学 位 論 文 の 要 旨

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学　位　論　文　名　A Novel Fusicoccin Derivative Preferentially Targets Hypoxic Tumor Cells and Inhibits Tumor Growth in Xenografts

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論 文 内 容 の 要 旨

INTRODUCTION

Solid tumor cells can survive and even grow in a hypoxic microenvironment. Tumor hypoxia has been shown to be a cause of malignant transformation and a source of resistance to current cancer therapies. Tumor hypoxia is also associated with a more malignant phenotype and poor survival in cancer patients. Recent progress in our understanding of the biology of tumor cells under hypoxia has led to increased attention on targeting hypoxia for cancer therapy.

Many human cancers contain cancer stem cells, which possess an enhanced tumor-initiating capacity, can self-renew, partially recreate the cellular heterogeneity of the parental tumor, and seem to be generally more resistant than other cancer cells to conventional anticancer therapeutics. Because of these properties, cancer stem cells have been linked to tumor recurrence and distant metastasis. It should be necessary to develop strategies to effectively target the cancer stem cell population.

Several diterpenes including fusicoccins have been shown to induce apoptosis in human cancer cells. In the present study we synthesized various fusicoccin derivatives with antitumor activity. Moreover, we surveyed these derivatives to identify novel hypoxia-targeting drugs.

MATERIALS AND METHODS

Human cancer cell lines were cultured in RPMI-1640 medium supplemented with 10%

fetal bovine serum and 80 μg/ml gentamicin at 37℃ in a humidified atmosphere of 5% CO_2 in air. Human umbilical vein endothelial cells (HUVEC) were cultured in Human Endothelial Cell Medium. To provide a hypoxic environment, cells were maintained in a humidified automatic O_2/CO_2 incubator that was set at 37℃, 1% O_2 and 5% CO_2. Hypoxia-inducible factor-1 (HIF-1) α-deficient cells were prepared using short hairpin RNA sequences against human HIF-1α and scrambled control oligonucleotides. Transplantation of human cancer cells into nude mice. Mice were subcutaneously inoculated with 2 x 10^6 cells. Tumor volume was measured with vernier calipers. Our protocol was approved by the animal ethics committee at Shimane University.

RESULTS AND DISCUSSION

We synthesized and various compounds from fusicoccin A and examined their growth-inhibiting effects on human pancreatic cancer MIAPaCa-2 cells under hypoxia (1% O_2) and normoxia (21% O_2). ISIR-042 was the most effective at inhibiting cell growth under hypoxic conditions. Similar results were obtained when the other human cancer cells.

HIF-1α is up-regulated in hypoxia, and activates the transcription of target genes to result in the adaptation of tumor cells to hypoxia. Some hypoxia-inducible genes have been shown to reduce responsiveness to certain anticancer drugs. Then, we investigated the effect of ISIR-042 on the hypoxia-induced accumulation of HIF-1α protein. ISIR-042 significantly inhibited the hypoxia-induced accumulation of HIF-1α protein. Next, we examined the growth-inhibitory effect of ISIR-042 on HIF-1α-deficient cells. HIF-1α-deficient cells were much more sensitive to the inhibition of cell growth by ISIR-042. These results suggest that the growth-inhibitory effect of ISIR-042 is partly related to HIF-1α expression.

The extracellular signal-regulated kinase (ERK) pathway mediates several cellular fates, including growth and survival. Akt also plays a pivotal role in cell proliferation and survival. We studied whether Akt or ERK signaling pathway was involved in the action of ISIR-042 under hypoxia. Hypoxia greatly increased the level of phosphorylated Akt and ISIR-042 effectively inhibited the Akt activation. On the other hand, ERK activation was not associated with the growth-inhibitory effect of ISIR-042 under hypoxia. To confirm the effect of hypoxia on Akt activation, we studied the effects of phosphoinositide 3 kinase/Akt inhibitors on the growth of MIAPaCa-2 cells under normoxia and hypoxia. ISIR-042 and the Akt inhibitors cooperatively inhibited the growth of MIAPaCa-2 cells. ISIR-042 preferentially inhibited the growth of hypoxic cells, and the growth inhibition by ISIR-042 was also observed under normoxia in the presence of PI3K/Akt inhibitors, suggesting that the hypoxia-targeting action of ISIR-042 is closely associated with the PI3K/Akt signaling pathway.

We compared the growth-inhibitory effect of ISIR-042 in normal endothelial HUVEC

cells with that in MIAPaCa-2 cells under the same culture conditions (normoxia). MIAPaCa-2 cells underwent morphological changes characteristic of apoptosis, whereas the morphology of HUVEC cells did not change, although there was a slight decrease in the cell number.

Hypoxia is particularly important for maintaining a cancer stem cell niche and leads to an increase in the proportion of cancer stem cells in a tumor. Therefore, we examined the effect of ISIR-042 on cancer stem cells. Pancreatic cancer stem cells from primary tumors express the cell surface markers CD24 and CD44. Gemcitabine, a first-line chemotherapeutic agent against pancreatic cancers, showed an increased percentage of $CD24^{+}CD44^{+}$ cells in MIAPaCa-2 cells, whereas ISIR-042 significantly reduced the percentage of $CD24^{+}CD44^{+}$ cells in a concentration-dependent manner.

To determine the potential for ISIR-042 in treating pancreatic cancer, we treated MIAPaCa-2 xenograft-bearing nude mice with ISIR-042 alone or in combination with the standard of care agent gemcitabine. ISIR-042 alone dose-dependently inhibited the *in vivo* growth of MIAPaCa-2 cells. ISIR-042 at 3 mg/kg inhibited the growth of MIAPaCa-2 xenograft tumors with a day 20 (end of treatment period) T/C value of 38 %. This dose of ISIR-042 was well-tolerated with no decrease in body weight. Our *in vitro* studies suggest that the combination of ISIR-042 and gemcitabine is more effective therapeutically than treatment with ISIR-042 alone. Therefore, we examined the combined effects of gemcitabine and ISIR-042 on the in *vivo* growth of MIAPaCa-2 cells. Gemcitabine alone significantly inhibited tumor growth with a T/C value of 27 %. Combined treatment caused tumor stasis at day 20 with a T/C value of 11 %. In this model, ISIR-042 exhibited significant single-agent activity in xenografts of MIAPaCa-2 cells, along with increased antitumor effects in combination with gemcitabine.

CONCLUSION

We report that a novel fusicoccin derivative (ISIR-042) is more cytotoxic to hypoxic cells than to normoxic cells. ISIR-042 inhibited the growth of human pancreatic cancer MIAPaCa-2 cells while sparing normal endothelial cells, and significantly inhibited the growth of MIAPaCa-2 cells as xenografts without apparent adverse effects. ISIR-042 preferentially inhibits stem/progenitors in pancreatic cancer cell lines compared with chemotherapeutic agents. These results suggest that ISIR-042 may be a potential therapeutic agent for hypoxic tumors such as pancreatic cancer. Further studies should provide useful information for the development of a new therapeutic strategy against pancreatic cancer.