Available online at www.sciencedirect.com
SCIENCE DIRECT®
ACADEMIC PRESS
Biochemical and Biophysical Research Communications 298 (2002) 178–183
BBRC
www.academicpress.com

Hygrolidin induces p21 expression and abrogates cell cycle progression at G1 and S phases

Manabu Kawada,[a] Ihomi Usami,[a] Shun-ichi Ohba,[a] Tetsuya Someno,[a] Jin Woo Kim,[b] Yoichi Hayakawa,[b] Kiyoshi Nose,[c] and Masaaki Ishizuka[a,*]

[a] *Institute for Chemotherapy, Microbial Chemistry Research Foundation, 18-24 Miyamoto, Numazu-shi, Shizuoka-ken 410-0301, Japan*
[b] *Institute of Molecular and Cellular Biosciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan*
[c] *Department of Microbiology, Showa University School of Pharmaceutical Sciences, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*

Received 12 September 2002

Abstract

Hygrolidin family antibiotics showed selective cytotoxicity against both cyclin E- and cyclin A-overexpressing cells. Among them, hygrolidin was the most potent and inhibited growth of solid tumor-derived cell lines such as DLD-1 human colon cancer cells efficiently more than that of hematopoietic tumor cells and normal fibroblasts. FACS analysis revealed that hygrolidin increased cells in G1 and S phases in DLD-1 cells. While hygrolidin decreased amounts of cyclin-dependent kinase (cdk) 4, cyclin D, and cyclin B, it increased cyclin E and p21 levels. Hygrolidin-induced p21 bound to and inhibit cyclin A–cdk2 complex more strongly than cyclin E–cdk2 complex. Furthermore, hygrolidin was found to increase p21 mRNA in DLD-1 cells, but not in normal fibroblasts. Thus, hygrolidin inhibited tumor cell growth through induction of p21. In respect to p21 induction, inhibition of vacuolar-type (H+)-ATPase by hygrolidin was suggested to be involved.

Keywords: Hygrolidin; Cell cycle; p21; V-ATPase; Tumor growth

We have previously reported that bactobolin and cytostatin-induced apoptosis strongly in B16 melanoma cells and EL-4 lymphoma cells, respectively [1]. The study of mechanism of their differential effect suggested that cyclin E could be suppressive factor for induction of apoptosis in solid tumor cells, while cyclin A and/or B could be another one for induction of apoptosis in hematopoietic tumor cells. We, therefore, hypothesized that an inhibitor of cyclin E function should be a selective inducer of apoptosis in solid tumor cells without or with less side effects such as hematopoietic cytotoxicity. We have then screened a compound from natural sources, which induces apoptosis selectively in solid tumor cells, using specific cyclin-overexpressing cells. As a result, we have found that hygrolidin family antibiotics selectively showed cytotoxicity against cyclin E- and cyclin A-overexpressing cells.

Hygrolidins are 16-membered macrolide antibiotics and are first reported to be anti-fungus agents against *Valsa ceratosperma*, the pathogen of apple canker disease [2]. Hygrolidins are also reported to inhibit growth of src- and ras-transformed cells without effect on untransformed cells [3]. In this paper, we described that hygrolidin effectively inhibited growth of various tumor cell lines and affected cell cycle through induction of a cdk inhibitor, p21 [4–6].

Materials and methods

Antibodies. Antibodies used for Western blotting and immunoprecipitation were the following: anti-Rb (sc-50), anti-cdk4 (sc-260), anti-cyclin D1 (sc-718), anti-cdk2 (sc-163), anti-cyclin A (sc-751), anti-cdc2 (sc-54), anti-cyclin B (sc-594), anti-p21 (sc-397), anti-p27 (sc-528), and anti-p53 (sc-99) were from Santa Cruz Biotechnology (Santa Cruz, CA); cyclin E (06-459) was from Upstate Biotechnology (Lake Placid, NY).

Cells. Rat1-R12 fibroblasts were obtained from ATCC. Human cyclin E and cyclin A genes were kindly provided by Dr. Steven I. Reed (Scripps Research Institute), and cyclin E- and cyclin A-overexpressing cells were prepared and maintained as described [7,8]. Expressions of human cyclin E and cyclin A were confirmed by Western blotting (data not shown). DLD-1 human colon cancer, DMS273 human lung cancer, LNCaP human prostate cancer cells, WI-38 human normal fibroblasts,

* Corresponding author. Fax: +81-55-922-6888.
E-mail address: imcic@shizuokanet.ne.jp (M. Ishizuka).

0006-291X/02/$ - see front matter © 2002 Elsevier Science (USA). All rights reserved.
PII: S0006-291X(02)02416-6

Lewis lung carcinoma cells, and v-H-ras-transformed rat 3Y1 fibroblasts (HR-3Y1) [9] obtained from JCRB Cell Bank were grown in Dulbecco's modified Eagle's medium and EL-4 mouse lymphoma and K562 human leukemia cells were grown in RPMI1640 medium, supplemented with 10% fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS), 100 U/ml penicillin G, and 100 μg/ml streptomycin at 37 °C with 5% CO_2.

Cell growth. Cells were inoculated into 96-well plates at 5000 cells/well and incubated with or without test chemicals for 3 days. The growth was determined by using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) as described [10].

Cell cycle analysis. Cells (5×10^5) were cultured in 60 mm dishes with or without hygrolidin for 24 h and then fixed with ice-cold 70% ethanol. The fixed cells were treated with 0.1% RNase (Sigma, St. Louis, MO) at 37 °C for 15 min and then resuspended in phosphate-buffered saline (PBS) containing 50 μg/ml propidium iodide (Sigma, St. Louis, MO). DNA fluorescence was measured with a FACSCalibur (Becton–Dickinson Immunocytometer Systems, San Jose, CA).

Preparation of cell lysates and Western blotting. Cells (3×10^5) were cultured in 35-mm dishes with or without test chemicals for the indicated times. The cells were washed twice with ice-cold PBS containing 100 μM Na_3VO_4 and then lysed in a lysis buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerophosphate, 1 mM Na_3VO_4, and 25 μg/ml each of antipain, leupeptin, and pepstatin). Equal protein extracts were separated by SDS–PAGE and transferred onto Immobilon PVDF membranes (Millipore, Bedford, MA). Enhanced chemiluminescence (Amersham, Arlington Heights, IL) was used to visualize the immunoblot signals.

Immunoprecipitation and cdk2 assay. Cell lysates were prepared as described above and equal amounts of protein were incubated with the indicated antibody. The immune complexes were collected on protein A–Sepharose beads (Pharmacia Biotech, Uppsala, Sweden) and washed four times in IP buffer (20 mM Hepes, pH 7.5, 150 mM NaCl, 1% Triton X-100, 1 mM EDTA, 50 mM NaF, 50 mM β-glycerophosphate, 0.1 mM PMSF, and 0.2 mM Na_3VO_4) and once in a kinase buffer (20 mM Hepes, pH 7.5, 10 mM $MgCl_2$, and 1 mM DTT). Cdk2 activity was determined using histone H1 (Boehringer–Mannheim, Mannheim, Germany) as a substrate as described previously [11].

RT-PCR analysis. Cells were cultured with or without hygrolidin for 24 h and total RNA was isolated using RNeasy (Qiagen, Hilden, Germany). cDNAs were synthesized using AMV reverse transcriptase (Promega, Madison, WI) with the same quantity of RNA (1 μg) and amplified using *Taq* DNA polymerase (Promega, Madison, WI). Specific primers of p21 (495-bp product) were 5′-ATGTCAGAAC CGGCTGG-3′ (sense) and 5′-TAGGGCTTCCTCTTGGA-3′ (antisense), and those of GAPDH were reported elsewhere [12]. PCRs were optimized for each set of primers and performed using different numbers of cycles to ensure that amplification occurred in a linear range. After amplification, the products were electrophoresed in a 2% agarose gel and detected by ethidium bromide.

V-ATPase activity. Intracellular acidic organelles were stained with acridine orange for V-ATPase activity as described previously [13–15]. DLD-1 cells were cultured on coverslips and then incubated at 37 °C for 1 h with test chemicals. The cells were further incubated for 30 min with 10 μM acridine orange. After three washes with PBS, the coverslips were examined with a fluorescence microscope.

Results

Effect of hygrolidins on cyclin-overexpressing cells and various tumor cells

In the course of our screening, we found an activity of selective cytotoxicity against cyclin E- and A-overexpressing cells in a cultured microbial broth. The active materials were purified and identified as hygrolidin family antibiotics including hygrolidin [2], oxohygrolidin [16], and L-155,175 [17] (Fig. 1A). As shown in Fig. 1B, hygrolidin at 0.01 μg/ml selectively killed both cyclin E- and A-overexpressing cells, but not Rat1-R12 parental cells. Oxohygrolidin and L-155,175 also showed the same effect as hygrolidin (data not shown).

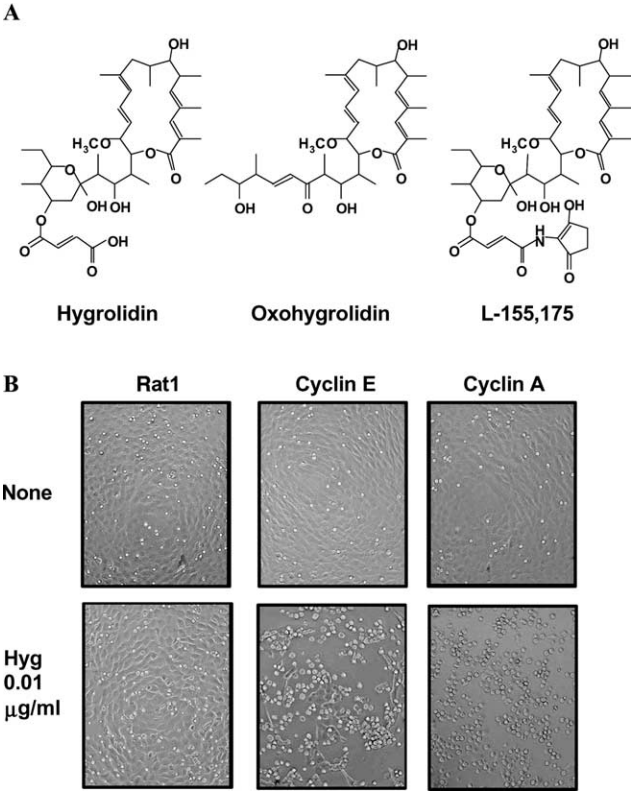

Fig. 1. (A) Structures of hygrolidin family antibiotics. (B) Effect of hygrolidin on cyclin-overexpressing cell lines. Cyclin E (Cyclin E)- or cyclin A (Cyclin A)-overexpressing cells or Rat1-R12 parental cells (Rat1) were cultured with 0.01 μg/ml hygrolidin for 2 days.

Growth inhibitory effects of hygrolidin family antibiotics against various cell lines are summarized in Table 1. Among the three compounds, hygrolidin was the most potent. Although hygrolidin inhibited growth of various tumor cell lines, at the lower concentrations it strongly inhibited solid tumor-derived cell lines such as DLD-1 colon cancer and DMS273 lung cancer rather than hematopoietic cancer cell lines such as K562 leukemia and EL-4 lymphoma. Furthermore, it only weakly affected growth of WI-38 normal fibroblasts even at a high dose of 10 μg/ml. Because hygrolidin significantly inhibited the growth of DLD-1 human colon cancer cells, we used DLD-1 cells for its mechanistic study.

Effect of hygrolidin on cell cycle progression

We first examined the effect of hygrolidin on cell cycle progression using DLD-1 cells. As a result, FACS

Table 1
Effect of hygrolidins on growth of various cell lines

Cell line	Hygrolidin	Oxohygrolidin	L155,175
DLD-1 colon cancer	0.0029	1.5	0.13
DMS273 lung cancer	0.0010	0.54	0.042
LNCaP prostate cancer	0.0052	0.50	0.11
Lewis lung carcinoma	0.0019	N.D.	N.D.
K562 leukemia	0.033	2.9	0.45
EL-4 lymphoma	0.015	3.1	0.35
WI-38 fibroblast	~10	2.9	~10

Cells were incubated with the indicated compounds for 3 days. Cell growth was determined using MTT. Values are means of duplicate determinations (IC_{50}, μg/ml). N.D., not determined.

analysis showed that hygrolidin increased both G1 and S phase populations and decreased M phase population (Fig. 2A). It is first expected that hygrolidin could induce apoptosis in DLD-1 cells. But cells in subG1 did not increase by hygrolidin treatment; moreover, degradation of DNA, one of the apoptotic markers, was not detected (data not shown). Thus, hygrolidin affected the cell cycle progression at G1 and S phases.

When we determined the levels of various cell cycle-related molecules, we found that hygrolidin decreased the amounts of cdk4, cyclin D, and cyclin B. In contrast, it increased the amounts of cyclin E and p21 (Fig. 2B). Other hygrolidin family antibiotics including oxohygrolidin and L-155,175 also decreased cdk4, cyclin D, and cyclin B levels and increased cyclin E and p21 levels (Fig. 2C). Since cdk2 is one of the targets for p21 [4–6], the cdk2 activity was also inhibited by hygrolidins in accordance with the increase in p21 levels (Fig. 2C).

Hygrolidin-induced p21 preferentially inhibits cyclin A–cdk2 complex

To explore the mechanism by which hygrolidin inhibits the cell cycle progression, we next examined what kind of cdk complex is inhibited by hygrolidin-induced p21. Cell lysates from hygrolidin-treated cells were immunoprecipitated by anti-cdk2, cyclin E, or cyclin A antibody and their kinase activities and co-immunoprecipitated p21 levels were determined. As a result, hygrolidin-induced p21 was found to associate with cyclin A–cdk2 complex and inhibit it more strongly than cyclin E–cdk2 complex (Fig. 3). Thus, hygrolidin-induced p21 preferentially associates with cyclin A–cdk2 complex and inhibits it.

Selective induction of p21 by hygrolidin

To ascertain whether hygrolidin induces p21 in other cell lines, we examined its effect on WI-38 normal

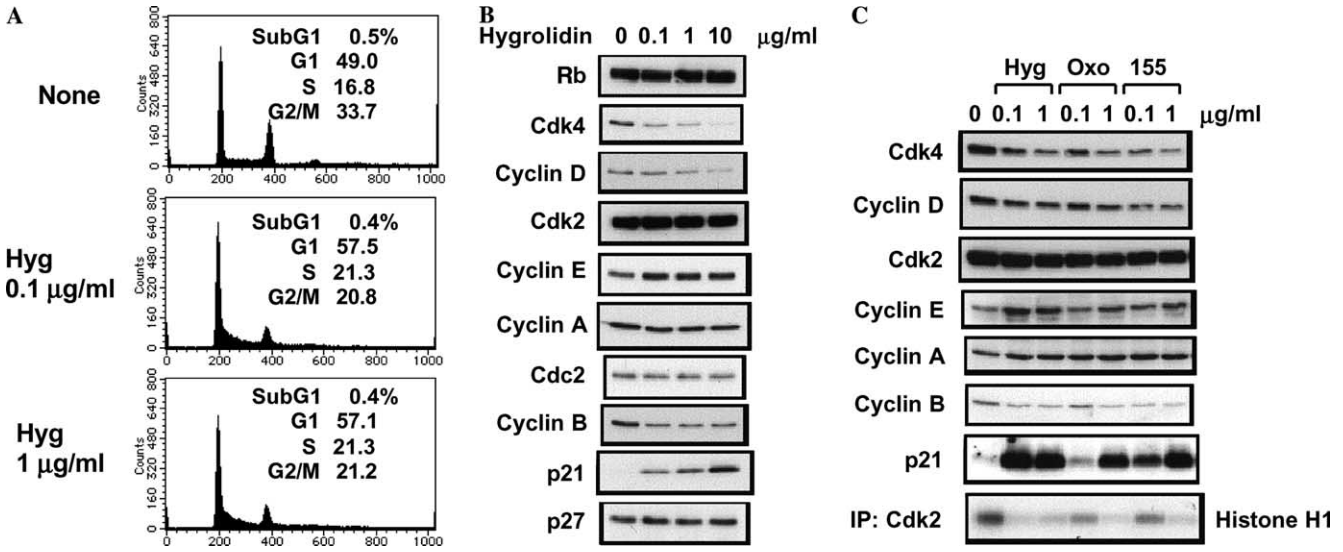

Fig. 2. Effect of hygrolidin on cell cycle progression. (A) DLD-1 cells were cultured with the indicated concentrations of hygrolidin for 24 h. The cell cycle distribution in subG1, G1, S, or G2/M phase was determined by FACS analysis. The *x*-axis shows DNA content; and *y*-axis shows the number of cells. (B and C) DLD-1 cells were cultured with the indicated concentrations of hygrolidin family antibiotics for 24 h. The protein extracts were applied to Western blot using the indicated antibodies. Cdk2 activity in (C) was assessed using immunoprecipitates with anti-cdk2 antibody and histone H1 as a substrate. Hyg, hygrolidin; Oxo, oxohygrolidin; 155, L-155,175.

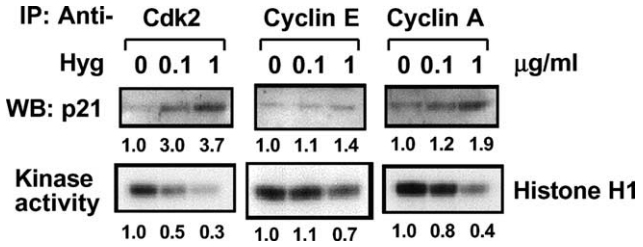

Fig. 3. Hygrolidin-induced p21 preferentially inhibited cyclin A–cdk2 complex. DLD-1 cells were cultured with hygrolidin for 24 h. The same amounts of protein extracts were immunoprecipitated with anti-cdk2, cyclin E, or cyclin A antibody and applied to Western blot using anti-p21 antibody or cdk2 kinase assay. Numbers are relative amount of p21 or kinase activity to that in each control (1.0).

fibroblasts and other tumor cell lines. Time course experiment revealed that hygrolidin decreased the amounts of cdk4, cyclin D, and cyclin B, and concomitantly increased cyclin E and p21 levels after 6-h treatment in DLD-1 cells (Fig. 4A). Although expressions of cyclin E, A, and B in control WI-38 fibroblasts were very low compared to DLD-1 cells and under detectable levels, hygrolidin apparently affected cyclin D level in WI-38 fibroblasts and but it did not increase p21 level. We then examined the effect of hygrolidin on p21 expression at mRNA level. RT-PCR experiment showed that hygrolidin increased PCR product of p21 in DLD-1 cells, but not in WI-38 fibroblasts (Fig. 4B). It increased p21 expression about 3 times as compared with control (Fig. 4C). Thus, hygrolidin selectively induced p21 in DLD-1 cells at mRNA level. To ascertain whether the p21 induction is common mechanism of hygrolidin, we examined the p21 levels in other tumor cell lines. As a result, hygrolidin also increased p21 levels in Lewis lung carcinoma cells and slightly in DMS273 cells, but it increased the amount of p27, another cdk inhibitor [18,19], in LNCaP and DMS273 cell lines (Fig. 4D).

V-ATPase inhibitors induce p21

Bafilomycin A1, a structurally related compound of hygrolidin, is a potent inhibitor of vacuolar-type (H+)-ATPase (V-ATPase) [20]. As shown in Fig. 5A, bafilomycin A1 also increased p21 levels in DLD-1 cells. V-ATPase maintains low pH of intracellular acidic organelles and the activity is detected as orange fluorescent pigments by staining with acridine orange [14,15]. V-ATPase activity was detected in DLD-1 cells and hygrolidin was found to inhibit the V-ATPase completely as

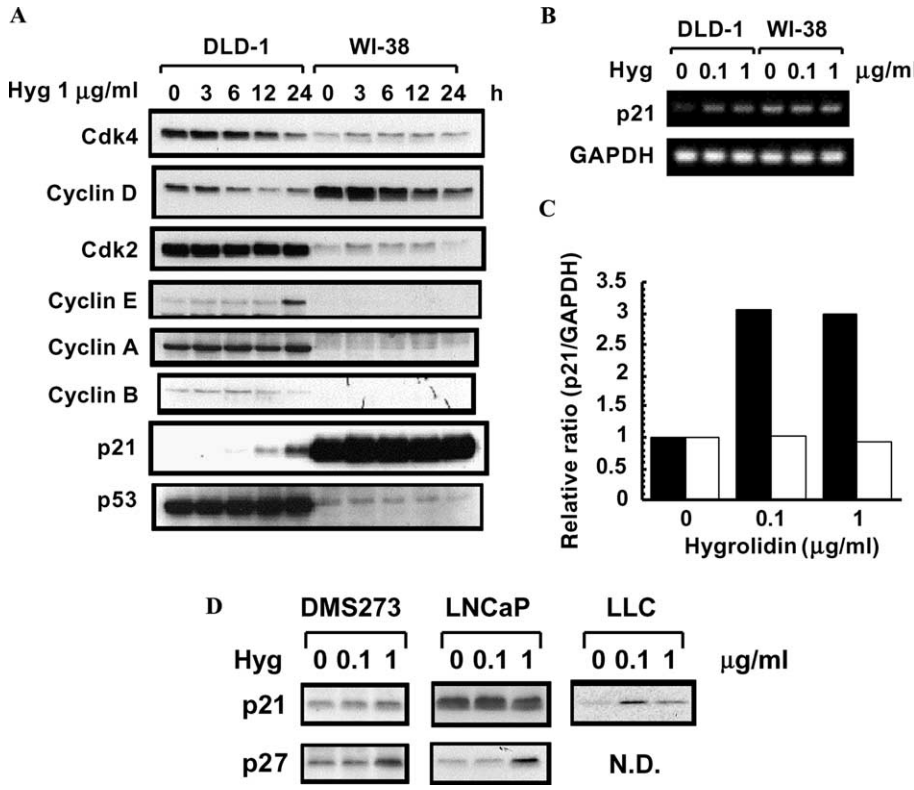

Fig. 4. Hygrolidin selectively induced p21 expression in DLD-1 cells. (A) DLD-1 or WI-38 cells were cultured with 1 μg/ml hygrolidin for the indicated times. The protein extracts were applied onto Western blot using the indicated antibodies. (B) DLD-1 or WI-38 cells were cultured with hygrolidin for 24 h. p21 expression was assessed by RT-PCR. (C) Relative amount of p21 to GAPDH. Data in (B) were evaluated by an image analyzer and expressed as relative ratios to control (1.0), which were dividing each value of p21 by respective value of GAPDH. (D) Cells were cultured with hygrolidin for 24 h. p21 and p27 levels were determined by Western blot. LLC, Lewis lung carcinoma; N.D., not determined.

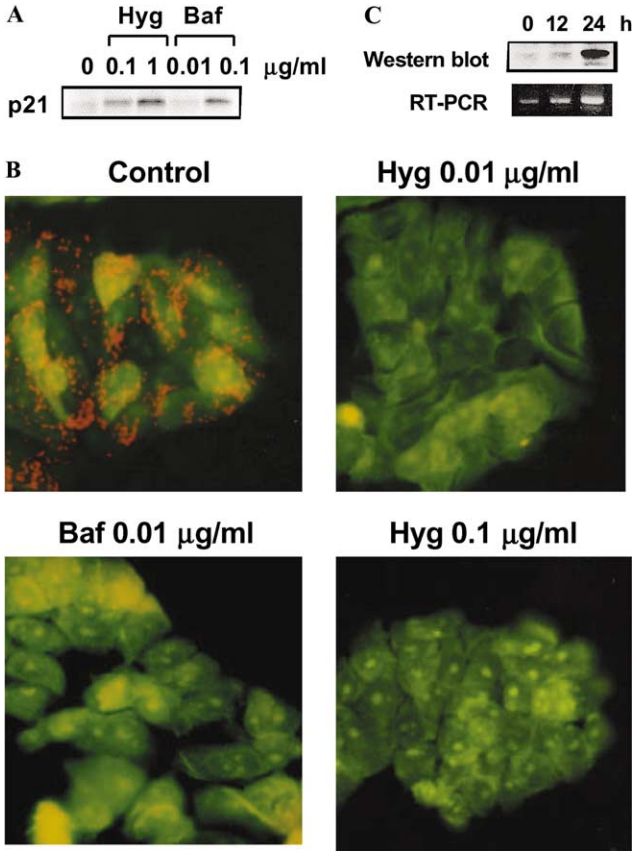

Fig. 5. V-ATPase inhibitors induced p21 expression. (A) DLD-1 cells were cultured with hygrolidin (Hyg) or bafilomycin A1 (Baf) for 24 h. The protein extracts were applied onto Western blot using an anti-p21 antibody. (B) DLD-1 cells were plated on cover slips and the cells were treated with Hyg or Baf and stained with acridine orange. (C) HR-3Y1 cells were cultured with 0.1 μg/ml oximidine I for the indicated times. Expression of p21 was determined by Western blot or RT-PCR.

well as bafilomycin A1 (Fig. 5B). To determine whether V-ATPase inhibitors can induce p21, we employed another V-ATPase inhibitor, oximidine I [21,22], which is not structurally related to hygrolidin. Oximidine I selectively inhibited the growth of rat 3Y1 cells transformed with ras or src oncogene [21] and arrested the cell cycle at G1 phase (data not shown). Oximidine I was found to increase p21 at protein and mRNA levels in ras-transformed cells (Fig. 5C). Thus, it is suggested that the inhibition of V-ATPase could induce p21 expression.

Discussion

We first hypothesized that an inhibitor of cyclin E function would be a selective apoptosis inducer of solid tumor cells. Whereas hygrolidins killed cyclin E-overexpressing cells as well as cyclin A-overexpressing cells in our screening (Fig. 1), hygrolidins showed rather selective cytotoxicity against solid tumor-derived cell lines (Table 1). Furthermore, hygrolidin only weakly affected the growth of normal fibroblasts. Because adriamycin and mitomycin C only weakly inhibited DLD-1 cells (data not shown), the effect of hygrolidin was not considered to be merely drug sensitivity of DLD-1 cells. It is expected that hygrolidin would efficiently induce apoptosis in solid tumor cells, but FACS analysis showed that hygrolidin inhibited cell cycle progression both at G1 and S phases (Fig. 2). Western blotting of cell cycle-related molecules revealed that hygrolidin decreased amounts of cyclin D and B and increased amounts of cyclin E, correlating with the result of FACS analysis (Fig. 2). In respect to a mechanism for cell cycle inhibition, we have found that hygrolidins increased p21 levels (Fig. 2). p21 is a cdk inhibitor and inhibits cyclin–cdk complex kinases through binding to them [4–6]. Expectedly, cdk2 activity was inhibited by hygrolidin treatment (Fig. 2). Compared these activities of three hygrolidins, the degree of cdk2 activity inversely correlated with the increase in p21 as well as growth inhibition by each compound (Table 1 and Fig. 2). Therefore, it is considered that hygrolidins inhibit tumor cell growth through the induction of p21. However, hygrolidin increased the amount of p27, another cdk inhibitor [18,19], instead of p21 in some tumor cell lines (Fig. 4D). Thus, there exists another mechanism of hygrolidin action on p27 expression and which cdk inhibitor is induced by hygrolidin depends on used cell lines.

Immunoprecipitation experiments showed that hygrolidin-induced p21 bound to and inhibited cyclin A–cdk2 complex more strongly than cyclin E–cdk2 complex (Fig. 3). Unlike DLD-1 cells, hygrolidin significantly increased S phase population without increase in G1 phase and increased amounts of cyclin A and p21 in Lewis lung carcinoma cells (data not shown and Fig. 4D). Although the precise mechanism of differential distribution of the induced p21 in each cyclin–cdk complex is still unknown, hygrolidin-induced p21 tends to associate with cyclin A–cdk2 complex.

Induction of p21 is known to be critical for either tumor suppressor p53-mediated G1 arrest or apoptosis. However, it is reported that overexpression of p21 itself only induces a cell-cycle arrest at G1 phase in p53-null or mutant tumor cells, and that p21 expression alone is not sufficient to induce the apoptosis [23]. Because DLD-1 cells has mutant p53 gene [24,25], it is, therefore, explained that hygrolidin-induced p21 inhibited cell cycle progression without commitment of apoptosis.

Hygrolidin increased p21 at mRNA level in DLD-1 cells, but not in WI-38 normal fibroblasts (Fig. 4). Bafilomycin A1, a structurally related compound of hygrolidin, is reported to inhibit growth of tumor cells in vitro and in vivo [26] and induced apoptosis through increase in p53 and p21 levels [27,28]. Bafilomycin A1 also increased amount of p21 in DLD-1 cells (Fig. 5). However, hygrolidin did not increase mutant p53 level in DLD-1 cells or wild-type p53 in WI-38 fibroblasts

(Fig. 4). It is reported that FR901228, a histone deacetylase inhibitor, induces p21 in a p53-independent manner through activation of its transcription [29]. We tested whether hygrolidin directly activates transcription of p21 using p21 promoter assays based on Saos-2 p53-null cells [30], but hygrolidin failed to increase the promoter activity (data not shown). Bafilomycin A1 is a potent inhibitor of vacuolar-type (H+)-ATPase (V-ATPase) [20]. The V-ATPase is an ATP-dependent proton pump and plays an important role in the acidification of intracellular acidic compartments and in the extrusion of certain protons from the cells [31]. Oximidine I [21], which is another inhibitor of V-ATPase [22], also increased p21 level in ras-transformed p53 mutant cells (Fig. 5), but not in Saos-2 p53-null cells (data not shown). Because hygrolidin inhibited V-ATPase in DLD-1 cells (Fig. 5B), it is suggested that V-ATPase inhibitors could increase p21 only in p53 mutant cell lines. In fact, hygrolidin failed to increase p21 level in LNCaP cells with wild-type p53 (Fig. 4D). Glycerol is reported to restore mutant p53 to normal p53 function through acting as a chemical chaperone [32,33]. Furthermore, the recent paper showed that PRIMA-1, a low-molecular-weight compound, can reactivate mutant p53 [34]. Although the precise mechanism by which inhibition of V-ATPase triggers induction of p21 is still unknown, V-ATPase inhibitors including hygrolidin possibly increase p21 expression through normalizing or activating mutant p53 function selectively.

Acknowledgments

We thank Dr. Y. Yoshimoto for helpful discussions and K. Miyaji for secretarial assistance. This work was supported by a grant-in-aid from the Japanese Ministry of Science, Education and Art.

References

[1] M. Kawada, M. Amemiya, M. Ishizuka, T. Takeuchi, Jpn. J. Cancer Res. 90 (1999) 219–225.
[2] H. Seto, H. Akao, K. Furihata, N. Otake, Tetrahedron Lett. 23 (1982) 2667–2670.
[3] K.S. Tsuchiya, Y. Moriya, H. Kawai, M. Hori, Y. Uehara, H. Iinuma, H. Naganawa, T. Takeuchi, J. Antibiot. 44 (1991) 344–348.
[4] W.S. El-Deiry, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, B. Vogelstein, Cell 75 (1993) 817–825.
[5] J.W. Harper, G.R. Adami, N. Wei, K. Keyomarsi, S.J. Elledge, Cell 75 (1993) 805–816.
[6] Y. Xiong, G.J. Hannon, H. Zhang, D. Casso, R. Kobayashi, D. Beach, Nature 366 (1993) 701–704.
[7] D. Resnitzky, M. Gossen, H. Bujard, S.I. Reed, Mol. Cell. Biol. 14 (1994) 1669–1679.
[8] D. Resnitzky, L. Hengst, S.I. Reed, Mol. Cell. Biol. 15 (1995) 4347–4352.
[9] H. Shimura, T. Mitsudomi, A. Matsuzaki, M. Kabemura, A. Okuda, G. Kimura, Cell Struct. Funct. 15 (1990) 211–219.
[10] H. Fukazawa, S. Mizuno, Y. Uehara, Anal. Biochem. 228 (1995) 83–90.
[11] M. Kawada, A. Kuwahara, T. Nishikiori, S. Mizuno, Y. Uehara, Exp. Cell Res. 249 (1999) 240–247.
[12] D. Yang, O.M. Howard, Q. Chen, J.J. Oppenheim, J. Immunol. 163 (1999) 1734–1741.
[13] M.J. Geisow, G.H. Beaven, P.D. Hart, M.R. Young, Exp. Cell Res. 126 (1980) 159–165.
[14] T. Yoshimori, A. Yamamoto, Y. Moriyama, M. Futai, Y. Tashiro, J. Biol. Chem. 266 (1991) 17707–17712.
[15] Y. Yoshimoto, M. Imoto, Exp. Cell Res., in press, 2002.
[16] A. Kretschmer, M. Dorgerloh, M. Deeg, H. Hagenmaier, Agric. Biol. Chem. 49 (1985) 2509–2511.
[17] M.A. Goetz, P.A. McCormick, R.L. Monaghan, D.A. Ostlind, O.D. Hensens, J.M. Liesch, G.A. Schonberg, J. Antibiot. 38 (1985) 161–168.
[18] K. Polyak, M. Lee, H. Erdjument-Bromage, A. Koff, J.M. Roberts, P. Tempst, J. Massague, Cell 78 (1994) 59–66.
[19] H. Toyoshima, T. Hunter, Cell 78 (1994) 67–74.
[20] B.P. Crider, X.S. Xie, D.K. Stone, J. Biol. Chem. 269 (1994) 17379–17381.
[21] J.W. Kim, K. Shin-ya, K. Furihata, Y. Hayakawa, H. Seto, J. Org. Chem. 64 (1999) 153–155.
[22] M.R. Boyd, C. Farina, P. Belfiore, S. Gagliardi, J.W. Kim, Y. Hayakawa, J.A. Beutler, T.C. Mckee, B.J. Bowman, E.J. Bowman, J. Pharm. Exp. Ther. 297 (2001) 114–120.
[23] S. Kagawa, T. Fujiwara, A. Hizuta, T. Yasuda, W. Zhang, J. Roth, N. Tanaka, Oncogene 15 (1997) 1903–1909.
[24] S. Shirasawa, M. Furuse, N. Yokoyama, T. Sasazuki, Science 260 (1993) 85–88.
[25] T. Waldman, C. Lengauer, K.W. Kinzler, B. Vogelstein, Nature 381 (1996) 713–716.
[26] T. Ohta, H. Arakawa, F. Futagami, S. Fushida, H. Kitagawa, M. Kayahara, T. Nagakawa, K. Miwa, K. Kurashima, M. Numata, Y. Kitamura, T. Terada, S. Ohkuma, J. Pathol. 185 (1998) 324–330.
[27] T. Nishihara, S. Akifusa, T. Koseki, S. Kato, M. Muro, N. Hanada, Biochem. Biophys. Res. Commun. 212 (1995) 255–262.
[28] X. Long, M.T. Crow, S.J. Sollott, L. O'Neill, D.S. Menees, M.L. Hipolito, M.O. Boluyt, T. Asai, E.G. Lakatta, J. Clin. Invest. 101 (1998) 1453–1461.
[29] V. Sandor, A. Senderowicz, S. Mertins, D. Sackett, E. Sausville, M.V. Blagosklonny, S.E. Bates, Br. J. Cancer 83 (2000) 817–825.
[30] K. Egawa, H. Nishigori, S. Kunimoto, T. Takeuchi, K. Nose, Biol. Pharm. Bull. 21 (1998) 899–904.
[31] M.E. Finbow, M.A. Harrison, Biochem. J. 324 (1997) 697–712.
[32] T. Ohnishi, K. Ohnishi, X. Wang, A. Takahashi, K. Okaichi, Radiat. Res. 151 (1999) 498–500.
[33] T. Ohnishi, H. Matsumoto, X. Wang, A. Takahashi, T. Tamamoto, K. Ohnishi, Int. J. Radiat. Biol. 75 (1999) 1095–1098.
[34] V.J.N. Bykov, N. Issaeva, A. Shilov, M. Hultcrantz, E. Pugacheva, P. Chumakov, J. Bergman, K.G. Wiman, G. Selivanova, Nature Med. 8 (2002) 282–288.