DOCTORAL THESIS

Anticancer Effects and Mechanisms of Action of a Chinese Medicine Formula Comprising Sophorae Flos and Gardeniae Fructus

LI, Junkui

Date of Award:
2021

ABSTRACT

Colorectal cancer (CRC) and melanoma are major causes of cancer-related death worldwide. Signal transducer and activator of transcription 3 (STAT3) plays a pivotal role in cancer development. Inhibition STAT3 signaling is a promising strategy for treating CRC and melanoma. *Huai-Hua-San* (HHS), a traditional Chinese medicine (TCM) formula comprising Sophorae Flos (SF) and Gardeniae Fructus (GF), was documented in a Chinese medicine book *Jing-Yan-Liang-Fang* written 600 years ago. One of HHS's traditional indications is *Zangdu* that is nowadays diagnosed as cancer. HHS together with other herbs is commonly used for treating cancers including CRC and melanoma, but the chemical and pharmacological basis is unknown. Several compounds, such as quercetin and genipin, occurring in SF and/or GF have been reported to have anti-CRC and anti-melanoma properties and inhibit STAT3 signaling. In this study, we evaluated the anti-CRC and anti-melanoma effects of an ethanolic extract of HHS (HHSE), and investigated the involvement of STAT3 signaling in these effects.

We found that HHSE reduced viability of and induced apoptosis in CRC and melanoma cells, triggered S phase cell cycle arrest in CRC cells, and dampened invasion of melanoma cells. In mouse models, the human equivalent dose of HHSE inhibited growth of HCT116 CRC and B16F10 melanoma. Mechanistic studies revealed that HHSE lowered protein level of phopho-STAT3 (Tyr705) in CRC/melanoma cells and tumors. Also, HHSE lowered protein level of phospho-EGFR (Tyr1068) in CRC cells, and inhibited phosphorylation/activation of JAK2 (Tyr1007/1008) and Src (Tyr416), decreased STAT3 nuclear protein level, and down-regulated protein levels of STAT3 target genes in CRC and melanoma cells. Over-

activation of STAT3 in HCT116 and A375 cells attenuated the cytotoxic effects of HHSE.

Network pharmacology studies showed that 17 bioactive compounds are potential anti-CRC components of HHS. To determine which of the 17 compounds can inhibit Src/STAT3 signaling, we performed molecular docking to identify Src binding compounds. Results showed that ISO exhibited high affinity of binding to Src kinase domain. ISO has been reported to possess anti-CRC properties and to induce autophagy in Caco2 CRC cells. Immunoblotting showed that ISO inhibited Src/STAT3 signaling in CRC cells and tumors, and over-activation of STAT3 diminished the cytotoxic effects of ISO in HCT116 cells. Moreover, ISO induced autophagy via inhibiting AKT/mTOR signaling in CRC cells. Blocking autophagy using an autophagy inhibitor chloroquine (CQ) or 3-methyladenine (3-MA) enhanced the cytotoxic effect of ISO in CRC cells. Compared to ISO alone, ISO plus CQ or 3-MA exerted more potent apoptotic effects in CRC cells. In a HCT116 cell-bearing mouse model, 3-MA enhanced the effects of ISO in suppressing tumor growth.

In summary, our results demonstrated that HHSE has anti-CRC and anti-melanoma effects in cell and mouse models. Inhibition of STAT3 signaling contributes to the anti-CRC and anti-melanoma mechanisms of HHSE. ISO is one of the active components responsible for the anti-CRC effects of HHSE. Blocking autophagy with 3-MA or CQ in cell models, or with 3-MA in mice, enhances the anti-CRC effects of ISO. This study provides pharmacological and chemical justifications for the traditional use of the formula HHS in treating cancer, and suggests that HHSE and HHSE-derived compounds have potential to be developed into alternative and/or complementary drugs for cancer management.

Keywords: Traditional Chinese medicine; *Huai-Hua-San*; Sophorae Flos; Gardeniae Fructus; Colorectal cancer; Melanoma; Isorhamnetin; STAT3 signaling; AKT/mTOR signaling

Table of contents

List of figures

List of tables