学 位 論 文 の 要 旨

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学 位 論 文 名　Menin-MLL Inhibitors Induce Ferroptosis and Enhance Antiproliferative Activity of Auranofin in Several Types of Cancer Cells

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論 文 内 容 の 要 旨

INTRODUCTION

Menin-mixed-lineage leukemia (MLL) inhibitors have potential as therapeutic agents for MLL-rearranged leukemia. The inhibition of the menin-MLL interaction with small-molecule inhibitors, such as MI-463, MI-503, MI-2-2 and VTP50469, inhibited proliferation and induced differentiation in MLL1-rearranged and nucleophosmin 1-mutated leukemia. They are also effective against solid cancers, such as breast cancer. Ferroptosis, a recently discovered form of regulated cell death, is dependent on the presence of intracellular iron and the accumulation of reactive oxygen species (ROS). Due to the enhanced dependence of cancer cells on iron, the induction of ferroptosis is becoming a promising therapeutic strategy particularly for eradicating aggressive malignancies that are resistant to traditional therapies. The purpose of this study is to find new ferroptosis inducers against aggressive cancer cells such as triple-negative breast cancer cells and to develop effective combination therapies including MI-463 which induces ferroptosis.

MATERIALS AND METHODS

Human ovarian cancer cell lines (OVCAR-8, OVCAR-3 and OVCAR-4), triple negative

breast carcinoma cell lines (BT-549, MDA-MB-231 and MDA-MB-468), luminal A breast carcinoma cell lines (MCF-7 and T47D), pancreatic carcinoma cell lines (MIAPaCa-2, PANC-1, BxPC-3 and CFPAC-1) and lung carcinoma cell lines (A549, LU99, LU65 and PC-7) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 80 μg/ml gentamicin at 37 °C in a humidified atmosphere of 5% CO_2 in air. Cells were seeded at $1\text{-}2\times10^4$ cells/ml in a 24-well multi-dish. After culturing with or without the test compounds for the indicated times, viable cells were examined by Methyl thiazolyl tetrazorium (MTT) assay or trypan blue dye exclusion test using the automated cell counter model R1 (Olympus). 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). Expressions of specific proteins were analyzed by Western blotting. Measurement of mRNA levels was performed by a reverse transcriptase-quantitative polymerase chain reaction (qPCR) using the SYBER Green method with the Thunderbird SYBER qPCR Mix (Toyobo) on a Thermal Cycler Dice Real-time PCR instrument (Takara Bio).

RESULTS AND DISCUSSION

MI-463, MI-503 and MI-2-2 dose-dependently decreased the OVCAR-8 viable cell numbers. The IC_{50} (concentration of the drug required for 50% inhibition of cell growth) values of MI-463, MI-503 and MI-2-2 were 0.06, 0.04 and 4.5 μM, respectively. Ferrostatin-1(Ferr-1, a ferroptosis inhibitor) almost completely abrogated the MI-463-, MI-503- and MI-2-2-induced decrease in the viable number of OVCAR-8 cells. These results suggested that these menin-MLL inhibitors induced ferroptotic cancer cell death and that both MI-463 and MI-503 were new potent ferroptosis inducers of OVCAR-8 cell death. However, VTP50469, another recently reported potent menin-MLL inhibitor, could not induce the death of OVCAR-8 cells even at a high concentration.

Treatment with MI-463 induced an approximately 2-fold increase in the ROS high cell population at 8 h and an approximately 5-fold increase in the ROS high cell population at 24 h. The cancer cell-killing activity was inhibited by the ROS scavenger (N-acetylcysteine), ferroptosis inhibitor (Ferr-1) and iron-chelators (deferoxamine (DFO) and ciclopirox (CPX)), but not by the apoptosis inhibitor (Z-VAD-FMK). Consistent with the established role of lipoxygenase-catalyzed lipid hydroperoxidation in ferroptosis, it was found that treatment with the specific inhibitor of arachidonate 15-lipoxygenase, PD146176, protected the cancer cells against MI-463-induced cell death. Furthermore, the cancer cell-killing activity of MI-463 was inhibited by idebenone (a lipophilic antioxidant and membrane-permeable analog of CoQ_{10}) and oleic acid [a monounsaturated fatty acid and one of the end products of stearoyl-CoA desaturase 1 (SCD1)]. These results collectively indicate that MI-463 induces cancer cell death through the induction of ferroptosis in OVCAR-8 cells.

Furthermore, MI-463 induced cell death of BT-549 triple-negative breast cancer cells, and significantly enhanced the anticancer effect of various types of anticancer agents, with auranofin being highly effective. MI-463 in combination with auranofin synergistically increased the death of breast, ovarian, pancreatic and lung cancer cell lines (88%, 14/16 cell lines). The synergistic induction of cell death was abrogated by ferroptosis inhibitors and DFO. Inhibitors of SCD1 (CAY10566 and MF-438), similar to MI-463, also enhanced cancer cell death synergistically with auranofin. On the other hand, CAY10566 could not enhance the MI-463-induced death of BT-549 cells, but rather CAY10566 interfered with the cell death-inducing activity of MI-463. These results suggest that the MI-463-induced decrease in cell viability may be due at least in part to the inhibition of SCD1 activity. Previous studies have demonstrated that heme oxygenase-1 (HO-1) accelerates erastin-induced ferroptotic cell death and that HO-1 is a major intracellular source of iron. HO-1gene expression was markedly induced (~60 or 70-fold) in the MI-463 plus auranofin-treated BT-549 cells at 12 or 24 h, respectively. HO-1 protein expression was also markedly increased (~16-fold) in the MI-463 plus auranofin-treated BT-549 cells at 24 h. Treatment with zinc protoporphyrin-9, a specific inhibitor of HO-1, markedly attenuated the cell death induced by MI-463 plus auranofin. These results suggest that the induction of HO-1 is associated at least in part with the induction of ferroptotic cancer cell death by combined treatment with MI-463 plus auranofin. It was shown that treatment with MI-463 (45 mg/kg, twice daily, p.o. to mice) could maintain ~1 μM MI-463 in plasma, and that auranofin concentrations of 1-3 μM in plasma are achievable without obvious side-effects in patient or volunteer subject who received the recommended dose of 6 mg/day for rheumatoid arthritis. These reports suggest that combination therapy of MI-463 and auranofin seems to be clinically applicable.

CONCLUSION

MI-463 induced ferroptotic cell death in ovarian cancer and breast cancer cells and MI-463 in combination with auranofin synergistically increased cancer the death of breast, ovarian, pancreatic and lung cancer cell lines. The results suggest that menin-MLL inhibitors, such as MI-463, in combination with auranofin have potential for use as an effective therapeutic approach for several cancers via the induction of ferroptosis.