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Efficacy of a combination therapy targeting CDK4/6 and autophagy in a mouse xenograft model of t(8;21) acute myeloid leukemia

Hidemasa Matsuo [a], Kana Nakatani [a], Yutarou Harata [a], Moe Higashitani [a], Yuri Ito [a], Aina Inagami [a], Mina Noura [a], Tatsutoshi Nakahata [b], Souichi Adachi [a,*]

[a] Human Health Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[b] Drug Discovery Technology Development Office, Center for iPS Cell Research and Application, Kyoto University, Kyoto, Japan

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ABSTRACT

One of the most frequent cytogenetic abnormalities in acute myeloid leukemia (AML) is t(8;21). Although patients with t(8;21) AML have a more favorable prognosis than other cytogenetic subgroups, relapse is still common and novel therapeutic approaches are needed. A recent study showed that t(8;21) AML is characterized by *CCND2* deregulation and that co-inhibition of CDK4/6 and autophagy induces apoptosis in t(8;21) AML cells. In this study, we examined the *in vivo* effects of co-inhibiting CDK4/6 and autophagy. We used a mouse model in which t(8;21)-positive Kasumi-1 cells were subcutaneously inoculated into NOD/Shi-scid IL2Rg^{null} mice. The mice were treated with the autophagy inhibitor chloroquine (CQ), a CDK4/6 inhibitor (either abemaciclib or palbociclib), or a CDK4/6 inhibitor plus CQ. After 20 days of treatment, tumor volume was measured, and immunostaining and transmission electron microscopy observations were performed. There was no change in tumor growth in CQ-treated mice. However, mice treated with a CDK4/6 inhibitor plus CQ had significantly less tumor growth than mice treated with a CDK4/6 inhibitor alone. CDK4/6 inhibitor treatment increased the formation of autophagosomes. The number of single-strand DNA-positive (apoptotic) cells was significantly higher in the tumors of mice treated with a CDK4/6 inhibitor plus CQ than in mice treated with either CQ or a CDK4/6 inhibitor. These results show that CDK4/6 inhibition induces autophagy, and that co-inhibition of CDK4/6 and autophagy induces apoptosis in t(8;21) AML cells *in vivo*. The results suggest that inhibiting CDK4/6 and autophagy could be a novel and promising therapeutic strategy in t(8;21) AML.

1. Introduction

Acute myeloid leukemia (AML) is a genetically and clinically heterogeneous disease, characterized by the expansion of undifferentiated myeloid precursor cells [1]. One of the most common chromosomal abnormalities in AML is t(8;21)(q22;q22)*/RUNX1-RUNX1T1* (also known as *AML1-ETO*, hereafter referred to as t(8;21)) [2]. Genetic risk stratification classifies the prognosis of patients with t(8;21) AML as favorable [3]. Although most patients with t(8;21) AML achieve complete remission after intensive chemotherapy, relapse occurs in 30–40% of patients and is associated with a poor prognosis [4]. Therefore, novel therapeutic approaches are needed.

Frequent mutations in the *CCND2* gene (which encodes cyclin D2 protein) in t(8;21) AML were reported by several groups including ours [5–7]. D-type cyclins, including cyclin D2, form complexes with cyclin-dependent kinase 4 (CDK4) and the closely related CDK6 to induce cell cycle progression from G1 to S phase [8]. *CCND2* mutations stabilize cyclin D2 and promote cell proliferation [5]. *CCND2* is a crucial transcriptional target of the RUNX1–RUNX1T1 fusion protein, which also contributes to the deregulation of *CCND2* in t(8;21) AML [9]. Patients with deregulated D-type cyclins, such as t(8;21) AML patients, may benefit from treatment with CDK4/6 inhibitors [10].

Recently, we studied the effectiveness of CDK4/6 inhibitors in t(8;21) AML cells [11]. CDK4/6 inhibition suppressed cell proliferation by inducing cell cycle arrest in the G1 phase; however, CDK4/6 inhibition also induced autophagy in t(8;21) AML cells. Autophagy is an intracellular degradation mechanism that recycles cellular components by engulfing them into double-membrane vesicles called autophagosomes [12]. In cancer cells, autophagy is associated with resistance to chemotherapy, and autophagy inhibition is a promising strategy to

* Corresponding author. Human Health Sciences, Graduate School of Medicine, Kyoto University, Address: 53 Kawahara-cho, Shogoin, Sakyoku, Kyoto, 606-8507, Japan.
E-mail address: adachiso@kuhp.kyoto-u.ac.jp (S. Adachi).

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overcome chemotherapy resistance [13]. Chloroquine (CQ) is a well-known autophagy inhibitor that blocks autophagosome-lysosome fusion (late stage of autophagy), resulting in the accumulation of autophagosomes in the cytoplasm [14]. We examined the effect of the combination of a CDK4/6 inhibitor and CQ, and found that the two inhibitors acted synergistically to induce apoptosis in t(8;21) cells [11]. However, that study did not analyze the *in vivo* efficacy of the combination therapy. Here, we evaluated the efficacy of the combination therapy targeting CDK4/6 and autophagy in a mouse xenograft model of t(8;21) AML.

2. Materials and methods

2.1. Cell line

The Kasumi-1 cell line was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). The cell line was cultured in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 5% CO_2 and 37 °C.

2.2. Mice

NOD/Shi-scid IL2Rg^{null} (NOG) mice were purchased from the Central Institute for Experimental Animals, Japan.

2.3. Compounds

We selected the commercially available CDK4/6 inhibitors abemaciclib and palbociclib for use in this study because many clinical trials use these drugs [15]. Abemaciclib was obtained from MedChemExpress (HY-16297) and dissolved in 1% hydroxyethyl cellulose solution in phosphate-buffered saline (PBS). Palbociclib was obtained from Pfizer (La Jolla, CA) and suspended in 0.5% methyl cellulose solution. CQ was obtained from Sigma-Aldrich (C6628) and dissolved in PBS.

2.4. In vivo study

Kasumi-1 cells (5 × 10^6/mouse) were injected subcutaneously into NOG mice (25–30 weeks old; male) as described previously [16]. Once tumor volume reached 100 mm^3, the mice were randomized into four groups and treated once daily for the next 20 days with the following treatments: (1) control vehicle (via intraperitoneal injection and orally), (2) CQ (40 mg/kg via intraperitoneal injection) and control vehicle (orally), (3) abemaciclib or palbociclib (75 mg/kg orally) and control vehicle (via intraperitoneal injection), and (4) CQ (40 mg/kg via intraperitoneal injection) and abemaciclib or palbociclib (75 mg/kg orally). The doses of CQ, abemaciclib, and palbociclib used were determined from previous studies [17,18]. The number of mice for each group was five to six, except for the control group and the CQ-treated group in the palbociclib study, which contained three mice to avoid the unnecessary use of mice. Tumor volume was measured daily using a caliper and calculated using the equation: length × (width^2)/2. On day 20, mice were euthanized and the tumors were collected for further analysis. A schematic illustration of the *in vivo* study is presented in Fig. 1.

All animal studies were properly conducted in accordance with the Regulation on Animal Experimentation at Kyoto University, based on the International Guiding Principles for Biomedical Research Involving Animals. All procedures used in this study were approved by the Kyoto University Animal Experimentation Committee (permit number: Med Kyo 20021).

2.5. Transmission electron microscopy (TEM)

Tumor samples were fixed in 4% paraformaldehyde, 2% glutaraldehyde, and 0.1 M phosphate buffer at 4 °C, washed in isotonic phosphate-buffered sucrose, re-fixed in phosphate-buffered 1% OsO_4, dehydrated through a graded series of ethanol solutions, and embedded in Luveak 812 reagent (Nacalai Tesque, Kyoto, Japan). Thin sections (70–90 nm thick) were cut with a diamond knife on an EM UC6 ultramicrotome (Leica, Wetzlar, Germany), stained with uranyl acetate and

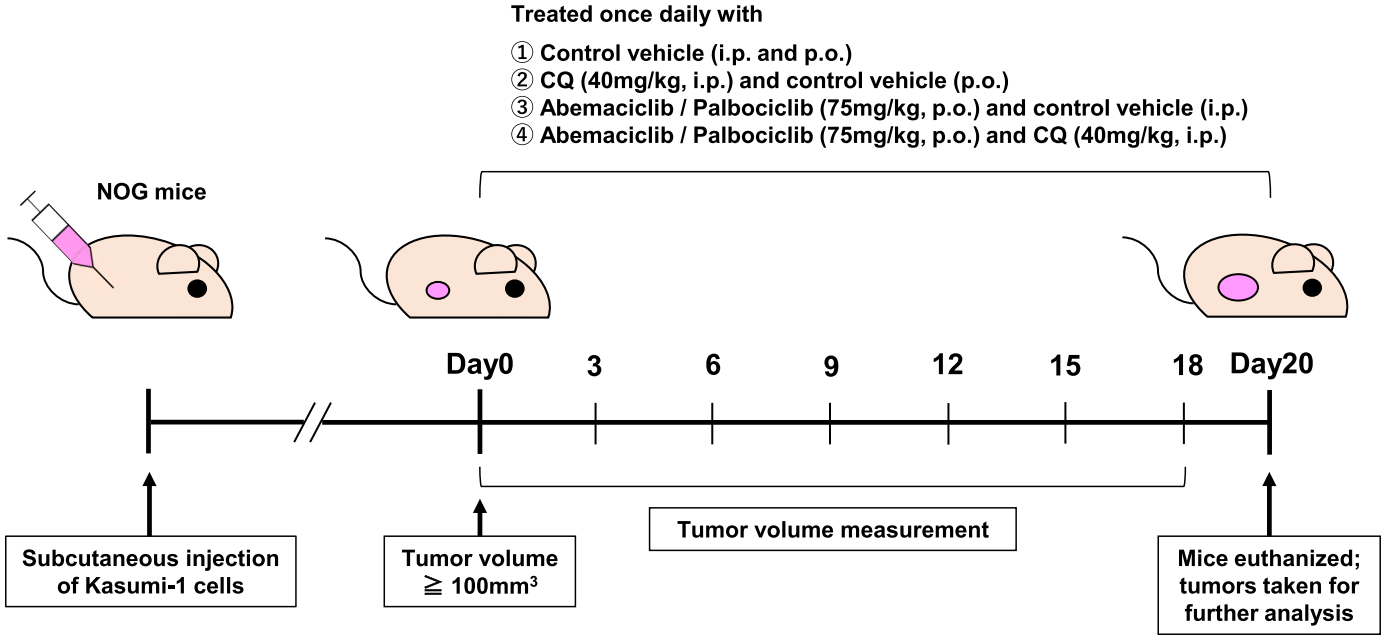

Fig. 1. A schematic illustration of the *in vivo* study. Kasumi-1 cells were injected subcutaneously into NOG mice. Once tumor volume reached 100 mm^3, the mice were randomized into four groups and treated once daily for the next 20 days with the following: (1) control vehicle (via intraperitoneal (i.p.) injection and oral delivery (p.o.)), (2) chloroquine (CQ) (40 mg/kg, i.p. injection) and control vehicle (orally), (3) abemaciclib or palbociclib (75 mg/kg, p.o.) and control vehicle (i.p. injection), or (4) CQ (40 mg/kg, i.p. injection) and abemaciclib or palbociclib (75 mg/kg p.o.). Tumor volume was measured daily using a caliper and calculated using the equation: length × (width^2)/2. On day 20, mice were euthanized and the tumors were collected for further analysis.

lead citrate, and observed using a Hitachi H-7650 electron microscope (Hitachi, Tokyo, Japan). The number of autophagosomes in each cell was counted as in our previous study (50 cells per sample) [19].

2.6. Immunohistochemical (IHC) staining

Tumor samples were paraffin-embedded and cut into 3–4 μm sections. After deparaffinization and antigen retrieval using proteinase K, endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide dissolved in methyl alcohol for 30 min. The glass slides were washed in PBS (six times, 5 min per wash) and mounted in PBS containing 1% horse normal serum for 30 min. Subsequently, an anti-single stranded DNA (ssDNA) rabbit IgG antibody (18731, IBL, Gunma, Japan) (primary antibody; dilution 1 : 600) was applied overnight at 4 °C. The slides were then incubated with biotinylated horse anti-mouse serum (secondary antibody; dilution 1 : 300) in PBS for 40 min, and then washed in PBS (six times, 5 min per wash). An avidin–biotin–peroxidase complex (ABC; ABC-Elite; Vector Laboratories, Burlingame, CA, USA) diluted 1 : 100 in BSA was applied for 50 min. After washing in PBS (six times, five per wash), a diaminobenzidine-based color reaction was conducted for 7 min and cell nuclei were counterstained with hematoxylin. Unless otherwise specified, all incubation and washing procedures were carried out at room temperature. The number of ssDNA-positive cells was counted in five randomly selected high power fields (HPF, × 400) in each sample (3–4 samples per group). The average number of ssDNA-positive cells per HPF was compared between the treatment groups as described previously [20].

2.7. Statistical analysis

Continuous variables were compared using the Student's t-test. A *P*-value of <0.05 was considered statistically significant. Statistical significance was defined as follows: $P < 0.05$ (*), $P < 0.01$ (**), or $P < 0.001$ (***).

3. Results

3.1. Co-inhibition of CDK4/6 and autophagy suppresses t(8;21) AML cell growth in vivo

First, we examined whether co-inhibition of CDK4/6 and autophagy suppresses t(8;21) AML cell growth in a mouse xenograft model. Kasumi-1 cells were injected subcutaneously into NOG mice, and then mice were treated with control vehicle, CQ, abemaciclib, or CQ and abemaciclib (Fig. 1). CQ treatment did not significantly affect tumor growth (mean tumor volume on day 18: 2201 mm^3 (control) versus 2077 mm^3 (CQ), $P = 0.67$). However, tumors treated with abemaciclib plus CQ treatment were significantly smaller than abemaciclib-treated tumors (1224 mm^3 (abemaciclib) versus 789 mm^3 (abemaciclib plus CQ), $P = 0.03$) (Fig. 2A and C). Similar results were found when palbociclib was studied: CQ treatment did not significantly affect tumor growth (2499 mm^3 (control) versus 2849 mm^3 (CQ), $P = 0.36$). Tumor growth in the palbociclib plus CQ-treated group was lower than that in the palbociclib-treated group, but this effect was not statistically significant (1112 mm^3 (palbociclib) versus 352 mm^3 (palbociclib + CQ), $P = 0.18$) (Fig. 2B and D). These results suggest that co-inhibition of CDK4/6 and autophagy suppresses t(8;21) AML cell growth *in vivo*.

3.2. CDK4/6 inhibitors induce autophagy in t(8;21) AML cells in vivo

Next, we examined whether CDK4/6 inhibitors induce autophagy. Tumor samples collected from euthanized mice were examined by TEM. The formation of autophagosomes in the cytoplasm of leukemia cells increased in the CDK4/6 inhibitor-treated groups compared with controls (Fig. 3A and B). The mean number of autophagosomes was significantly higher in both abemaciclib-treated cells and palbociclib-treated cells than in control cells (Fig. 3C and D). We also examined the number of autophagosomes in CQ-treated cells and palbociclib plus CQ-treated cells, which revealed that the number of autophagosomes was significantly higher in palbociclib plus CQ-treated cells than in palbociclib-treated cells (Supplementary Fig.1, $P = 0.03$). Compared with CQ-treated cells, the number of autophagosomes was also significantly higher in palbociclib plus CQ-treated cells ($P = 0.004$). These results suggest that autophagy was induced by CDK4/6 inhibitors and inhibition of the last stage of autophagy by CQ caused autophagosome accumulation.

3.3. CDK4/6 and autophagy inhibitors synergistically induce apoptosis in t(8;21) AML cells in vivo

Finally, we examined whether co-inhibition of CDK4/6 and autophagy induces apoptosis in AML cells in a mouse xenograft model. Tumor samples collected from euthanized mice were examined by IHC staining of ssDNA, a marker of apoptosis. Representative images of IHC staining with an anti-ssDNA antibody in control samples, CQ-treated samples, CDK4/6 inhibitor-treated samples, and CDK4/6 inhibitor plus CQ-treated samples are shown in Fig. 4A and B. In both control samples and CQ-treated samples, few ssDNA-positive cells were observed. By contrast, the number of ssDNA-positive cells was significantly higher in samples treated with a CDK4/6 inhibitor plus CQ than in CDK4/6 inhibitor-treated samples (Fig. 4C and D). The results suggest that CDK4/6 inhibitors and autophagy inhibitors synergistically induce apoptosis in t(8;21) AML cells *in vivo*.

4. Discussion

In this study, we evaluated the efficacy of a combination therapy targeting CDK4/6 and autophagy in a mouse xenograft model of t(8;21) AML. CQ treatment alone did not significantly affect tumor growth, suggesting that autophagy inhibition does not modulate tumor growth. By contrast, tumor growth in the CDK4/6 inhibitor plus CQ-treated group was significantly lower than in the CDK4/6 inhibitor-treated group. Together with the data showing that CDK4/6 inhibitors enhance autophagy, our results suggest a mechanism by which an autophagy inhibitor suppresses tumor growth by inhibiting CDK4/6 inhibitor-mediated autophagy.

In this study, abemaciclib and palbociclib were selected as CDK4/6 inhibitors because many clinical trials use these drugs [15]. A recent study investigated the spectrum of CDK/cyclin activity mediated by CDK4/6 inhibitors [21]. The study showed that although both abemaciclib and palbociclib inhibit CDK4/6, abemaciclib more broadly inhibits other CDKs (e.g., CDK2) and affects the cell cycle differently. To the best of our knowledge, published *in vivo* experiments investigating co-inhibition of CDK4/6 and autophagy have used only palbociclib [22,23]. In our study, similar effects on tumor growth were observed when either abemaciclib or palbociclib was used in combination with an autophagy inhibitor, indicating that the effect of the combined treatment was independent of the specificity of the CDK4/6 inhibitor.

In a preliminary experiment of this study, we examined the tail-vein injection of Kasumi-1 cells. However, the cells did not engraft in the bone marrow, and so we used subcutaneous injection of Kasumi-1 cells (as described previously [16]). Subcutaneous tumors are not exposed to the bone marrow microenvironment, which has profound effects on treatment responses in both patients and mouse models. The effects of co-inhibiting CDK4/6 and autophagy on tumor growth in models that use leukemia cells in the bone marrow should be examined in future studies. Since we previously showed that t(8;21) AML cells are sensitive to co-inhibition of CDK4/6 and autophagy *in vitro*, we selected Kasumi-1 cells in this study. Future studies will be required to confirm *in vivo* sensitivity to the combination therapy using AML cells with t(8;21) and other cytogenetic abnormalities.

We identified a mechanism of tumor suppression that involves co-

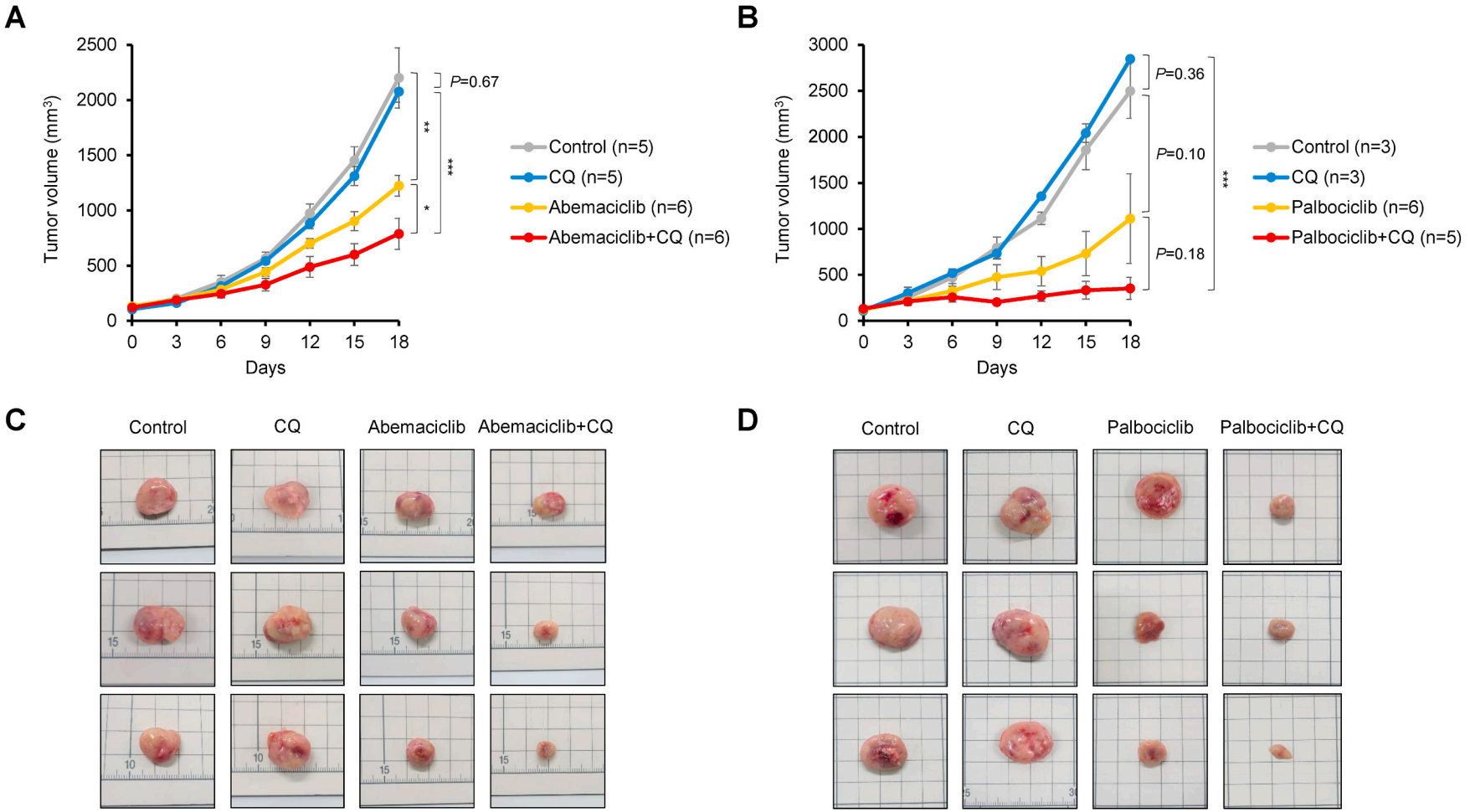

Fig. 2. Co-inhibition of CDK4/6 and autophagy suppresses t(8;21) AML cell growth *in vivo*. (A) Tumor volume curves in mice treated with control vehicle, CQ, abemaciclib, and abemaciclib plus CQ. (B) Tumor volume curves in mice treated with control vehicle, CQ, palbociclib, and palbociclib plus CQ. (C) (D) Representative images of tumors collected at day 20 from each group. Tumor volumes are presented as the mean ± SE. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

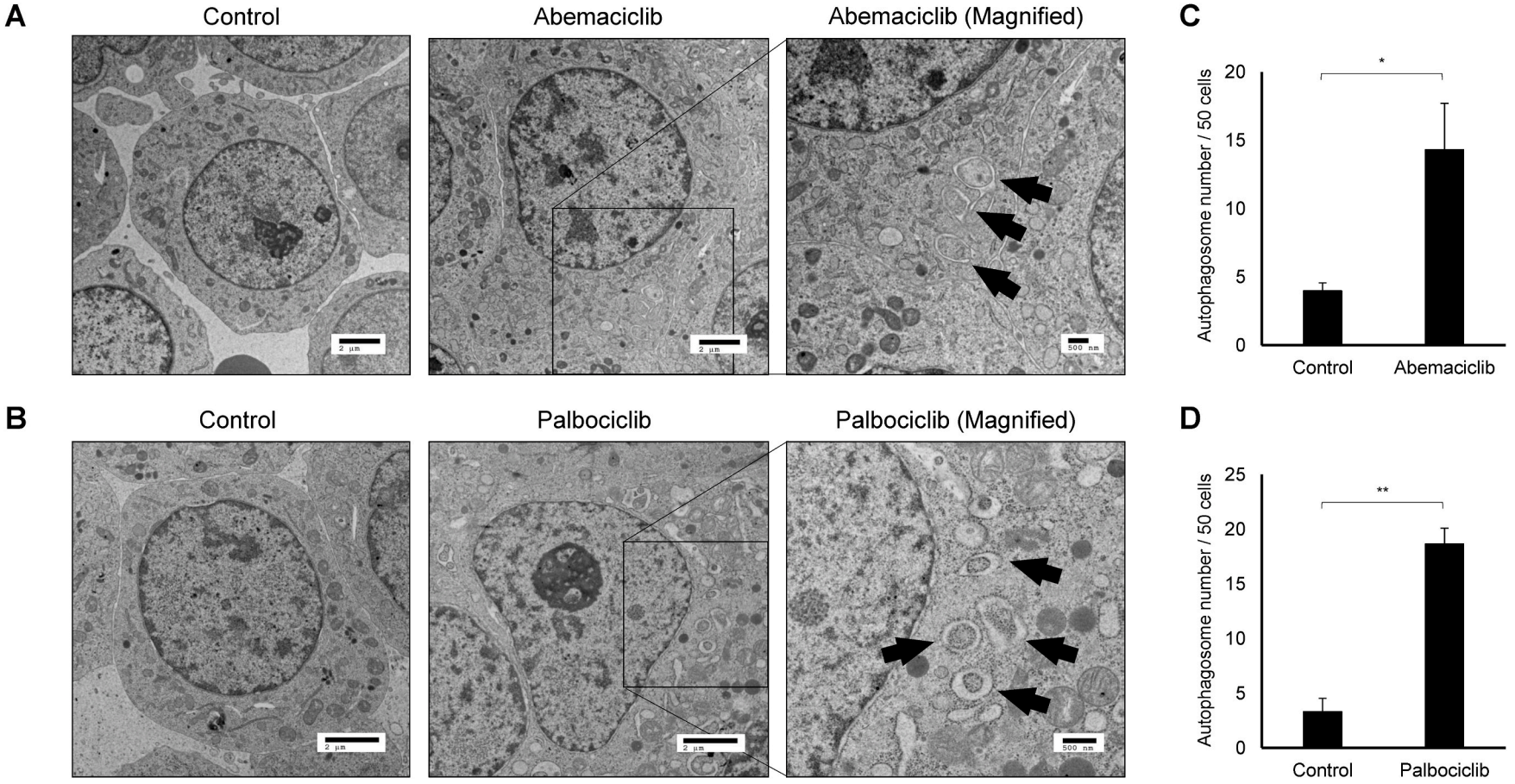

Fig. 3. CDK4/6 inhibition induces autophagy in t(8;21) AML cells *in vivo*. (A) Representative images of control and abemaciclib-treated samples observed by TEM. (B) Representative images of control and palbociclib-treated samples observed by TEM. The black arrows in the magnified images indicate autophagosomes (double-membrane). (C) (D) The number of autophagosomes per 50 cells in each group. Data are presented as the mean ± SE of three independent samples. $*P < 0.05$, $**P < 0.01$.

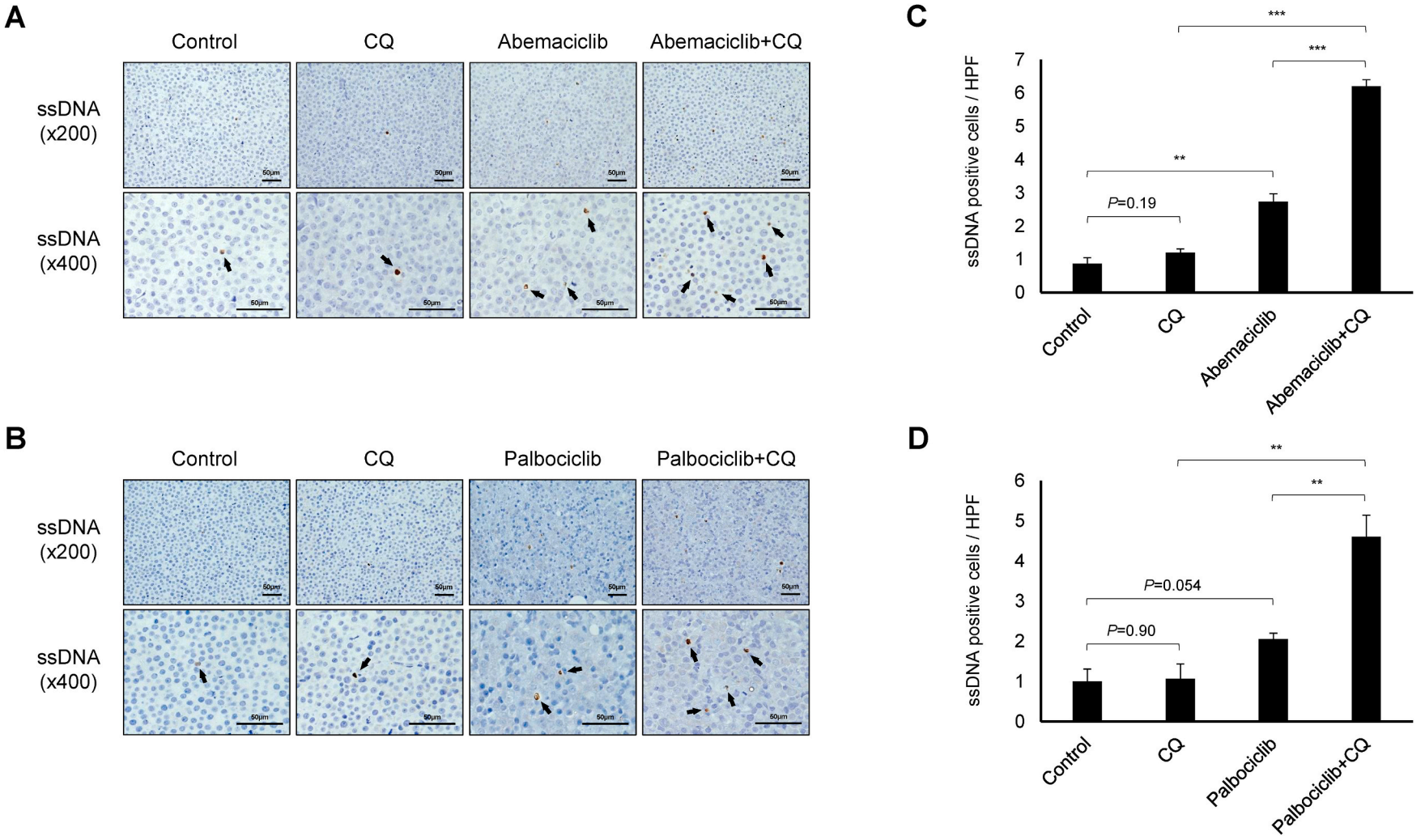

Fig. 4. Co-inhibition of CDK4/6 and autophagy synergistically induces apoptosis in t(8;21) AML cells *in vivo*. (A) Representative images of IHC staining with an anti-ssDNA antibody in a control sample, a CQ-treated sample, an abemaciclib-treated sample, and a sample treated with abemaciclib plus CQ. (B) Representative images of IHC staining with an anti-ssDNA antibody in a control sample, a CQ-treated sample, a palbociclib-treated sample, and a sample treated with palbociclib plus CQ. The black arrows indicate ssDNA-positive (apoptotic) cells. (C) (D) The number of ssDNA-positive cells per high power field (HPF, × 400) in each group. The number of ssDNA-positive cells was counted in five randomly selected HPFs per sample. Data are presented as the mean ± SE of 3–4 independent samples. $^{**}P < 0.01$, $^{***}P < 0.001$.

inhibiting CDK4/6 and autophagy to enhance apoptosis; this mechanism is compatible with previously reported *in vitro* data [11]. A synergistic effect of CDK4/6 inhibition and autophagy inhibition is also reported in solid tumors [22,23], suggesting that such a treatment strategy may be applicable for a broad range of tumors. Identifying cancer types predicted to be sensitive to this combination therapy will be important future work.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2021.101099.

Author contributions

H.M. designed the project. H.M., K.N., Y.H., M.H., Y.I., A.I., and M.N. contributed to animal experiments. H.M., K.N., Y.H., M.H., Y.I., A.I., M.N., and S.A. contributed to the interpretation of data. H.M. and K.N. prepared the figures. H.M. wrote the manuscript. H.M., T.N., and S.A. supervised the project.

References

[1] E. Papaemmanuil, M. Gerstung, L. Bullinger, et al., Genomic classification and prognosis in acute myeloid leukemia, N. Engl. J. Med. 374 (2016) 2209–2221.
[2] D. Grimwade, R.K. Hills, A.V. Moorman, et al., Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials, Blood 116 (2010) 354–365.
[3] H. Döhner, E. Estey, D. Grimwade, et al., Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel, Blood 129 (2017) 424–447.
[4] E. Jourdan, N. Boissel, S. Chevret, et al., Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia, Blood 121 (2013) 2213–2223.
[5] Z.J. Faber, X. Chen, A.L. Gedman, et al., The genomic landscape of core-binding factor acute myeloid leukemias, Nat. Genet. 48 (2016) 1551–1556.
[6] A.K. Eisfeld, J. Kohlschmidt, S. Schwind, et al., Mutations in the CCND1 and CCND2 genes are frequent events in adult patients with t(8;21)(q22;q22) acute myeloid leukemia, Leukemia 31 (2017) 1278–1285.
[7] H. Matsuo, K. Yoshida, K. Fukumura, et al., Recurrent CCND3 mutations in MLL-rearranged acute myeloid leukemia, Blood Adv 2 (2018) 2879–2889.
[8] S. Bates, D. Parry, L. Bonetta, et al., Absence of cyclin D/cdk complexes in cells lacking functional retinoblastoma protein, Oncogene 9 (1994) 1633–1640.
[9] N. Martinez-Soria, L. McKenzie, J. Draper, et al., The oncogenic transcription factor RUNX1/ETO corrupts cell cycle regulation to drive leukemic transformation, Canc. Cell 34 (2018) 626–642.
[10] X. Gong, L.M. Litchfield, Y. Webster, et al., Genomic aberrations that activate D-type cyclins are associated with enhanced sensitivity to the CDK4 and CDK6 inhibitor abemaciclib, Canc. Cell 32 (2017) 761–776.
[11] K. Nakatani, H. Matsuo, Y. Harata, et al., Inhibition of CDK4/6 and autophagy synergistically induces apoptosis in t(8;21) acute myeloid leukemia cells, Int. J. Hematol. 113 (2021) 243–253.
[12] N. Mizushima, Autophagy: process and function, Genes Dev. 21 (2007) 2861–2873.
[13] J.M.M. Levy, C.G. Towers, A. Thorburn, Targeting autophagy in cancer, Nat. Rev. Canc. 17 (2017) 528–542.
[14] N. Mizushima, T. Yoshimori, B. Levine, Methods in mammalian autophagy research, Cell 140 (2010) 313–326.
[15] M.E. Klein, M. Kovatcheva, L.E. Davis, et al., CDK4/6 inhibitors: the mechanism of action may not Be as simple as once thought, Canc. Cell 34 (2018) 9–20.
[16] L.T. Chen, C.T. Chen, W.T. Jiaang, et al., BPR1J373, an oral multiple tyrosine kinase inhibitor, targets c-KIT for the treatment of c-KIT-driven myeloid leukemia, Mol. Canc. Therapeut. 15 (2016) 2323–2333.
[17] T. Dhir, C.W. Schultz, A. Jain, et al., Abemaciclib is effective against pancreatic cancer cells and synergizes with HuR and YAP1 inhibition, Mol. Canc. Res. 17 (2019) 2029–2041.
[18] B. Cao, L.A. Parnell, M.S. Diamond, et al., Inhibition of autophagy limits vertical transmission of Zika virus in pregnant mice, J. Exp. Med. 214 (2017) 2303–2313.
[19] H. Matsuo, H. Itoh, N. Kitamura, et al., Intravenous immunoglobulin enhances the killing activity and autophagy of neutrophils isolated from immunocompromised patients against multidrug-resistant bacteria, Biochem. Biophys. Res. Commun. 464 (2015) 94–99.
[20] K. Ito, T. Nakazato, K. Yamato, et al., Induction of apoptosis in leukemic cells by homovanillic acid derivative, capsaicin, through oxidative stress: implication of phosphorylation of p53 at Ser-15 residue by reactive oxygen species, Cancer Res 64 (2004) 1071–1078.
[21] M. Hafner, C.E. Mills, K. Subramanian, et al., Multiomics profiling establishes the polypharmacology of FDA-approved CDK4/6 inhibitors and the potential for differential clinical activity, Cell Chem Biol 26 (2019) 1067–1080, e8.
[22] S. Vijayaraghavan, C. Karakas, I. Doostan, et al., CDK4/6 and autophagy inhibitors synergistically induce senescence in Rb positive cytoplasmic cyclin E negative cancers, Nat. Commun. 8 (2017) 15916.
[23] Y. Ji, X. Liu, J. Li, et al., Use of ratiometrically designed nanocarrier targeting CDK4/6 and autophagy pathways for effective pancreatic cancer treatment, Nat. Commun. 11 (2020) 4249.