Antitumor activity and pharmacologic characterization of the depsipeptide analog as a novel histone deacetylase/ phosphatidylinositol 3-kinase dual inhibitor

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Histone deacetylase (HDAC)/phosphatidylinositol 3-kinase (PI3K) dual inhibition is a promising strategy for the treatment of intractable cancers because of the advantages of overcoming potential resistance and showing synergistic effects. Therefore, development of an HDAC/PI3K dual inhibitor is reasonably attractive. Romidepsin (FK228, depsipeptide) is a potent HDAC inhibitor. We previously reported that depsipeptide and its analogs have an additional activity as PI3K inhibitors and are defined as HDAC/PI3K dual inhibitors. Subsequently, we identified FK-A11 as the most potent analog and reported its biochemical, biological, and structural properties as an HDAC/PI3K dual inhibitor. In this study, we reveal the *in vitro* and *in vivo* efficacy of FK-A11 in HT1080 fibrosarcoma and PC3 prostate cancer cell xenograft mouse models. FK-A11 showed *in vivo* antitumor activity by both i.v. and i.p. administration in a dose-dependent manner. In both xenograft models, FK-A11 showed superior antitumor effects compared to other depsipeptide analogs in accordance with *in vitro* anti-cell proliferation effects and the potency of HDAC/PI3K dual inhibition. In addition, we showed evidence of HDAC/PI3K dual inhibition accompanying antitumor efficacy in xenograft tumor tissues by immunohistochemistry. We also detailed pharmacokinetic characterization of FK-A11 in mice. These findings will be essential for guiding further preclinical and clinical studies.

The phosphatidylinositol 3-kinase (PI3K) family encompasses lipid kinases that convert phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate, which in turn activates protein kinase B (AKT) and downstream molecules, leading to the promotion of cell growth, proliferation, and survival.[1,2] Class I PI3K is heterodimeric complex composed of a catalytic subunit p110 and a regulatory subunit p85, p101, or p84. The catalytic subunit p110 consists of four isoforms: p110α, β, γ, and δ. In particular, p110α is known to play an important role in tumorigenesis through the gain of function mutations in the *PIK3CA* gene that encodes p110α.[3] Therefore, p110α has been considered a potential drug target for cancer therapy.[4] However, antecedent to p110α inhibitors, the p110δ selective inhibitor idelalisib has been successful in the treatment of chronic lymphocytic leukemia.[5] The efficacy of p110α inhibitor is considered to be limited by compensatory or concurrent activation of other signaling pathways,[6,7] indicating a potential mechanism by which cancer cells evade single-targeted kinase inhibitors. One of the promising strategies to overcome the limitation of single-targeted kinase inhibitor is to block the multiple pathway networks through inhibition of an epigenetic regulator, such as

histone deacetylase (HDAC).[8] Indeed, the combination therapy of a kinase inhibitor and an HDAC inhibitor was reported to not only overcome the kinase inhibitor resistance but also induce synergistic antitumor effects.[8–11]

CUDC-907, an oral dual inhibitor of HDAC and PI3K, showed a promising response in patients with refractory lymphoma or multiple myeloma in a phase I trial.[12] Thus, the dual blockade of HDAC and PI3K is a promising therapeutic strategy for intractable cancers.

Romidepsin (depsipeptide, FK228) is an HADC inhibitor with US FDA approval for the treatment of cutaneous and peripheral T-cell lymphoma.[13–15] We have previously shown that FK228 and depsipeptide analogs directly inhibit PI3K activity and potently induce apoptosis through HDAC/PI3K dual inhibition.[16] In other words, FK228 and depsipeptide analogs have been identified as novel HDAC/PI3K dual inhibitors. Furthermore, we have identified and reported an analog FK-A11 with potent HDAC/PI3K dual inhibitory activity.[17] Here, we describe the *in vitro* and *in vivo* antitumor activity of FK-A11 in human cancer cell line xenograft mouse models. Furthermore, we report the pharmacokinetic profile of FK-A11 in mice.

Materials and Methods

Reagents. FK-A11 was produced by Hamari Chemicals (Osaka, Japan). FK228 and FK-A3 were provided by Tohoku Medical and Pharmaceutical University (Sendai, Japan).[18] GDC-0941 was purchased from LC Laboratories (Woburn, MA, USA). Suberoylanilidehydroxamic acid (SAHA) was purchased from Cayman Chemical Company (Ann Arbor, MA, USA). Reagents were dissolved in DMSO for the *in vitro* assay and in saline with 1.3% DMSO and 20% 4-hydroxypropyl β-cyclodextrin (ChemScene, Monmouth Junction, NJ, USA) for the *in vivo* experiments.

Cell lines. The human fibrosarcoma cell line HT1080 and the prostate cancer cell line PC3 were used in the present study. HT1080 was obtained from ATCC (Manassas, VA, USA). PC3 was obtained from the Cell Resource Center for Biomedical Research (Institute of Development, Aging, and Cancer, Tohoku University, Sendai, Japan). Both cell lines were cultured in RPMI-1640 medium containing 10% FBS.

Enzyme assay. Phosphatidylinositol 3-kinase (p110α/p85α) activity was measured by mobility shift assay (Carna Biosciences, Kobe, Japan). Compound solutions (1000 nM phosphatidylinositol 4,5-bisphosphate, 50 μM ATP, 5 mM MgCl, and 21 nM PI3K [p110α/p85α]) were prepared with assay buffer containing 2 mM DTT and incubated in a 384-well microplate for 5 h. The reaction mixtures were then applied to the LabChip (Caliper Life Sciences, Hopkinton, MA, USA), and the product and substrate peaks were quantified. The PI3K reaction was evaluated by the product ratio calculated from peak heights of the product and substrate. The other 312 cellular kinase activities were also evaluated using non-radioisotopic methods such as the mobility shift assay, ELISA, or IMAP (Carna Biosciences).[19] A kinase inhibition profiling panel was produced based on the results of 313 kinase inhibition rates. Histone deacetylase 1 inhibitory activities were evaluated by the Screening Committee of Anticancer Drugs (Tokyo, Japan).

Cell proliferation assay. Cell proliferation assays were carried out with the Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan) as previously described.[16] A total of 8×10^3 cells were seeded and incubated in 96-well plates for 24 h at 37°C. Cells were treated with test compounds and further cultured for 24 h. The ratios of surviving cells to control cells treated with 0.1% DMSO were calculated.

Western blot analysis and immunohistochemistry. Western blot analysis was carried out as previously described[16] using monoclonal antibodies of phospho-AKT (Ser473 and Thr308), AKT, phospho-S6, S6, acetyl-histone H3, and β-actin.[16] All of the above antibodies, except for β-actin (Sigma-Aldrich, St. Louis, MI, USA), were purchased from Cell Signaling Technology (Danvers, MA, USA). For immunohistochemical staining, tumor tissues fixed in 10% formalin were embedded in paraffin. Tissue sections were deparaffinized in xylene and in a series of (100–50%) ethanol solutions. Tissues were then autoclaved at 120°C for antigen retrieval. Specific antibodies against phospho-AKT (Ser-473), phospho-S6, and acetyl-histone 3 (Cell Signaling Technology) were used.
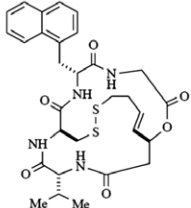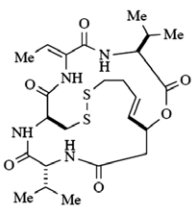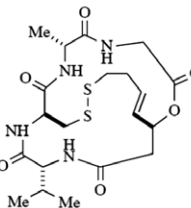
Xenograft model and *in vivo* antitumor analysis. Six- to eight-week-old female BALB/cAJcl-nu/nu mice were used in the present study. A total of 1×10^6 HT1080 cells or PC3 cells in 100 μL PBS were s.c. injected into the flank of each mouse. After approximately 10 days, when tumor formation was confirmed, mice were divided into each treatment group. Each test compound was then given. The length (L), width (W), and height (H) of each s.c. tumor mass were measured by digital calipers. Tumor volumes were calculated as $(L \times W \times H)/2$. In addition, body weights of mice were measured.

Pharmacokinetic study in mice. FK-A11 was given to healthy BALB/cAJcl-nu/nu mice i.v. or i.p. at a dose of 4 mg/kg; they were also given orally with two doses of 12 or 24 mg/kg. Nine mice were used for each administration route and dose. After treatment with FK-A11, blood samples were taken at each of nine time points (15 min, 30 min, and 1, 3, 6, and 24 h) per dose, and three mice were used for each time point. All blood samples were frozen at −80°C until analysis by liquid chromatography–tandem mass spectrometry.

Results

FK-A11 is the most potent HDAC/PI3K dual inhibitor among depsipeptide analogs. We have designed and synthesized 24 depsipeptide analogs to date. Although HDAC inhibitory activity is equally potent, the PI3K inhibitory activity is different among these analogs. We previously compared their inhibitory activities against HDAC and PI3K and identified FK-A11 as the most potent HDAC/PI3K dual inhibitor.[17] In the present study, we researched three compounds: FK-A11, FK228, and FK-A3. The chemical structures and their IC$_{50}$ values for PI3K (p110α) and HDAC1 are indicated in Figure 1. FK228 is the original compound known as romidepsin. FK-A3 is an analog with poor PI3K inhibitory activity retaining potent HDAC inhibitory activity.

Kinase inhibition selectivity of FK-A11. To evaluate the kinase inhibition profile, we previously reported inhibitory activities of FK-A11 for 22 kinases.[17] In the present study, inhibitory activities of FK-A11 were assayed further using a panel including 313 kinases. The percent inhibition of each kinase by FK-A11 at a concentration of 10 μM was calculated and

Compound		**FK-A11**	**FK228**	**FK-A3**
IC$_{50}$	HDAC1	0.64 ± 0.3 nM	3.6 ± 0.46 nM	4.2 ± 0.14 nM
	p110α	6.7 μM	57.1 μM	>100 μM
Structure				

Fig. 1. Chemical structures of depsipeptide analogs and their IC$_{50}$ values for histone deacetylase 1 (HDAC1) and phosphatidylinositol 3-kinase (PI3K) (p110α). Chemical structures of three depsipeptide analogs, FK-A11, FK228, and FK-A3, used in the present study are shown. IC$_{50}$ values for HDAC1 were assayed and determined as the mean ± SD from three independent dose–response curves by the Screening Committee of Anticancer Drugs (Japan). Inhibition of PI3K activity was evaluated by a mobility shift assay. Data are the mean of two experiments performed in duplicate.

indicated as the corresponding bubble plotted in the phylogenetic tree of the human protein kinome (Fig.2). The actual inhibition rate for each kinase by FK-A11 is shown in Table S1. Although PI3K was not included in the tree because of its feature of lipid kinase, 10 μM FK-A11 inhibited PI3K activity by 60.4%, shown as the largest bubble in Figure 2. In contrast, FK-A11 inhibited other kinases by only 0–23.8%. These result indicated that FK-A11 is a highly selective PI3K inhibitor.

Anti-cell proliferation activity of FK-A11 against human cancer cells. Anti-cell proliferation effects were examined in the fibrosarcoma cell line HT1080 and the prostate cancer cell line PC3. These cell lines have different genetic backgrounds leading to activation of the PI3K/AKT pathway. PC3 lacks phosphatase and tensin homolog (PTEN), the functional counterpart of PI3K, and it shows constitutively activated AKT.[20] HT1080 has mutant N-ras (Q61K) that activates its downstream PI3K/AKT pathway.[21,22] Regarding the *PIK3CA* gene, we analyzed the base sequences in exons 9 and 20 by the direct sequencing method. We confirmed that both cell lines contain no hotspot *PIK3CA* mutation, such as E542K, E545K, or H1047R (data not shown). HT1080 and PC3 cells were cultured for 24 h with depsipeptides (FK-A11, FK228, and FK-A3), an HDAC inhibitor SAHA, a PI3K inhibitor GDC-

0941, or a combination of SAHA and GDC-0941. The effects on cell proliferation were then evaluated. In both cell lines, FK-A11 showed stronger anti-cell proliferative effects than any other compound. In HT1080 cells, the effect of 100 nM FK-A11 was almost equivalent to that of a combination of SAHA and GDC0941 (Fig.3a). Furthermore, the effect of 1 μM FK-A11 exceeded that of combination therapy. FK-A11 greatly potentiated the anti-cell proliferative effect between concentrations of 10 nM and 100 nM, whereas FK228 potentiated the effect between 100 nM and 1 μM (Fig. 3a). The concentration ranges at which the effects strengthened were 10-fold lower in FK-A11 than in FK228, mostly in accordance with the scale of PI3K IC_{50} values of these two compounds. In contrast, FK-A3 with poor PI3K inhibitory activity very slightly potentiated the effect, even at a concentration of 1 μM. A similar result was shown in PC3 cells (Fig. 3b). These results were consistent with data we previously reported in several colon cancer cell lines.[16,17]

Inhibition of HDAC and PI3K by FK-A11 *in vitro*. HT1080 and PC3 cells were cultured with test compounds as with the cell proliferation assay. The effects on the PI3K pathway and histone acetylation status were evaluated by Western blot analysis (Fig. 3), and were densitometrically quantified (Fig. S1). In HT1080 cells, similar to the PI3K inhibitor GDC0941,
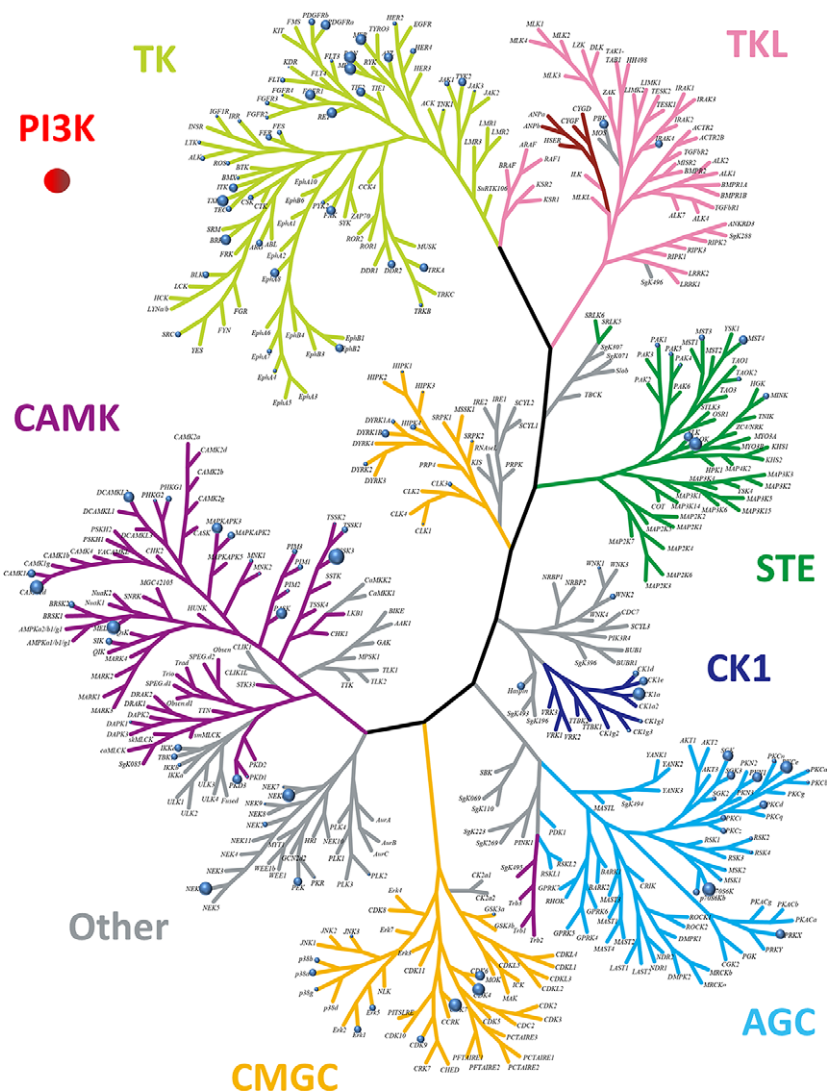
Fig. 2. Kinome inhibition plot by FK-A11. A total of 313 cellular kinase activities were evaluated by non-radioisotopic methods. Percent inhibition of each kinase by FK-A11 at a concentration of 10 μM was calculated and is indicated as a corresponding bubble plotted in the phylogenetic tree of the human protein kinome. The size of the bubble indicates the inhibition rate. Phosphatidylinositol 3-kinase (PI3K) is shown as the largest bubble. AGC, kinases from the protein kinase A, G, and C families; CAMK, calcium/calmodulin-dependent protein kinases; CK1, casein kinase 1; CMGC: kinases from the cyclin-dependent kinase, MAPK, glycogen synthase kinase, and casein kinase II families; STE, homologs of yeast sterile 7, sterile 11, and sterile 20 kinases; TK, tyrosine kinases; TKL, tyrosine kinase-like kinases.
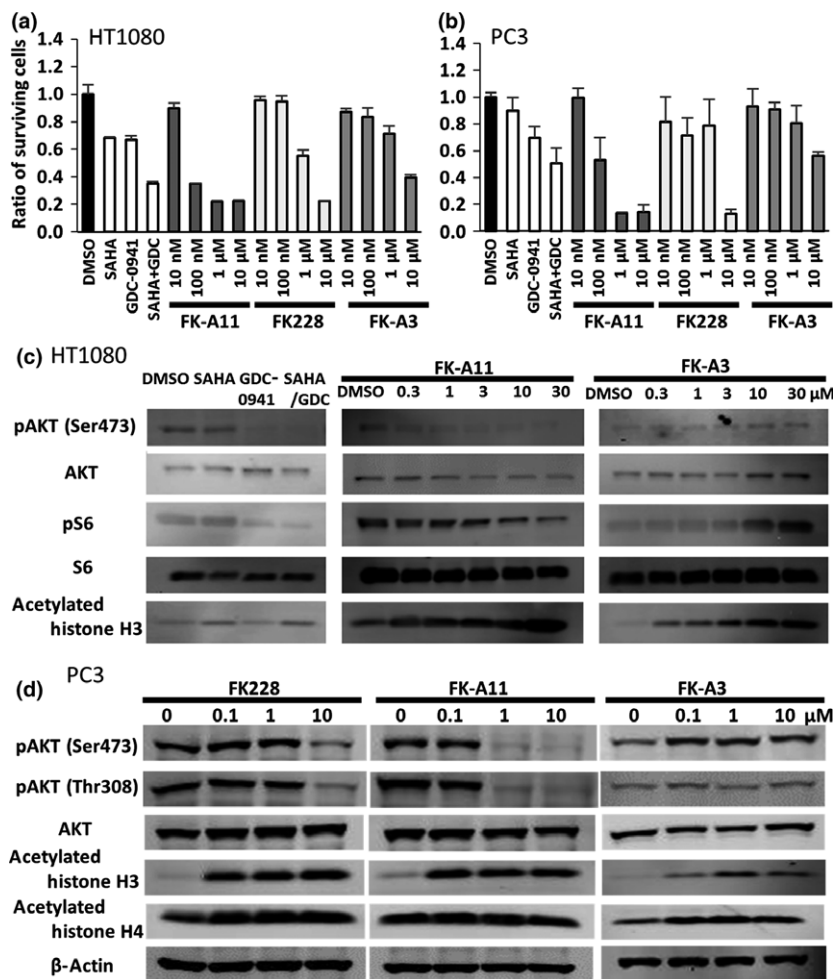
Fig. 3. *In vitro* anti-cell proliferation activity and histone deacetylase/phosphatidylinositol 3-kinase dual inhibition by FK-A11. (a,b) HT1080 and PC3 cells were used for cell proliferation assays. Cells were treated for 24 h with DMSO, 2.5 μM suberoylanilidehydroxamic acid (SAHA), 1 μM GDC-0941, a combination of 2.5 μM SAHA and 1 μM GDC-0941, or FK-A11, FK228, or FK-A3 at concentrations of 10 nM, 100 nM, 1 μM, or 10 μM. The ratio of surviving cells to the control treated with 0.1% DMSO was calculated. (c) Western blot analysis of phosphorylated protein kinase B (pAKT), phosphorylated S6 (pS6), and acetylated histone H3 in HT1080 cells treated for 3 h with 2.5 μM SAHA, 1 μM GDC-0941, a combination of 2.5 μM SAHA and 1 μM GDC-0941, various concentrations of FK-A11, or FK-A3. (d) Western blot analysis of pAKT, and acetylated histone H3 and H4 in PC3 cells. Cells were treated for 3 h with 0.1, 1, or 10 μM FK-A11, FK228, or FK-A3.

FK-A11 inhibited the phosphorylation of AKT and S6 ribosomal protein, both of which are downstream components of the PI3K pathway (Fig. 3c). In contrast, FK-A3 with poor PI3K inhibitory activity did not decrease, but rather increased the phosphorylation of AKT and S6 (Fig. 3c, Fig. S1a,b). Similar effects were also shown by SAHA. This result corresponded with previous reports that HDAC inhibitors activate the PI3K/AKT pathway in a cell-specific manner.[23–25] In regards to HDAC inhibition, FK-A11 and FK-A3 as well as SAHA promoted acetylation of histone H3 (Fig. 3c, Fig. S1c). Similar effects were observed in PC3 cells. FK-A11 inhibited the phosphorylation of AKT at lower concentrations than that observed with FK228 (Fig. 3d). FK-A3 did not change the phosphorylation of AKT. These three depsipeptides showed similar effects on acetylation of histones H3 and H4, indicating that they have equally potent HDAC inhibitory activity (Fig. 3d). These results in cellular assays corresponded to those of cell-free enzymatic inhibition assays.

Pharmacokinetics of FK-A11 in mice. FK-A11 was given to healthy female BALB/cAJcl-nu/nu mice. The pharmacokinetics following treatment with 4 mg/kg i.v., 4 mg/kg i.p., or two doses (12 or 24 mg/kg) orally were analyzed. The pharmacokinetic parameters are shown in Table1. The concentration in plasma reached the peak (Cmax) 15 min after dosing i.p. or orally. The biological half-life was within 1 h by every dosing route. Calculated area under the time–concentration curve (AUC)$_{0-t}$ values was slightly higher by i.p. dosing than by i.v. dosing (15.01 and 10.36 ng h/mL, respectively). However,

their AUC values were clearly higher than those of oral administration (0.2716–2.222 ng h/mL). This is consistent with the results of a previous report in which FK228 given orally to rats produced lower and more erratic drug levels than i.v. treatment, with a possibility of low bioavailability.[26]

Antitumor efficacy of FK-A11 in vivo. Antitumor efficacy and toxicity were evaluated using HT1080 and PC3 xenograft mouse models. In HT1080 fibrosarcoma tumor-bearing BALB/cAJcl-nu/nu mice, 4 mg/kg FK-A11 and 4 mg/kg FK-A3 were given i.p. three times every 3 days. Only FK-A11 significantly inhibited tumor growth (Fig.4a). To assess toxicity, body weights of tumor-bearing mice were measured. Although their weights were slightly reduced by FK-A11, the reduction was readily recovered (Fig. 4b). Furthermore, to compare the antitumor activities among different doses and different routes of administration, two doses (2 and 4 mg/kg) of FK-A11 were given i.v. or i.p. five times every 3 days. Except for the group given 2 mg/kg i.v., FK-A11 significantly inhibited HT1080 tumor growth (Fig. 4c). Tumor volumes tended to be smaller in i.p. treatment groups than those in i.v. treatment groups (Fig. 4c). In PC3 prostate cancer tumor-bearing mice, FK-A11 inhibited tumor growth without changes in body weight (Fig. 4d,e). In the efficacy comparison of FK-A11 with FK228 or FK-A3 at an identical dose of 2 mg/kg, growth inhibition with FK-A11 was superior to those of the other two compounds (Fig. 4f).

Inhibition of HDAC and PI3K by FK-A11 in vivo. To confirm that FK-A11 inhibits both HDAC and PI3K *in vivo*, the

Table 1. Pharmacokinetic parameters of FK-A11 in mice

	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (h)	C_0 (ng/mL)	AUC_{0-t} (ng·h/mL)	AUC_{inf} (ng·h/mL)	$t_{1/2}$ (h)
i.v.	4	–	–	46.76	10.3600	11.130	0.3
i.p.	4	36.6200	0.25	–	15.0100	16.340	1.0
p.o.	12	0.8254	0.25	–	0.2716	0.557	0.4
	24	7.1140	0.25	–	2.2220	3.495	0.2

–, Not calculated; AUC, area under the concentration–time curve; C_0, initial concentration; C_{max}, maximum concentration; $t_{1/2}$, half-life; T_{max}, time of maximum concentration.

acetylation status of histone H3, phosphorylation status of AKT, and phosphorylation status of S6 were determined immunohistochemically by the specific antibodies. The s.c. HT1080 and PC3 tumors were excised from mice 4 h after i.p. treatment with FK-A11. In both cell tumor tissues, FK-A11 accelerated acetylation of histone H3, indicating HDAC inhibition. In addition, FK-A11 decreased phosphorylation of S6 or AKT, indicating PI3K inhibition (Fig. 5). These results verified the pharmacodynamics effects of FK-A11, which are consistent with HADC/PI3K dual-target inhibition.

Discussion

Histone deacetylase inhibitors have pleiotropic mechanisms for antitumor activity because HDACs have many protein substrates, not only histone but also other molecules that are involved in the regulation of gene expression, cell proliferation, and cell death.[27] If used together, the pleiotropic mechanisms of HDAC inhibitors often help other drugs, such as kinase inhibitors, to evade or overcome resistance.[10] Moreover, the combination of an HDAC and a kinase inhibitor, for example, a PI3K inhibitor, was reported to improve efficacy or induce synergistic antitumor effects.[9,28] In this regard, single-molecule dual inhibitors targeting HDAC and kinase have been developed.[29] One of the inhibitors is CUDC-907, an oral HDAC/PI3K dual inhibitor, that showed promising efficacy for hematological cancer patients in a phase I trial.[12] Development of an HDAC/PI3K dual inhibitor would provide an attractive novel anticancer agent, stimulating antitumor effects in intractable
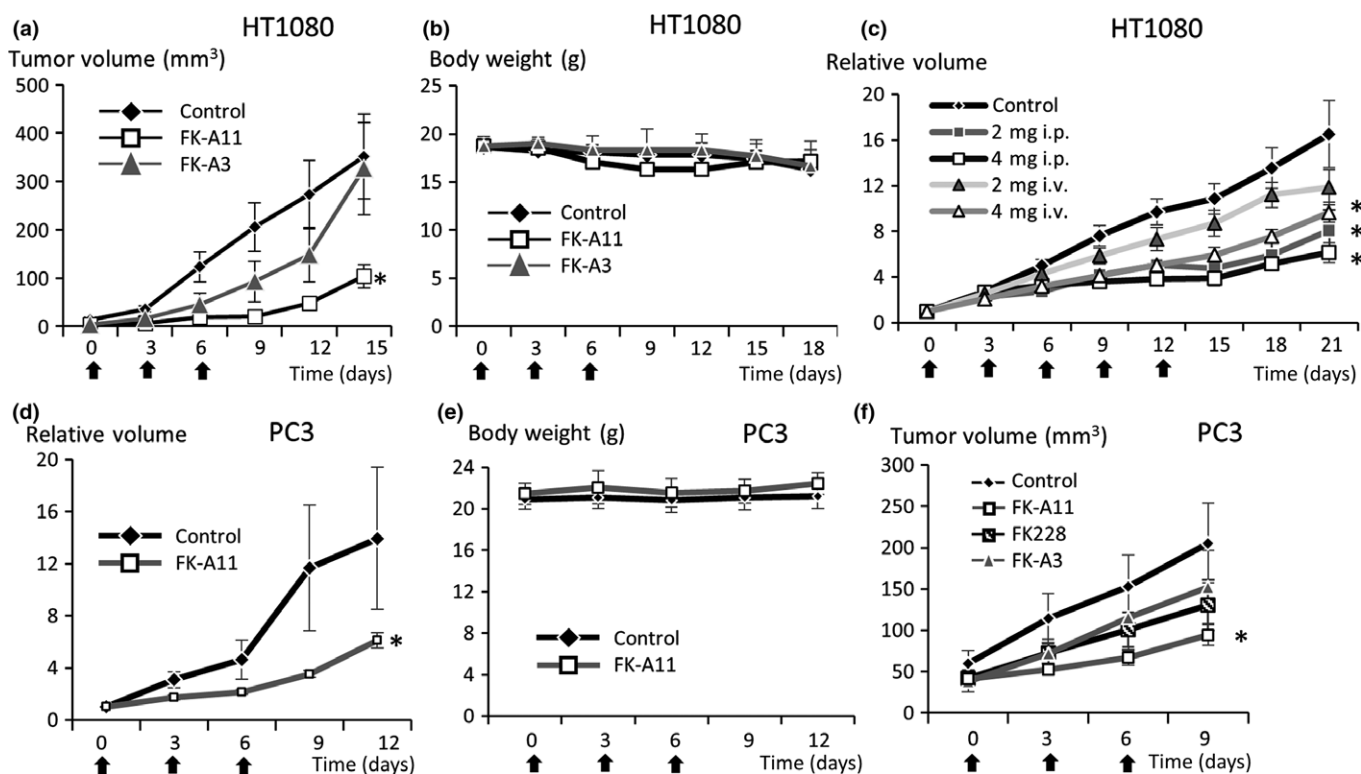

Fig. 4. *In vivo* antitumor activity of FK-A11. Antitumor activity of FK-A11 against HT1080 tumor (a–c) and PC3 tumor (d–f). (a,b) 1×10^6 HT1080 cells were s.c. injected into 7-week-old female BALB/c nu/nu nude mice. After tumor formation was confirmed, mice were randomly divided into test groups, each with six mice. Solvent alone (Control), 4 mg/kg FK-A11, or 4 mg/kg FK-A3 was given i.p. three times every 3 days on days 0, 3, and 6. The length (L), width (W), and height (H) of the s.c. tumor mass were measured, and the tumor volumes were calculated as (L × W × H)/ 2. Body weight was also measured. Data are the means ± SE from six mice. (c) In HT1080 tumor-bearing nude mice, FK-A11 was given i.p. or i.v. with two doses of 2 or 4 mg/kg, five times every 3 days. Six mice were used for each treatment group. (d,e) 1×10^6 PC3 cells were s.c. inoculated into nude mice. After tumor formation, 4 mg/kg FK-A11 was given i.p. three times every 3 days. Tumor volume and body weight were measured. (f) Against PC3 tumor, 2 mg/kg FK-A11, FK228, or FK-A3 was given i.p. three times every 3 days. Two-tailed Student's *t*-test compared with respective control was carried out for the *in vivo* study. *$P < 0.05$.
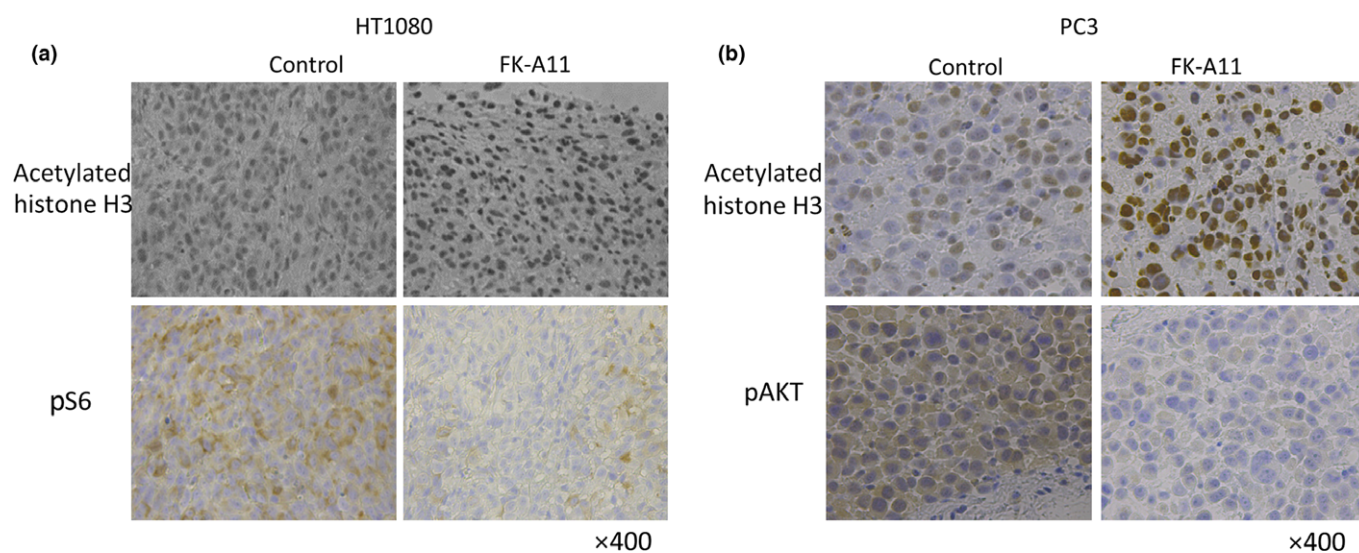
Fig. 5. Histone deacetylase/phosphatidylinositol 3-kinase dual inhibition by FK-A11 *in vivo*. HT1080 and PC3 subcutaneous tumors were excised from mice 4 h after i.p. treatment with 4 mg/kg FK-A11. The acetylation status of histone H3 and phosphorylation (p) status of protein kinase B (AKT) and S6 were determined immunohistochemically by specific antibodies.

cancers by overcoming their refractory mechanisms for existing drugs.

We have previously reported depsipeptide analogs as HDAC/PI3K dual inhibitors.[16] Subsequently, we identified FK-A11 as the most potent analog, and reported its biochemical, biological, and structural properties as an HDAC/PI3K dual inhibitor.[17,30] In the present study, we reported the efficacy of FK-A11 in solid cancer cells derived from carcinoma and sarcoma with intractability and unmet medical needs. In addition, we showed evidence of HDAC/PI3K dual inhibition accompanying antitumor efficacy in xenograft tumor tissues. We also detailed pharmacokinetic characterization of FK-A11 in mice.

Information on the kinase inhibition profile or inhibition selectivity is important to elucidate a property of a kinase inhibitor.[31,32] We previously reported that FK-A11 inhibits all four class I PI3K isoforms and that FK-A11 selectively inhibits PI3K among 22 kinases, including mammalian target of rapamycin.[17] In the present study, inhibitory activities of FK-A11 were assayed for a further 313 kinases. FK-A11 has proved to be a highly selective PI3K inhibitor.

In vitro and *in vivo* anti-cell proliferative activity and antitumor activity were evaluated in two human cancer cell lines with abnormal activation of the PI3K/AKT pathway through different mechanisms. The HT1080 fibrosarcoma cell line contains the N-ras mutation, and the PC3 prostate cancer cell line is PTEN null. We showed that FK-A11 has therapeutic efficacy against these human cancer cell xenografts in mice. By comparing the antitumor activities of FK-A11 using different doses and different routes of administration, we found dose-dependent efficacies in both i.p. and i.v. treatment. Intraperitoneal treatment tended to inhibit tumor growth to a larger degree than i.v. (Fig. 4c). Pharmacokinetic analysis of FK-A11 in mice showed that i.p. treatment results in slightly higher AUC than i.v. treatment (Table1). This difference in AUC might make a difference in the efficacy. In both xenograft models, FK-A11 showed superior antitumor effects compared to both FK228 and FK-A3, in accordance with *in vitro* anti-cell proliferation effects and the potency of HDAC/PI3K dual

inhibition (Fig. 4a,f). We have not evaluated the efficacy of FK-A11 against cancer cells in which the PI3K/AKT pathway is not constitutively activated, although it is reasonable to consider that blockade of an activated signaling pathway is more efficient than blockade of an non-activated pathway. Identification of biomarkers predicting the efficacy of FK-A11 will be an important problem.

FK-A11 is a poorly soluble compound. It could be dissolved into saline with 1.3% DMSO and 20% 4-hydroxypropyl β-cyclodextrin and used for *in vivo* study. Hence, in this study, not only FK-A11 but also FK228 were dissolved into the same solvent. In previous studies analyzing the pharmacokinetics of FK228 in mice or rats, FK228 was formulated by dissolving in 40% ethanol, 5% PEG 400, and 55% normal saline.[25,26] One of those studies reported that the AUC value following 4.4 mg/kg i.v. injection of FK228 reached 1123 ng h/mL in SCID mice,[25] which is much higher than that of FK-A11 in our study. We have not examined the pharmacokinetics of FK228 dissolved in our solvent. The difference in solvents might affect the pharmacokinetics. Although there was no report about toxicity in the previous studies, solvents used in those studies included a very high percentage of ethanol that is considered unsuitable for clinical use. Optimization of solvents or improvement of the solubility of FK-A11 is needed.

Because HDAC/PI3K dual inhibition is a promising strategy for the treatment of intractable cancers, development of an HDAC/PI3K dual inhibitor is reasonably attractive. In the present study, we reported novel findings of FK-A11 regarding evidence of antitumor effects, pharmacodynamic effects on HDAC/PI3K dual inhibition *in vivo*, and pharmacokinetic profiling in mice. These findings will be essential for guiding further preclinical studies and for leading to clinical studies.

Disclosure Statement

The authors have no conflict of interest.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Fig. S1. Densitometric quantification of Western blot data shown in Figure 3.

Table S1. Percent inhibition of FK-A11 for 313 kinases.