学 位 論 文 の 要 旨

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学 位 論 文 名　Piperlongumine Rapidly Induces the Death of Human Pancreatic Cancer Cells Mainly Through the Induction of Ferroptosis

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論 文 内 容 の 要 旨

INTRODUCTION

Pancreatic cancer is one of the most lethal types of cancer with a mortality rate of almost 95%. Treatment with current chemotherapeutic drugs has limited success due to poor responses. Therefore the development of novel drugs or effective combination therapies is urgently required. Ras transformation tenders cells sensitive to reactive oxygen species (ROS)-induced cell death and pancreatic cancers exhibit an extremely high mutation rate of K-ras (>90%). Piperlongumine (PL) is a natural product with cytotoxic properties restricted to cancer cells by significantly increasing intracellular ROS levels. Ferroptosis has recently emerged as a new form of programmed cell death that has been characterized as non-apoptotic peroxidation-induced cell death contingent on the availability of iron and ROS. Cotylenin A (CN-A; a plant growth regulator) exhibits potent antitumor activities in several cancer cell lines, including pancreatic cancer cell lines. In this study, we found PL as a new ferroptosis inducer against pancreatic cancer cells and further investigated the effects of combined treatment with PL, CN-A and sulfasalazine (SSZ, one of the known ferroptosis inducers).

MATERIALS AND METHODS

The human pancreatic cancer cell lines, MIAPaCa-2, PANC-1, CFPAC-1, and BxPC-3,

were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (FBS) at 37℃ in humidified atmosphere of 5% CO_2 in air. Mouse embryonic fibroblasts (MEFs) were cultured in Dulbecco's modified Eagle's medium containing 0.1 mM non-essential amino acids, 0.05 mM 2-mercaptoethanol and 10% FBS at 37℃ in a humidified atmosphere of 5% CO_2 in air.

Cell viability was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded at $3x10^4$ cells/ml in a 24-well multidish. After being cultured with or without test compounds for 16 h, 500 μl of dimethyl sulfoxide was added to each well to solubilize formazan in viable cells. Plates were analyzed by measuring optical density at 540 nm. Cellular glutathione (GSH) contents were measured using a GSH-Glo Glutathione Assay Kit (Promega) according to the instructions provided by manufacturer. Expressions of specific proteins were analyzed by Western blotting. The production of ROS was monitored using a Muse cell analyzer (Millipore), and the experimental protocol followed the description provided with Muse Oxidative Stress Kit (Millipore). Annexin V staining and the detection of Annexin V-positive cells were performed using the Muse cell analyzer, and the experimental protocol followed the description provided with Muse Annexin V & Dead Cell Assay kit (Millipore).

RESULTS AND DISCUSSION

PL dose-dependently decreased cell viabilities of PANC-1 and MIAPaCa-2 cells. The cancer cell-killing activity was inhibited by the ROS scavenger (N-acetylcysteine), ferroptosis inhibitors (ferrostatin-1 and liproxstatin-1) and iron-chelator (deferoxamine), but not by the apoptosis inhibitor (Z-VAD-FMK), or the necroptosis inhibitor (necrostatin-1). Consistent with the established role for lipoxygenase-catalyzed lipid hydroperoxidation for ferroptosis, we observed that treatment with the lipoxygenase inhibitor, PD146176, prevented the cancer cells from undergoing PL-induced cell death. The content of cellular GSH in the cells treated with 10 μM PL for 4 h was markedly depleted. PL did not induce the expression of typical apoptotic markers, such as cleaved poly (ADP-ribose) polymerase (PARP) and cleaved caspase-3. PL also did not affect the LC3-II/LC3-I ratio, an indicator of autophagy induction. These results suggest that PL alone induces cancer cell death through, at least in part, the induction of ferroptosis in pancreatic cancer cells.

PL and CN-A synergistically induced the death of pancreatic cancer MIAPaCa-2 and PANC-1 cells for 16 h. CN-A enhanced the induction of ROS by PL for 4 h. The synergistic induction of cell death was also abrogated by ferroptosis inhibitors and the iron-chelator but not by the apoptosis inhibitor or the necroptosis inhibitor.

SSZ enhanced the death of pancreatic cancer cells induced by PL and the combined effects were abrogated by ferroptosis inhibitors and the iron-chelator. SSZ further enhanced the cancer cell-killing activities induced by combined treatment with PL plus CN-A. We found that PL alone at 2 μM, CN-A alone at 15 μg/ml, or SSZ alone at 100 μM did not influence the viability of the MIAPaCa-2 cells, and PL (2 μM) plus CN-A (15 μg/ml) or PL (2 μM) plus SSZ (100 μM) only slightly reduced viability (~90%). Triple combined treatment with PL (2 μM), CN-A (15 μg/ml) and SSZ (100 μM) synergistically reduced viability of the MIAPaCa-2 cells to ~20%. The synergistic induction of cell death induced by triple combined treatment with PL, CN-A and SSZ was also observed in the PANC-1 cells. This triple combined treatment-induced cancer cell death was also largely canceled by the pretreatment with the ferroptosis inhibitor ferrostatin-1, suggesting that this combined treatment induced ferroptotic cell death in pancreatic cancer cells. Therapy-resistant cancer cells are characterized by a dysregulated apoptotic cascade and exhibit an enhanced ability to undergo ferroptosis. Then the triple combined treatment with PL, CN-A, and SSZ may also be effective against therapy-resistant pancreatic cancer cells.

On the other hand, the synergistic induction of cell death by PL and CN-A was not observed in non-transformed MEFs, and SSZ did not enhance the death of MEFs induced by PL plus CN-A. Moreover, SSZ-induced death of the MEFs was completely abolished by PL. Therefore, PL prevents cytotoxic effect induced by SSZ against normal cells, and triple combined treatment with PL, CN-A and SSZ may be effective for inducing pancreatic cancer cell death.

CONCLUSION

PL induced ferroptotic cell death in pancreatic cancer cells, and which was further effectively induced by the combined treatment with PL, CN-A and SSZ. Treatment that are combined with PL, CN-A and SSZ, may be promising for patients with pancreatic cancer.