The Cyclic Pentapeptide d-Arg3FC131, a CXCR4 Antagonist, Induces Apoptosis of Somatotrope Tumor and Inhibits Tumor Growth in Nude Mice

Jeong Mo Kim, Yong-ho Lee, Cheol Ryong Ku, and Eun Jig Lee

Endocrinology (J.M.K., Y.-h.L., C.R.K., E.J.L.), Severance Hospital Integrative Research Institute for Cerebral & Cardiovascular Diseases and Department of Internal Medicine, Brain Korea 21 Project for Medical Science (J.M.K., E.J.L.), Yonsei University College of Medicine, Seoul 120-752, Korea; and Endocrinology (E.J.L.), Northwestern University, Feinberg School of Medicine, Chicago, Illinois 60611

The interaction between the chemokine stromal cell-derived factor 1 and its receptor CXCR4 plays an important role in GH production and cell proliferation in normal and tumorous pituitary somatotrope cells. Therefore, the chemokine receptor CXCR4 could be an attractive target for anti-tumor drugs in patients with acromegaly. A synthetic antagonist of CXCR4, cyclic pentapeptide d-Arg3FC131 ([Gly1-d-Tyr2-d-Arg3-Arg4-Nal5]) significantly inhibited GH production and proliferation of GH3 somatotrope tumor cells in vitro. It also induced apoptosis of GH3 cells through activation of the caspase-3 pathway. Systemic administration of d-Arg3FC131 inhibited the growth of GH3 cell xenografts in immunodeficient nude mice by inducing apoptosis and suppressing the proliferation of tumor cells. These results indicate that d-Arg3FC131 might have potential for the treatment of pituitary tumors producing excess GH in patients with acromegaly. (Endocrinology 152: 536–544, 2011)

Although pituitary tumors associated with acromegaly are rarely malignant, elevated levels of GH and IGF-I cause various cardiovascular, respiratory, endocrine, and metabolic morbidities (1, 2). Surgery is the only way to remove all tumor tissues and completely cure the acromegalic patient; however, second-line therapies are usually necessary to postoperatively manage patients with bulky tumors or to deal with recurrences (3). Three drug classes are currently available for the treatment of acromegaly: dopamine agonists, somatostatin receptor ligands (SRLs), and a GH receptor antagonist. Therapy using SRLs alone induced 60–70% biochemical control of serum IGF-I (4). Combined treatment of SRLs with GH receptor antagonist achieved 95% IGF-I normalization although high cost is an issue (3, 5, 6). If a new pharmacological agent targeting different signaling pathways in somatotrope tumor is developed, it would provide a multimodal approach in conjunction with current medical agents for the medical treatment of GH-producing tumors.

The chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 are reported to be involved in trafficking of hematopoietic precursor cells, infection with HIV, migration and metastasis of cancer cells, and the development of rheumatoid arthritis (7–11). SDF-1 has been demonstrated to be expressed in many organs in mice, including the brain, heart, lung, thymus, liver, kidney, spleen, stomach, intestine, and bone marrow. CXCR4 is widely expressed like SDF-1, particularly in neuronal tissue (12). SDF-1 was originally cloned from a murine bone marrow stromal cell line (13), and its amino acid sequences are highly conserved during the evolution, implying that this substance might play an important biological role. SDF-1/CXCR4 interaction plays an essential role in organ-specific growth, survival, and metastasis of several malignancies, including those of the breast, kidney, prostate, lung and pancreas, melanomas, neuroblastomas, non-Hodgkin’s lymphoma, multiple myelomas, ovarian cancers, and malignant brain tumors (14–18). Several cel-
lular pathways, including increased cytosolic Ca\(^{2+}\), activated Pyk2, ERK1/2, and large-conductance Ca\(^{2+}\)-activated K\(^+\) channels have been reported to involve SDF-1/CXCR4-associated carcinogenesis (19). The expression of SDF-1 and CXCR4 in neuronal, astroglial, microglial, and endothelial cells of the central nervous system and anterior pituitary suggests a possible link between this chemokine and neuroendocrine regulation (12, 20, 21). We have identified the expression of SDF-1 and CXCR4 in normal and tumorous rat pituitary cells (22) and the human pituitary tumors (23). SDF-1/CXCR4 interaction stimulated GH3 cell proliferation and GH secretion through ERK1/2 phosphorylation (22), which is consistent with other studies (19, 24).

Until now, CXCR4 blockade for oncology has moved from preclinical research into clinical in some malignancies. However, only a few studies concerning the effect of CXCR4 antagonist on pituitary tumor have been reported. There are many species of CXCR4 antagonist, including AMD3100, T22, and T140. Each agent has a different pathway for blocking the CXCR4 with different pharmacokinetics and pharmacodynamics. In T140 [Arg1-Arg2-Nal3-c(Cys4-Tyr5-Arg6-Lys7-d-Lys8-Pro9-Tyr10-Arg11-Cit12-Cys13)-Arg14], which is one of the strong CXCR4 antagonists, residues such as Arg2, Nal3, Tyr5, and Arg14 are the most important for retaining antagonistic activity against CXCR4 (25). Because T140 is not stable in mouse serum or in rat liver, indispensable residues (Arg14 in serum; Arg2, Nal3, and Arg14 in liver homogenates) are inevitably deleted, leading to a dramatic decrease in the efficacy of degraded peptides (26, 27). To overcome structural instability of T140, the molecular size was minimized and cyclic pentapeptide libraries were designed. As a result, FC131 [cyclo(-Arg1-Arg2-Nal3-Gly4-DTyr5-)] was obtained and showed strong CXCR4-antagonistic activity similar to that of T140 (25, 28). Here, we hypothesized that the CXCR4 antagonist, cyclic peptidetide FC131 [c(Gly1-d-Tyr2-Arg3-Arg4-Nal5)] and its isomeric peptides would effectively inhibit proliferation in pituitary adenoma cell lines and suppress the secretion of GH.

**Materials and Methods**

**Cell culture and treatment**

GH3 cells, a rat pituitary tumor cell line from the American Type Culture Collection (Manassas, VA), were cultured in 5% CO\(_2\) at 37 C in DMEM (Life Technologies, Inc., Gaithersburg, MD), supplemented with 10% fetal bovine serum (GIBCO-BRL), 100 U/ml of penicillin and 100 mg/ml of streptomycin (Invitrogen, Carlsbad, CA). These cells are an established model for studying GH gene expression and its molecular mechanisms (22, 29, 30). Cells were treated with 18 nM SDF-1 (CXCL12; R&D Systems, Minneapolis, MN) (22), 100 nM of somatostatin-14 (SST, Sigma-Aldrich, St. Louis, MO), or 100 nM of FC131 analogs including FC131, cyclo[Gly1-d-Tyr2-Arg3-Arg4-Nal5]; d-Arg3FC131, cyclo[Gly1-d-Tyr2-d-Arg3-Arg4-Nal5]; and d-Arg3-d-Nal5FC131, cyclo[Gly1-d-Tyr2-d-Arg3-Arg4-d- Nal5] (Anygen, Daejeon, Korea; Fig. 1). To determine the concentration of FC131 analogs in cell culture study, GH3 cells were treated with 1, 10, and 100 nM FC131 analogs. The highest suppressive effect on tumor growth was observed at a concentration of 100 nM. Therefore, further studies were conducted using 100 nM FC131 analogs.

**Generation of recombinant adenoviral vectors**

A cassette containing a luciferase gene driven by the human GH promoter (610, +58; cloned from human genomic DNA;
performed at 1 h after treatment with antagonists (100 nM).

Administration of CXCR4 antagonists and SST. Real-time RT-PCR was
treated group. C, GH mRNA expression in GH3 cells after
averaged and plotted as the mean
control. Results of three independent experiments (B and C) were
0.001 compared with control. **,
normalization of the GH mRNA level. ***,
Glyceraldehyde-3-dehydrogenase served as a reference gene used for
reagent) were evaluated using a luciferase reporter system. *,
been performed serially for 3 d (100 nM for d-ArgFC131 and SST).

Treatment with d-ArgFC131 and SST decreased the concentration of
GH in GH3 cells cultured media. Western blot analysis for media had

Effect of CXCR4 antagonists on inhibition of GH gene

FIG. 2. A CXCR4 Antagonist Inhibits Somatotrope Tumor Growth

Quantitative real-time RT-PCR

The amplification primers for the GH gene were designed as follows: sense, 5'-gtcgcgcgtctgtcatcag-3'; and antisense, 5'-cggaggtaccaaatcagcagg-3'. The cDNAs containing SYBR Green I fluorescent dye and 1 × PCR Master Mix (Takara, Shuzo, Kyoto, Japan) were amplified using an ABI 7300 sequence detection system (PerkinElmer Applied Biosystems, Foster City, CA). The PCR cycling conditions were as follows: 95 C for 5 min, 40 cycles of 95 C for 15 sec, annealing at 56 C for 15 sec, and extension at 72 C for 1 min. Relative expression levels were calculated based on the cycle threshold method (22). Target gene expression levels were normalized to endogenous controls in all experiments, and data were plotted relative to the control (hormone free) mRNA.

Assessment of cell proliferation

GH3 cells (5 × 10^5 cells per well) were seeded on 96-well plates in serum-free DMEM, and on the next day, different antagonists were treated twice per day. On d 6, cell proliferation was assessed using a nonradioactive cell proliferation assay (Cell Titer 96 Aqueous Non-Radioactive Cell Proliferation Assay; Promega). Optical density was determined at 490 nm, and relative data are presented as percentages (mean ± sd) (34).

Terminal deoxynucleotidyl transferase-mediated
dUTP nick end labeling (TUNEL)

Cell apoptosis was evaluated using TUNEL assay kits (Roche Molecular Biochemicals, Indianapolis, IN) according to the manufacturer’s protocol. Briefly, GH3 cells were seeded onto coverslips and incubated in the presence or absence of CXCR4 antagonists (100 nm) for 16 h at 37 C. The cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100 in 0.1% sodium acetate for 2 min on ice, exposed to the DNA-labeling solution for 60 min at 37 C, and observed using an Olympus BX51 microscope (Olympus, Tokyo, Japan) with an Olympus DP71 camera (Olympus) using DP Controller Software (Olympus).

Flow cytometry analysis

We analyzed the effect of CXCR4 antagonists on the cell cycle and apoptosis in GH3 cells using fluorescence-activated cell sorting. After treating them with CXCR4 antagonists, GH3 cells (1 × 10^6 cells/ml) were harvested and resuspended with 70% ethanol at −20 C for 2 h. The cells were washed in PBS and repeatedly resuspended in 0.5 ml propidium iodide (PI, Sigma-Aldrich) solution containing 50 mg/ml ribonuclease A, 0.1 mM
EDTA, and 0.1% Triton X-100 at room temperature for 30 min in the dark. Fluorescence was detected using the channel for PI.

Western blot analysis

Antagonist-treated GH3 cells were lysed in radioimmune precipitation assay buffer (20 mM Tris, pH 7.5; 2 mM EDTA; 150 mM NaCl; 0.5% Triton X-100; 5% phosphatase inhibitors; 0.5% protease inhibitors). Furthermore, GH3 cell cultured media was collected, concentrated, and dialyzed. Equal amounts adjusted using a Bradford protein assay kit (Bio-Rad Laboratories, Inc., Richmond, CA) were separated on 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Blots were probed by using primary antibodies against phosphorylated ERK (1:1000; Cell Signaling Technology, Beverly, MA), phosphoprotein kinase B/Akt (1:1000, Cell Signaling), Bcl-2 (1: 1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), Bcl-6 (1:1000; Santa Cruz Biotechnology, Inc.), caspase-3 (1:1000; Cell Signaling), or β-actin (1:1000; Santa Cruz Biotechnology, Inc.) as an internal control. Protein bands were visualized using a second antibody conjugated with horseradish peroxidase and an enhanced chemiluminescence kit (Cell Signaling).

Animal experiments

Animal experiments were performed using 6-wk-old male athymic immunodeficient nude mice (Charles River Japan, Inc., Yokohama, Japan). Each group had five animals and was housed in an animal room controlled at 23 ± 2°C and 55 ± 5% room humidity, under a 12-h light, 12-h dark cycle and fed a standard unrestricted diet. GH-secreting tumors were collected, washed twice with PBS, resuspended in medium, and injected (2 x 10^6 cells) into the flanks of mice at d 0. Then, ip injection of either a CXCR4 antagonist (1.23 mg/kg body weight), octreotide [Sandostatin (Norvatis, Stein, Switzerland) 1.23 mg/kg body weight] or PBS was performed twice per day (35). The size of tumor mass (in mm^3) was measured with calipers in three dimensions (34) and calculated using the following formula: (3.14 x length x width x depth)/6. After 30 d of treatment, the mice were euthanized and tumor masses were removed. All animal studies were approved by the Animal Care and Use Committee of the Yonsei University College of Medicine. The collected tumors were fixed with 10% neutral buffered formalin and processed into paraffin blocks. Sections (4 µm) were subjected to hematoxylin and eosin (H&E), TUNEL, and Ki-67 staining. After incubation with fluorescent TUNEL reagent and a primary antibody for Ki-67, specific biotinylated secondary antibodies (1:100; Vector Laboratories, Burlingame, CA) and streptavidin-peroxidase (DAKO, Kyoto, Japan) were incubated sequentially. Diaminobenzidine (Vector Laboratories) was used as a chromogen, and counterstaining was performed using hematoxylin.

Statistical analysis

All statistical analyses of differences were performed using Student’s t test for comparison between two groups or for multiple comparisons (more than two groups) and compared using the linear-by-linear association test. P < 0.05 was considered significant. Statistical analyses were performed using SPSS for Windows software (version 15.0; SPSS Inc., Chicago, IL).

Results

CXCR4 antagonists inhibit the production and secretion of GH in GH3 pituitary tumor cells

In this study, we tested whether CXCR4 antagonists were involved in GH release and production in response to various peptides (SST, CXCR4 antagonists FC131, cyclo[Gly1-d-Tyr2-Arg3-Arg4-Nal5], d-Arg3FC131, cyclo[Gly1-d-Tyr2-d-Arg3-Arg4-Nal5], d-Arg3-d-Nal5FC131 cyclo[Gly1-d-Tyr2-d-Arg3-Arg4-d-Nal5]). We used GH3 cells transduced with adenovirus carrying GH promoter-driven luciferase gene (Fig. 2A). Luciferase assays revealed that d-Arg3FC131 (100 nm) and d-Arg3-d-Nal5FC131 (100 nm) significantly decreased the activity of GH promoter (0.02-fold) compared with the control (arbitrary value of 1), implying that the CXCR4 antagonists d-Arg3FC131 and d-Arg3-d-Nal5FC131 block GH production at the transcription level. Luciferase activities declined by 0.3-fold with antagonist FC131 and 0.4-fold with SST, respectively, compared with the control group (Fig. 2B).

We next examined the inhibitory effect of CXCR4 antagonists on GH production by quantitative real-time RT-PCR (RT-PCR) and Western blotting. Application of 100 nm d-Arg3FC131 decreased the GH transcription levels by 0.6 ± 0.1-fold and d-Arg3-d-Nal5FC131 by 0.67 ± 0.2-fold (Fig. 2C). Western blotting also showed that the protein levels of GH in GH3 cells were decreased in d-Arg3FC131-treated GH3 cells in a time-dependent manner (Fig. 2D). Quantitation of the results from Western blotting (Supplemental Fig. 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org) showed that d-Arg3FC131 (100 nm) significantly suppressed GH expression levels after 18 h. GH levels in GH3 cell culture media increased as time went by, whereas treatment with d-Arg3FC131 or SST inhibited GH secretion from GH3 (Fig. 2E). These results clearly demonstrate that d-Arg-FC131 inhibits GH production in GH3 pituitary tumor cells.

CXCR4 antagonists inhibit the proliferation of GH3 pituitary adenoma cells

To determine whether CXCR4 antagonists inhibited the proliferation of pituitary tumor cells, GH3 cells were treated with different doses (1–100 nm) of CXCR4 antagonists and SST (100 nm). Growth inhibition was observed after 6 d of treatment. As shown in Fig. 3A, d-Arg3-d-Nal5FC131 blocked the growth of GH3 cells in a dose-dependent manner (67.1 ± 13.2% at 10 nm, 59.3 ± 10.6% in 100 nm; P < 0.05 compared with control for both). The inhibitory effect of d-Arg3FC131 on GH3 cell proliferation was the most potent (100 nm, 30% ± 3.8; P < 0.01 compared with control), much stronger than that
of SST (100 nM, 65.3% ± 11; P < 0.05 compared with control) (Fig. 3A). Therefore, d-Arg3FC131 was used for the next experiments.

Several reports indicated that SDF-1/CXCR4 interaction activates cell proliferation and survival by stimulating the ERK1/2 and phosphatidylinositol 3-kinase/Akt pathways (35). Therefore, we examined whether the inhibitory effect of CXCR4 antagonists on cell proliferation was related to the phosphorylation of ERK1/2 and Akt. Western blotting clearly demonstrated that SDF-1 stimulated the activation of ERK1/2 at 5 and 10 min after treatment and of Akt at 10, 30, and 60 min after treatment (Fig. 3B). However, the antagonist d-Arg3FC131 (100 nM) inhibited these activations.

d-Arg3FC131 causes apoptosis

To elucidate the mechanism of CXCR4 antagonist-induced inhibition of cell proliferation, we quantified the cell population in different parts of the cell cycle and those undergoing apoptosis using flow cytometry. As shown in Fig. 4A, cell cycle analysis revealed that treatment with d-Arg3FC131 (100 nM) increased the percentage of cells in the G0–G1 phase, indicating partial G1 cell cycle arrest. This arrest lasted for at least 72 h. Our study also showed that d-Arg3FC131 induced apoptosis in GH3 cells in a time-dependent manner (Fig. 4B). The proportion of apoptotic cells (8 ± 3.4%; P < 0.05) was significantly increased after treatment with 100 nM d-Arg3FC131 compared with that of vehicle-treated GH3 cells (1.35 ± 0.8%) at 72 h.

d-Arg3FC131-induced apoptosis was assessed using TUNEL analysis (Fig. 4B). The number of apoptotic nuclei

FIG. 3. Effect of CXCR4 antagonists on GH3 cell proliferation. A, Proliferation of GH3 cells was assessed by nonradioactive cell proliferation assay. Cell numbers of GH3 cells are represented as a percentage of those in the nontreated control group. Among CXCR4 antagonists, d-Arg3FC131 (10, 100 nM) and d-Arg3-d-Nal5FC131 (10, 100 nM) inhibited cell proliferation by approximately 30–60%. Results of three independent experiments were averaged and plotted as the mean ± so for triplicate wells. *, P < 0.001 compared with control. ***, P < 0.001 compared with SST-treated group (100 nM). B, Inhibition of ERK1/2 and Akt phosphorylation by the CXCR4 antagonist, d-Arg3FC131, in GH3 cells. Western blot analysis was performed to examine the phosphorylation of ERK1/2 and Akt using specific antibodies after treatment of SDF-1 or SDF-1 with d-Arg3FC131.

FIG. 4. Effects of d-Arg3FC131 on cell cycle progression and apoptosis in GH3 cells. A, GH3 cells were exposed to d-Arg3FC131 (100 nM) for 72 h, and cell cycle distributions were analyzed by flow cytometry as described in Materials and Methods. The proportions of GH3 cells in specific cell cycle phases are shown. P values compared with control group at 0 h were calculated using linear-by-linear association. B, Induction of positive TUNEL reactions in GH3 cells after 72 h of treatment with d-Arg3FC131 (100 nM). Green fluorescent staining indicates TUNEL, and red fluorescence with PI shows the nuclei of GH3 cells counted to examine cell density. C, Expression of cleaved caspase-3 and BCL-6 in GH3 cells. Cells were treated with d-Arg3FC131 (100 nM) for 72 h, and Western blot analysis was performed to examine apoptotic pathways with specific antibodies; the gene for β-actin was used as a control. FITC, Fluorescein isothiocyanate.
markedly increased after treatment with 100 nM d-Arg3FC131 for 3 d. Treatment with d-Arg3FC131 induced approximately a 3-fold increase in apoptotic cells vs. 11.24 ± 4.8% in vehicle-treated cells and 33.4 ± 7.1% in antagonist-treated cells (P < 0.05). Western blot analyses of apoptosis-related genes were then performed to confirm this. Expression levels of BCL-6, BCL-2, BCL-XL, Bad, Bax, caspase-3, and cleaved caspase-3 were examined and compared with GH3 cells treated with or without d-Arg3FC131 (100 nM) for 72 h (Fig. 4C). The amount of the cleaved form of caspase-3 increased from 0% (control) to 0.8% at 6 h, 1.2% at 12 h, 1.5% at 18 h, 1.1% at 24 h, 0.8% at 48 h, and 0.5% at 72 h (P < 0.05 for all), and an increase of BCL-6 expression was observed at 18 h compared with control. However, treatment with d-Arg3FC131 did not induce any significant alteration in the expression levels of the BCL family of genes including BCL-2, BCL-XL, Bad, and Bax.

**d-Arg3FC131 inhibits growth and stimulates apoptosis of GH3 cells in a nude mouse xenograft tumor model**

Based on our findings that the CXCR4 antagonist d-Arg3FC131 inhibited cell growth and induced apoptosis in vitro, we hypothesized that it might suppress tumor formation by GH-producing pituitary adenoma cells in immunodeficient nude mice (Fig. 5). GH3 cells were injected sc into male athymic mice as described in Materials and Methods. Tumors developed within 15 d in mice injected with PBS (as a control). In the d-Arg3FC131-treated mice, similar to octreotide-treated ones, tumor formation was delayed by more than 10 d compared with the control group. The rate of growth and the size of the tumors were greatly reduced in mice treated with d-Arg3FC131 compared with those treated with PBS. Furthermore, the administration of d-Arg3FC131 significantly decreased both tumor and total body weights (84 ± 1.9 and 28.16 ± 0.5%, respectively; P < 0.05). There were no significant differences in the inhibition of tumor growth between octreotide- and d-Arg3FC131-treated mice.

We used histology to evaluate the effect of CXCR4 antagonists on tumor growth in the nude mice. H&E staining of tumor tissues demonstrated that microvessels filled with erythrocytes were prominent in control tumor tissue; however, this was rarely seen in d-Arg3FC131-treated tumors (Fig. 6 upper panel). We stained for Ki-67 to detect cell proliferation and performed TUNEL assays to examine apoptosis in tumor tissues (Fig. 6). Numbers of cells positive for Ki-67 expression and TUNEL were counted using computer-assisted image analysis software (Meta Morph, version 4.6; Universal Imaging Co., Downingtown, PA). Tumor tissues from mice treated with d-Arg3FC131 showed less number of cells stained positive for Ki-67 than those from control mice (10.3% vs. 4.4%, P < 0.05; Fig. 6 middle panel), and the proportion of TUNEL-positive cell nuclei was significantly increased in the d-Arg3FC131-injected mice (5.6% vs. 1.2% in controls, P < 0.05; Fig. 6, lower panel). Thus, d-Arg3FC131 can reduce xenograft tumor size by inducing apoptosis and inhibiting cell proliferation, consistent with its effects in vitro.

**Discussion**

CXCR4 is one of the most widely expressed chemokine receptors in human cancers, and SDF-1/CXCR4 interac-
tion causes proliferation, migration, invasion, and metastasis of cancer cells as well as promoting angiogenesis in tumors (7, 17, 18). Both CXCR4 and SDF-1 are expressed in rat pituitary primary cells and GH3 cell lines. SDF-1/CXCR4 interaction is also involved in pituitary cell proliferation and GH secretion and production (19, 22, 24). We also demonstrated CXCR4 expression in human pituitary tumors, including GH-producing and nonfunctioning tumors (23). These results indicate that CXCR4 antagonists inhibit pituitary cell proliferation and GH secretion, especially in GH-producing pituitary adenomas.

According to the present study, a CXCR4 antagonist, d-Arg3FC131, appears to have an important role in acromegalic tumor expansion and GH secretion. Among several synthetic peptides acting as CXCR4 antagonists, d-Arg3FC131 more strongly inhibited GH release and cell proliferation than SST in vitro. In immunodeficient nude mice, d-Arg3FC131 significantly inhibited xenograft tumor growth by inducing apoptosis and suppressing cell proliferation. A combined treatment of d-Arg3FC131 and octreotide (Sandostatin; Novartis, Pharma AG, Basel, Switzerland) was slightly more effective in inhibiting GH3-secreting pituitary tumor formation (Supplemental Fig. 2).

Our study demonstrates the putative mechanisms of d-Arg3FC131 on inhibiting pituitary tumor formation. First, the interruption of SDF-1/CXCR4 interaction by competitively binding d-Arg3FC131 blocked the proliferation of pituitary adenoma cells by stimulating phosphorylation of ERK1/2 and Akt (phosphatidylinositol 3-kinase pathway). Cell cycles of tumor cells were arrested at the G1 checkpoint, as shown by flow cytometry. Second, d-Arg3FC131 induced apoptosis of GH3 pituitary tumor cells through activation of the caspase-3, but not via the BCL family of genes. Third, tumor formation was delayed in the group of nude mice treated with the CXCR4 antagonist d-Arg3FC131 compared with the control group by suppressing proliferation and induction of apoptosis. Fourth, H&E staining of histology sections suggests that d-Arg3FC131 could play a role in antiangiogenesis and block tumor formation. Because there are several reports indicating the proangiogenic functions of the SDF-1/CXCR4 pathway (36, 37), further experiments would be interesting.

How CXCR4 antagonists and SDF-1 are associated with alterations in cell cycles and apoptosis has been investigated in several types of cells. According to previous reports, activation of PI3-K and the BCL family of genes are mainly involved in the intrinsic cell death pathway caused by treatment with CXCR4 antagonists (11, 38, 39). Our findings are consistent with previous studies indicating that CXCR4 antagonists have dual antiproliferative effects, leading to cytostatic (growth arrest) and cytotoxic (apoptosis) consequences.

Among three drugs that are currently used for the treatment of patients with acromegaly, somatostatin analogs are widely used as primary agents. However, more than one third of patients with acromegaly fail to achieve adequate biochemical control and suppression of tumor growth with current medications. Our in vivo results using an animal model suggest that a combined use of d-Arg3FC131 and somatostatin analogs would exert synergetic effects on pharmaceutical therapy for patients with acromegaly.

In this study, d-Arg3Fc131 was administered to mice twice daily because of its short half-life in the systemic circulation. To apply this new agent to patients with acromegaly, additional experiments related to pharmacodynamics, pharmacokinetics, and toxicity need to be conducted. In addition, the development of long-acting slow-releasing formulations such as sandostatin long acting repeatable is highly desirable.

In conclusion, here we demonstrated that d-Arg3FC131 effectively induced apoptosis and cell cycle arrest in GH3-secreting pituitary tumor cells in vitro and inhibited tumor growth in a xenograft mouse model. Together with somatostatin analogs, d-Arg3FC131 might have potential for the treatment of patients with acromegaly.
Acknowledgments

Address all correspondence and requests for reprints to: Eun Jig Lee, M.D., Ph.D., Endocrinology, Internal Medicine, Yonsei University College of Medicine, 250 Seongsanno, Seodaemun-gu, Seoul, 120-752, South Korea. E-mail: ejlee423@yuhs.ac.

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