P – 259 A novel anti-tumorigenic mechanism by herbal extract saikosaponin-d through p-STAT3/C/EBP*β* signaling suppression of COX-2 in liver cancer

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Introduction: Liver cancer ranks second worldwide in cancer-related deaths, advocating for more efficacious therapeutic strategies for hepatocellular carcinoma (HCC). Saikosaponin d (SSd), a natural compound and one of the most pharmacologically active saponin extracts from the root of Radix Bupleuri, holds promise as a new treatment for HCC. The aim of the study was to investigate the antitumorigenic effects of SSd, in two human hepatocellular carcinoma cell lines and define the signaling pathway of how SSd suppresses the expression of cyclooxygenase (COX)-2 in liver cancer.

Methods: Human hepatocellular carcinoma cell lines, SMMC-7721 and HepG2, were used to study the effect of SSd in liver cancer. The MTT assay was used to assess cancer cell proliferation. Apoptosis analysis was performed using annexin V-FITC/PI staining and flow cytometry. In situ protein expression of STAT3, p-STAT3 and COX-2 was examined by immunochemistry. Western blot and real-time quantitative PCR (RT-qPCR) were used to investigate the anti-tumor effect of SSd in the COX2, p-STAT3/C/EBPβ signaling pathway.

Results: SSd effectively inhibited the proliferation of hepatocellular cells in a dose-dependent manner. Apoptosis was significantly increased in cells treated with SSd with dosage as low as 2.5 μg/ml and the apoptotic rate increased with increasing treatment concentrations. SSd treatment led to an increase in the pro-apoptotic protein Bax and a decrease in the anti-apoptotic proteins Bcl-2. The protein expression of COX-2, confirmed by Western bot and immunochemical staining, was significantly decreased by SSd accompanied by a similar decrease in C/EBPβ and p-STAT3. The mRNA expression of STAT3, C/EBPβ and COX-2 was consistently decreased by SSd in a dose-dependent fashion. AG490, a JAK2 kinase inhibitor, produced similar effects on STAT3, C/EBPβ and COX-2 in both cell lines tested.

Conclusion: Our data demonstrated that SSd has a promising anti-tumorigenic property in the liver and these results suggest that this anti-tumourigenic effect is potentiated through inhibition of COX-2 via the p-STAT3/C/EBPβ signaling pathway. These findings further our understanding of the pharmacological action of SSd and provide new information on the potential of SSd as a natural liver cancer therapeutic agent.