the effect of largazole on p21-CDK2 interaction and found that in A549 cells p21 dissociated from CDK2, while treatment with largazole at 0.1 μM for 4 to 8 h markedly enhanced the p21-CDK2 binding affinity (Figure 3H). Taken together, our data indicate that largazole can increase p21 level by promoting its stability and activating its transcription, resulting in inhibition of CDKs and perturbation of cell cycle.

We reported that treatment with largazole at 0.1 μM for 6 to 12 h drastically reduced the phosphorylation of Rb (Figure 4A)

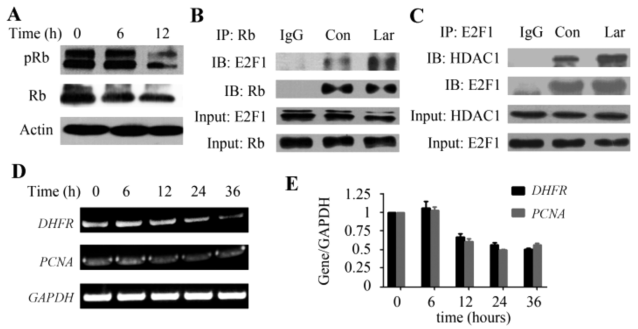

Figure 4. Largazole suppresses E2F1 activity. (A) A549 cells were treated with 0.1 μM largazole for indicated time points and lysed, and the lysates were analyzed by Western blot assay. (B,C) A549 cells were treated with largazole at 0.1 μM for 8 h and lysed, and the lysates were analyzed by immunoprecipitation and Western blot using indicated antibodies. (D,E) A549 cells were treated with largazole (0.1 μM) at indicated time points and lysed, and RNA was extracted for RT-PCR analysis using indicated primers (D). The expression of indicated genes was quantified by the densitometry analysis and normalized against GAPDH expression (E).

and strengthened the Rb-E2F1 binding affinity (Figure 4B). Furthermore, largazole enhanced the E2F1−HDAC1 interaction (Figure 4C). To evaluate the transcription activity of E2F1, we tested the expression of two E2F1 target genes, dihydrofolate reductase (DHFR) and proliferating cell nuclear antigen (PCNA) that are required for the transition of G1 to S, and found that both genes were transcriptionally suppressed (Figure 4D,E). These results demonstrate that the biological function of E2F1 is inhibited by largazole treatment in lung cancer cells.

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Acetylation can stabilize E2F1, while Rb-associated histone deacetylase can deacetylate E2F1.[25] By immunoprecipitation and Western blot assays, we showed that treatment of A549 cells with largazole caused deacetylation of E2F1 (Figure 5A),

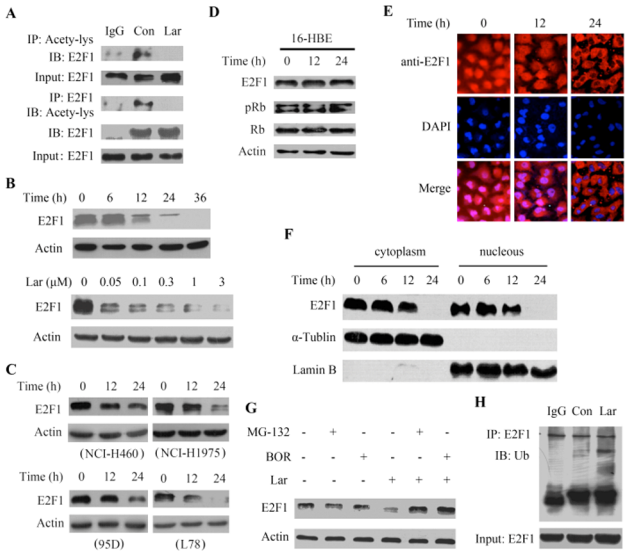

Figure 5. Largazole triggers proteasomal degradation of E2F1. (A) A549 cells were treated with largazole (0.1 μM) for 8 h and lysed, and the lysates were analyzed by immunoprecipitation and Western blot using indicated antibodies. (B) A549 cells were treated with largazole (0.1 μM) for indicated time points (upper panel) or indicated concentrations for 12 h (lower panel), lysed, and subjected to Western blotting. (C,D) The cells were treated with 0.1 μM largazole for indicated time points and lysed, and the lysates were analyzed by Western blot assay. (E) A549 cells were treated with 0.1 μM largazole for 12 to 24 h and assessed by immunofluorescence analysis using an anti-E2F1 antibody and DAPI. (F) A549 cells upon 0.1 μM largazole for indicated time points were lysed, proteins in cytoplasmic and nucleus fractions were separated and subjected to Western blot using indicated antibodies. (G) A549 cells were pretreated with proteasome inhibitor bortezomib (100 nM) or MG-132 (10 μM) for 2 h, followed by largazole (0.1 μM) treatment for 12 h, lysed, and analyzed by Western blot assay. (H) Lysates of A549 cells upon largazole (0.1 μM for 8 h) were analyzed by immunoprecipitation and Western blot using anti-E2F1 and antiubiquitin antibodies.

followed by its down-regulation in a time- and dose-dependent manner (Figure 5B). Largazole was also able to trigger E2F1 catabolism in NCI-H460, H1975, L78, and 95D lung cancer (Figure 5C) but not 16-HBE cells (Figure 5D). Immunofluorescence (Figure 5E) and Western blot (Figure 5F) assays revealed that largazole could, on one hand, cause translocation of E2F1 from nucleus into cytoplasm and, on the other hand, decrease the expression of E2F1 in both compartments. To test whether the degradation of E2F1 was mediated by proteasome, A549 cells were pretreated with proteasome inhibitor bortezomib (BOR, 100 nM) or MG-132 (10 μM) for 2 h followed by coincubation with largazole for an additional 12 h. The results showed that both bortezomib and MG-132 could inhibit largazole-induced E2F1 degradation (Figure 5G). By immunoprecipitation and Western blot assays, we reported that largazole caused ubiquitination of E2F1 (Figure 5H). These results indicate that largazole induces proteasomal degradation of E2F1.

We conducted Western blot analysis to evaluate the expression of E2F1 in tumor samples as well as adjacent normal tissues isolated from lung cancer patients whose baseline characteristics were shown in Supplementary Table 1. As compared to that in counterpart normal controls, E2F1 was overexpressed in 5 of 10 (50%) tumor samples (Supplementary Figure 1A). In only one patient, the expression of E2F1 in adjacent normal tissue is higher than in tumor specimen (Supplementary Figure 1A). E2F1 was also high in A549, H460, and H1975 lung cancer cell lines (Supplementary Figure 1B).

E2F1 has been identified as a tumor suppressor regulating the activities of p53 and promoting apoptosis by activating a plethora of death pathways including caspases and Apaf-1.[26,27] However, recent studies in knockout and transgenic mouse models indicate that E2F1-3 bear an unexpected pro-survival role in development and cell survival.[28,29] Moreover, E2F1 is reported to be overexpressed in many types of human cancers including stomach cancer,[30] B-cell chronic lymphocytic leukemia,[31] non-small-cell lung cancer,[14] metastatic colorectal cancer,[32] and sporadic Burkitt's lymphoma,[33] and is inversely associated with prognosis.[9] Consistent with these observations, our results confirm that E2F1 is overexpressed in lung cancer samples compared to paired normal controls (Supplementary Figure 1). These data indicate that E2F1 has oncogenic functions in some types of cancer and may represent a rational therapeutic target; therefore, E2F1-targeting agents are needed for the development of novel anticancer drugs.

We found that largazole significantly inhibits cell proliferation of lung cancer lines but not normal bronchial epithelial cells, demonstrating its selectivity to lung cancer cells. At an early stage (24 h) or at low concentration (0.06 to 0.1 μM), this compound arrests cell cycle at G1 phase and inhibits cell proliferation; and at late stage (48 h) or relatively high concentration (1 μM), it induces apoptosis of the lung cancer cells (Figure 2). At molecular level, largazole up-regulates p21 by activation of its transcription and prevention of its degradation, leading to sequestration of CDK2 (Figure 3). Moreover, largazole causes dephosphorylation of Rb, which sequesters E2F1 (Figure 3). Rb, E2F1, and HDAC1 can form a complex.[34] We show that, while HDAC1 recruits E2F1, largazole enhances this interaction (Figure 4), also contributing to the suppression of E2F1 trans-regulatory functions. In both cancerous and normal bronchial epithelial cells, largazole up-regulates acetyl H3, which may lead to up-regulation of p21 (Figure 3). While largazole is able to trigger E2F1 catabolism in lung cancer cells, it fails to affect E2F1 expression in 16-HBE cells (Figure 5). E2F1 can be degraded by the ubiquitin–proteasome pathway.[35−38] Interestingly, E2F1 can be stabilized by P/CAF-mediated acetylation, and the acetylated E2F1 can be deacetylated by Rb-bound deacetylase.[25] We report that upon largazole treatment, the acetylated E2F1 was drastically reduced (Figure 5), possibly due to enhancement of Rb-E2F1-HDAC1 binding affinity (Figure 5). Largazole induces a proteasomal degradation of E2F1 in lung cancer but not normal bronchial epithelial cells (Figure 5), partially explaining the relatively selective cytotoxicity of largazole (Figure 1 and Table 1). We show that E2F1 is overexpressed in 5 of 10 lung cancer samples compared to their counterpart controls (Supporting Information). Evidence suggests that HDAC inhibitors will be more effective in tumors with high E2F1 activity.[39] Therefore, our results demonstrate that E2F1 may represent a therapeutic target for lung cancer, and largazole could serve as a lead compound for the development of E2F1 inhibitor.

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■ ASSOCIATED CONTENT

Supporting Information

Supporting Information includes experimental procedures, one Table, and one Figure. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D. Global cancer statistics. *Cancer J. Clin.* **2011**, *61*, 69−90.

(2) Herbst, R. S.; Heymach, J. V.; Lippman, S. M. Lung cancer. *N. Engl. J. Med.* **2008**, *359*, 1367−1380.

(3) Pao, W.; Miller, V. A.; Politi, K. A.; Riely, G. J.; Somwar, R.; Zakowski, M. F.; Kris, M. G.; Varmus, H. Acquired resistance of lung adenocarcinomas to gefitinib or erlotinib is associated with a second mutation in the EGFR kinase domain. *PLoS Med.* **2005**, *2*, e73.

(4) Hanahan, D.; Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* **2011**, *144*, 646−674.

(5) Massague, J. G1 cell-cycle control and cancer. *Nature* **2004**, *432*, 298−306.

(6) Shah, M. A.; Schwartz, G. K. Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. *Clin. Cancer Res.* **2001**, *7*, 2168−2181.

(7) Sherr, C. J.; Roberts, J. M. Inhibitors of mammalian G1 cyclin-dependent kinases. *Genes Dev.* **1995**, *9*, 1149−1163.

(8) Weinberg, R. A. The retinoblastoma protein and cell cycle control. *Cell* **1995**, *81*, 323−330.

(9) Engelmann, D.; Putzer, B. M. The dark side of E2F1: In transit beyond apoptosis. *Cancer Res.* **2012**, *72*, 571−575.

(10) Chen, H. Z.; Tsai, S. Y.; Leone, G. Emerging roles of E2Fs in cancer: an exit from cell cycle control. *Nat. Rev. Cancer* **2009**, *9*, 785−797.

(11) Komiya, T.; Hosono, Y.; Hirashima, T.; Masuda, N.; Yasumitsu, T.; Nakagawa, K.; Kikui, M.; Ohno, A.; Fukuoka, M.; Kawase, I. p21 expression as a predictor for favorable prognosis in squamous cell carcinoma of the lung. *Clin. Cancer Res.* **1997**, *3*, 1831−1835.

(12) Shoji, T.; Tanaka, F.; Takata, T.; Yanagihara, K.; Otake, Y.; Hanaoka, N.; Miyahara, R.; Nakagawa, T.; Kawano, Y.; Ishikawa, S.; Katakura, H.; Wada, H. Clinical significance of p21 expression in non-small-cell lung cancer. *J. Clin. Oncol.* **2002**, *20*, 3865−3871.

(13) Zhao, Y. F.; Wang, C. R.; Wu, Y. M.; Ma, S. L.; Ji, Y.; Lu, Y. J. P21 (waf1/cip1) is required for non-small cell lung cancer sensitive to Gefitinib treatment. *Biomed. Pharmacother.* **2011**, *65*, 151−156.

(14) Huang, C. L.; Liu, D.; Nakano, J.; Yokomise, H.; Ueno, M.; Kadota, K.; Wada, H. E2F1 overexpression correlates with thymidylate synthase and survivin gene expressions and tumor proliferation in non small-cell lung cancer. *Clin. Cancer Res.* **2007**, *13*, 6938−6946.

(15) Eymin, B.; Gazzeri, S.; Brambilla, C.; Brambilla, E. Distinct pattern of E2F1 expression in human lung tumours: E2F1 is up-regulated in small cell lung carcinoma. *Oncogene* **2001**, *20*, 1678−1687.

(16) Suenaga, M.; Yamaguchi, A.; Soda, H.; Orihara, K.; Tokito, Y.; Sakaki, Y.; Umehara, M.; Terashi, K.; Kawamata, N.; Oka, M.; Kohno, S.; Tei, C. Antiproliferative effects of gefitinib are associated with suppression of E2F-1 expression and telomerase activity. *Anticancer Res.* **2006**, *26*, 3387−3391.

(17) Newman, D. J.; Cragg, G. M. Natural products as sources of new drugs over the last 25 years. *J. Nat. Prod.* **2007**, *70*, 461−477.

(18) Taori, K.; Paul, V. J.; Luesch, H. Structure and activity of largazole, a potent antiproliferative agent from the Floridian marine cyanobacterium Symploca sp. *J. Am. Chem. Soc.* **2008**, *130*, 1806−1807.

(19) Zeng, X.; Yin, B.; Hu, Z.; Liao, C.; Liu, J.; Li, S.; Li, Z.; Nicklaus, M. C.; Zhou, G.; Jiang, S. Total synthesis and biological evaluation of largazole and derivatives with promising selectivity for cancers cells. *Org. Lett.* **2010**, *12*, 1368−1371.

(20) Ying, Y.; Taori, K.; Kim, H.; Hong, J.; Luesch, H. Total synthesis and molecular target of largazole, a histone deacetylase inhibitor. *J. Am. Chem. Soc.* **2008**, *130*, 8455−8459.

(21) Ungermannova, D.; Parker, S. J.; Nasveschuk, C. G.; Wang, W.; Quade, B.; Zhang, G.; Kuchta, R. D.; Phillips, A. J.; Liu, X. Largazole and its derivatives selectively inhibit ubiquitin activating enzyme (E1). *PLoS One* **2012**, *7*, e29208.

(22) Law, M. E.; Corsino, P. E.; Jahn, S. C.; Davis, B. J.; Chen, S.; Patel, B.; Pham, K.; Lu, J.; Sheppard, B.; Norgaard, P.; Hong, J.; Higgins, P.; Kim, J. S.; Luesch, H.; Law, B. K. Glucocorticoids and histone deacetylase inhibitors cooperate to block the invasiveness of basal-like breast cancer cells through novel mechanisms. *Oncogene* **2013**, *32*, 1316−1329.

(23) Liu, Y.; Salvador, L. A.; Byeon, S.; Ying, Y.; Kwan, J. C.; Law, B. K.; Hong, J.; Luesch, H. Anticolon Cancer Activity of Largazole, a Marine-Derived Tunable Histone Deacetylase Inhibitor. *J. Pharmacol. Exp. Ther.* **2010**, *335*, 351−361.

(24) Jascur, T.; Brickner, H.; Salles-Passador, I.; Barbier, V.; El, K. A.; Smith, B.; Fotedar, R.; Fotedar, A. Regulation of p21(WAF1/CIP1) stability by WISp39, a Hsp90 binding TPR protein. *Mol. Cell* **2005**, *17*, 237−249.

(25) Martinez-Balbas, M. A.; Bauer, U. M.; Nielsen, S. J.; Brehm, A.; Kouzarides, T. Regulation of E2F1 activity by acetylation. *EMBO J.* **2000**, *19*, 662−671.

(26) Engelmann, D.; Putzer, B. M. Translating DNA damage into cancer cell death-A roadmap for E2F1 apoptotic signalling and opportunities for new drug combinations to overcome chemo-resistance. *Drug Resist. Updates* **2010**, *13*, 119−131.

(27) Muller, H.; Bracken, A. P.; Vernell, R.; Moroni, M. C.; Christians, F.; Grassilli, E.; Prosperini, E.; Vigo, E.; Oliner, J. D.; Helin, K. E2Fs regulate the expression of genes involved in differentiation, development, proliferation, and apoptosis. *Genes Dev.* **2001**, *15*, 267−285.

(28) Chen, D.; Pacal, M.; Wenzel, P.; Knoepfler, P. S.; Leone, G.; Bremner, R. Division and apoptosis of E2f-deficient retinal progenitors. *Nature* **2009**, *462*, 925−929.

(29) Chong, J. L.; Wenzel, P. L.; Saenz-Robles, M. T.; Nair, V.; Ferrey, A.; Hagan, J. P.; Gomez, Y. M.; Sharma, N.; Chen, H. Z.; Ouseph, M.; Wang, S. H.; Trikha, P.; Culp, B.; Mezache, L.; Winton, D. J.; Sansom, O. J.; Chen, D.; Bremner, R.; Cantalupo, P. G.; Robinson, M. L.; Pipas, J. M.; Leone, G. E2f1-3 switch from activators in progenitor cells to repressors in differentiating cells. *Nature* **2009**, *462*, 930−934.

(30) Suzuki, T.; Yasui, W.; Yokozaki, H.; Naka, K.; Ishikawa, T.; Tahara, E. Expression of the E2F family in human gastrointestinal carcinomas. *Int. J. Cancer* **1999**, *81*, 535−538.

(31) Korz, C.; Pscherer, A.; Benner, A.; Mertens, D.; Schaffner, C.; Leupolt, E.; Dohner, H.; Stilgenbauer, S.; Lichter, P. Evidence for distinct pathomechanisms in B-cell chronic lymphocytic leukemia and mantle cell lymphoma by quantitative expression analysis of cell cycle and apoptosis-associated genes. *Blood* **2002**, *99*, 4554−4561.

(32) Iwamoto, M.; Banerjee, D.; Menon, L. G.; Jurkiewicz, A.; Rao, P. H.; Kemeny, N. E.; Fong, Y.; Jhanwar, S. C.; Gorlick, R.; Bertino, J. R. Overexpression of E2F-1 in lung and liver metastases of human colon cancer is associated with gene amplification. *Cancer Biol. Ther.* **2004**, *3*, 395−399.

(33) Molina-Privado, I.; Rodriguez-Martinez, M.; Rebollo, P.; Martin-Perez, D.; Artiga, M. J.; Menarguez, J.; Flemington, E. K.; Piris, M. A.; Campanero, M. R. E2F1 expression is deregulated and plays an oncogenic role in sporadic Burkitt's lymphoma. *Cancer Res.* **2009**, *69*, 4052−4058.
(34) Robertson, K. D.; It-Si-Ali, S.; Yokochi, T.; Wade, P. A.; Jones, P. L.; Wolffe, A. P. DNMT1 forms a complex with Rb, E2F1 and HDAC1 and represses transcription from E2F-responsive promoters. *Nat. Genet.* **2000**, *25*, 338−342.
(35) Hateboer, G.; Kerkhoven, R. M.; Shvarts, A.; Bernards, R.; Beijersbergen, R. L. Degradation of E2F by the ubiquitin-proteasome pathway: regulation by retinoblastoma family proteins and adenovirus transforming proteins. *Genes Dev.* **1996**, *10*, 2960−2970.
(36) Hofmann, F.; Martelli, F.; Livingston, D. M.; Wang, Z. The retinoblastoma gene product protects E2F-1 from degradation by the ubiquitin-proteasome pathway. *Genes Dev.* **1996**, *10*, 2949−2959.
(37) Peart, M. J.; Poyurovsky, M. V.; Kass, E. M.; Urist, M.; Verschuren, E. W.; Summers, M. K.; Jackson, P. K.; Prives, C. APC/C(Cdc20) targets E2F1 for degradation in prometaphase. *Cell Cycle* **2010**, *9*, 3956−3964.
(38) Campanero, M. R.; Flemington, E. K. Regulation of E2F through ubiquitin-proteasome-dependent degradation: Stabilization by the pRB tumor suppressor protein. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 2221−2226.
(39) Zhao, Y.; Tan, J.; Zhuang, L.; Jiang, X.; Liu, E. T.; Yu, Q. Inhibitors of histone deacetylases target the Rb-E2F1 pathway for apoptosis induction through activation of proapoptotic protein Bim. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 16090−16095.