DOCTORAL THESIS

Anti-hepatocellular carcinoma mode and mechanism of action of antrodia camphorata mycelia

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ABSTRACT

Hepatocellular carcinoma (HCC), the major form of primary liver cancer, is a common cause of cancer-related death worldwide. Signal transducer and activator of transcription 3 (STAT3) plays a pivotal role in the pathogenesis of HCC. Inhibition of STAT3 signaling has been proposed as a promising strategy for treating HCC. Due to the limitations of conventional therapeutics, increasing attention has been paid to complementary and alternative medicines (CAM) including traditional Chinese medicine (TCM) for the management of HCC. *Antrodia camphorata* (AC), a medicinal mushroom, is historically used for treating HCC. Pharmacological data showed that extracts and constituents of AC are able to inhibit STAT3 activation. Natural AC is scarce, cultured AC mycelia are becoming alternatives. AC mycelia have been demonstrated to possess anti-HCC properties. We hypothesize that inhibition of the STAT3 signaling pathway contributes to the anti-HCC mechanisms of AC mycelia. To test our hypothesis, we evaluated the safety and investigated the anti-HCC effects of the ethyl acetate fraction of an ethanolic extract of AC mycelia (EEAC); and we further explored the involvement of STAT3 signaling in EEAC's anti-HCC effects.

Acute and repeated dose 28-day oral toxicity studies showed that EEAC had no toxicity in rats. The maximum tolerable dose for acute oral toxicity and the no-observed-adverse effects level for repeated dose 28-day oral toxicity of EEAC were higher than 5,000 mg/kg body weight and 1,000 mg/kg body weight, respectively, in rats. In cultured cells, EEAC is less toxic in normal liver-derived cells than in HCC cells. In HepG2 and SMMC-7721 cells, EEAC reduced viability, induced apoptosis, and retarded migration and invasion. In SMMC-7721 cell-bearing mice, EEAC significantly suppressed tumor growth. EEAC inhibited cell proliferation,

induced apoptosis and suppressed angiogenesis in tumors. Mechanistic studies showed that EEAC downregulated protein levels of phosphorylated and total STAT3 and JAK2 (an upstream kinase of STAT3) in HCC cells and tumors. In cultured HCC cells, EEAC lowered the protein level of nuclear STAT3, decreased the transcriptional activity of STAT3, and downregulated protein levels of STAT3 targeted molecules. Over-activation of STAT3 in HCC cells diminished the cytotoxic effects of EEAC. STAT3 can be activated by receptor tyrosine kinases (RTKs). Phospho-RTK array assays showed that EEAC significantly inhibited the tyrosine phosphorylation of platelet-derived growth factor receptor-beta (PDGFR-β) in HepG2 cells. EEAC dose-dependently lowered mRNA levels of PDGF BB (a ligand of PDGFR-β) and protein levels of p-PDGFR-β and PDGFR-β in HCC cells. Activating PDGFR-β enhanced STAT3 activation, and inhibiting PDGFR-β blocked STAT3 activation in HCC cells. EEAC reversed PDGF BB induced STAT3 activation in HCC cells. Our data indicate that EEAC exerts anti-HCC effects, and inhibition of PDGFR-β/STAT3 signaling is, at least in part, responsible for these effects.

In summary, we have demonstrated that EEAC exerts anti-HCC effects without significant toxicity *in vitro* and *in vivo*. We have also demonstrated that inhibition of PDGFR-β/STAT3 signaling contributes to the anti-HCC mechanisms of EEAC. Our findings provide a pharmacological basis for the development of EEAC as a modern anti-HCC agent and for the traditional use of AC in treating HCC. In addition, our data suggest that the PDGFR-β/STAT3 pathway plays a pathogenic role and presents a novel therapeutic target in HCC.

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