DOCTORAL THESIS

Anticancer efficacy and mechanism of action studies of the potent plant cycloheptapeptide compounds mavacyocines

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ABSTRACT

Background

Over the past 200 years, much attention has been paid to natural products for their great contribution in the industry of drug development as many of them have been shown effective against various diseased conditions in humans by virtue of their structural diversity and biological potency. Therefore, they are undeniably a rich resource for the discovery of novel bioactive compounds. To date, many of the mainstay anticancer agents often lead to undesirable side effects and/or develop rapid emergence of drug resistance. Therefore, new therapeutic remedies are desperately needed.

In fact, many active compounds are derived from macrocyclic natural products. The identification of their molecular targets may assist researchers to synthesize biological agents for combating particular diseased conditions. Cycloheptapeptides that modulate specific molecular pathways in suppressing the proliferation of cancer cells are potential candidates for anticancer therapeutics and/or chemopreventive agents. In the current research project, we have demonstrated that MV-A, a novel cycloheptapeptide with the unique amino acid DMCPA isolated from *Maytenus variabilis* (Loes.) C. Y. Cheng (Celastraceae), showed potent cytotoxic activities against a panel of human cancer cell lines, and is worthy for further investigation.

Objectives

The objectives of this study were to i) evaluate the anticancer effect, ii) elucidate the mechanism of action, and iii) identify the binding target(s) of the natural cycloheptapeptide MV-A.

Methods

We first carried out various kinds of cellular and animal studies for validating the *in vitro* and *in vivo* anticancer efficacy of MV-A. Next, we performed a number of

bioassays to ascertain the inhibitory effect of MV-A on several major cancer-associated pathways, including apoptosis, cell cycle arrest, senescence and metastasis. The biochemical assays included sulforhodamine B colorimetric assay, flow cytometric analyses of apoptosis and cell cycle arrest, Western blotting, real-time polymerase chain reactions (qPCR) arrays, senescence-associated β-galactosidase staining, phospho-specific protein arrays, as well as migration and invasion staining experiments. Lastly, we also identified the potential protein targets of MV-A by biochemical means, particularly the drug affinity responsive target stability (DARTS) approach.

Results

MV-A is a potent anti-proliferative agent against a variety of cancer cells. It inhibited the proliferation of the human colorectal carcinoma (CRC) HCT116 cells with an IC_{50} value of 2.28 nM. However, the application of MV-A at 2.68 nM did not induce significant apoptosis; rather it caused a notable cell-cycle arrest at the G1 phase. Moreover, the treatment with this compound (0.68 to 2.68 nM) led to a remarkable senescence in cancer cells as well as a mitigated cellular migration. Meanwhile, the expression levels of some components of the p16 cascade and PI3K-AKT pathway, so as several epithelial-to-mesenchymal transition (EMT) molecules were suppressed by MV-A. Furthermore, HSP90, calnexin, EF2, 14-3-3 and annexin A1 were identified as the direct binding targets of MV-A in our DARTS analysis.

Conclusions

In the present study, our results indicated that the novel cycloheptapeptide MV-A inhibited proliferation and migration of CRC HCT116 cells via the induction of cellular senescence and modulation of multiple pathways, including the p16/Rb, PI3K-AKT and EMT signaling pathways. These results revealed a potential role of MV-A in cancer therapy. The direct binding targets of MV-A further uncovered its molecular actions

against different diseased conditions. Our findings strongly support the development of MV-A as a therapeutic agent for combating cancerous pathologies, explicitly CRC.

Keywords: Plant natural products, colorectal carcinoma, cycloheptapeptide, anticancer, cellular senescence, PI3K-AKT pathway, EMT, binding targets.

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