DOCTORAL THESIS

A mechanistic study on the use of the TCM formula si-jun-zi-tang as an adjuvant anti-melanoma agent

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ABSTRACT

Melanoma is among the most aggressive and treatment-resistant cancers. Currently, available therapies for melanoma are not satisfactory, and the prognosis for patients with metastatic melanoma is still poor. Vemurafenib, a BRAF kinase inhibitor (BRAFi), provides an approximately 50% response rate in patients with metastatic melanoma, but eventually, relapse occurs due to acquired resistance to the drug. Novel therapeutics and BRAFi adjuvants for treating melanoma are needed. *Si-Jun-Zi-Tang* (SJZT) is a traditional Chinese medicine formula used to treat chronic and debilitating diseases including melanoma. SJZT-based therapies alone or in combination with chemotherapies have achieved good clinical outcomes in melanoma management. However, the pharmacological basis of SJZT for its clinical use in melanoma treatment is not fully understood. c-Met is a receptor tyrosine kinase (RTK), and hepatocyte growth factor (HGF) is the only known ligand of c-Met. Abnormal activation of HGF/c-Met has been implicated in melanoma progression. HGF/c-Met has been proposed as a therapeutic target for melanoma. Some bioactive constituents in SJZT have been shown to inhibit c-Met signaling. In this study, we investigated the anti-melanoma effects and c-Met signaling-related action mechanisms of SJZT.

Our *in vivo* study showed that SJZT-A, an ethanolic extract of SJZT, inhibited B16 tumor growth in mice without overt toxicity. Mechanistic investigations revealed that SJZT-A elevated miR-34b (a tumor-suppressing miRNA), and lowered c-Met (a miR-34b target gene) and β-catenin (a downstream molecule of c-Met signaling) expression levels in the B16 tumors. But SJZT-A failed to reduce the viability of B16 and A375 melanoma cells. Active component-enriched SJZT-B, which was prepared from SJZT-A using macroporous resin column chromatography, exhibited potent anti-proliferation effect and inhibited miR-34b/c-Met/β-catenin signaling in cultured melanoma cells. Overexpression of constitutively active β-catenin partially diminished the inhibitory effect of SJZT-B on cell proliferation. SJZT-B also exerted anti-proliferative effects, inhibited c-met signaling, and induced ER stress in vemurafenib resistance melanoma cells. *In vivo* experiments showed that intragastric administration of SJZT-B for consecutive 14 days overcame vemurafenib resistance in melanoma-bearing mice without observable toxicities. Overall, these results indicate that SJZT has anti-melanoma effects and is relatively safe.

Also, we found that licochalcone A, a flavonoid presented in SJZT-B, overcame vemurafenib resistance both *in vitro* and *in vivo*, as well as inhibited c-Met signaling and induced ER stress in A375-VR cells, which is in line with the effects of SJZT-B in melanoma. The role of triggering ER stress in licochalcone A's effects in overcoming vemurafenib resistance effects has also been established. Overall, these results suggest that licochalcone A is one of the active compounds responsible for the anti-melanoma effects of SJZT-B.

In conclusion, our results demonstrated that SJZT has anti-melanoma effects and is safe in cell and mouse melanoma models. Licochalcone A has been identified to be one of the active components responsible for the anti-melanoma effects of SJZT. This study provides a pharmacological and chemical basis for the traditional use of the formula SJZT in treating melanoma, and suggests that SJZT and SJZT-derived compounds have the potential to be developed as modern alternative and/or complementary agents for melanoma management.

Key words: *Si-Jun-Zi-Tang*; traditional Chinese medicine; melanoma; miR-34b; c-Met; β-catenin, ER stress, licochalcone A

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