

DOCTORAL THESIS

Anti-melanoma effects and mechanism of action of a herbal formula comprising Sophorae flos and Lonicerae Japonicae flos

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ABSTRACT

A herbal formula (SL) comprising edible *Sophorae Flos* and *Lonicerae Japonicae Flos* was used to treat melanoma in ancient China. In current Chinese medicine practice, the two ingredient herbs of SL are commonly prescribed by Traditional Chinese medicine (TCM) doctors for treating melanoma. However, there is no modern clinical or experimental evidence about the anti-melanoma actions of this formula. Signal transducer and activator of the transcription (STAT3), which is constitutively activated in melanoma, has been proposed as one of the anti-melanoma targets. Some natural compounds in SL have been shown to assault cancers including melanoma *via* inhibiting STAT3 signaling. In this study, we investigated the anti-melanoma effects and explored STAT3 signaling-related mechanism of action of SL. We also identified bioactive components responsible for SL's anti-melanoma effects.

Our *in vitro* and *in vivo* studies showed that SLE, an ethanolic extract of SL, induced apoptosis, inhibited proliferation, migration and invasion in melanoma cells, inhibited melanoma growth, angiogenesis and prolonged host survival in melanoma-bearing mice. SLE significantly suppressed the activation of STAT3 and its upstream kinase Src in both mouse melanoma tissues and cultured melanoma cells. In melanoma cells, we also found that SLE restrained STAT3 nuclear localization and inhibited the expression of STAT3-regulated genes related to melanoma growth, metastasis and angiogenesis. Overactivation of STAT3 in A375 human melanoma cells diminished the anti-proliferative, pro-apoptotic and anti-invasive effects of SLE. RNA-seq and small RNA sequencing analyses showed that SLE altered both the gene expression profile and miRNA signature in B16F10 melanoma tissues. Based on the RNA-seq data, we further validated that SLE inhibited the IL-17-IL-6-STAT3 axis in melanoma. Verification assays for the candidate miRNAs suggested that the significantly upregulated miR-205-5p is a possible target of SLE. Enforced miR-205 expression has been shown to suppress EMT in melanoma cells. In this study, we demonstrated that SLE inhibited melanoma cell EMT, and miR-205-5p knockdown diminished this effect of SLE. In addition, we computationally

demonstrated that luteolin, a naturally occurring edible flavone abundant in *Lonicerae Japonicae Flos*, could directly bind to Src kinase domain. Experimentally, we verified that luteolin inhibited the Src/STAT3 signaling in both melanoma cells and tissues. In addition to inhibit STAT3 activation, luteolin promoted ubiquitin-proteasome pathway-mediated degradation of STAT3. Luteolin also exerted evident *in vitro* and *in vivo* anti-melanoma effects, and overactivation of STAT3 diminished its anti-melanoma effects.

In conclusion, we demonstrated that SLE exerted *in vivo* and *in vitro* anti-melanoma effects, and inhibition of Src/STAT3 signaling and elevation of miR-205-5p expression contributed to these effects. Luteolin was identified to be one of the active components responsible for the inhibitory effects of SLE on STAT3 signaling and the anti-melanoma effects of SLE. This study provides a pharmacological and chemical basis for the traditional use of the formula SL in treating melanoma, and suggests that SLE and SLE-derived compounds have the potential to be developed as modern alternative and/or complimentary agents for melanoma management.

Key words: *Sophorae Flos*; *Lonicerae Japonicae Flos*; *Sophora japonica*; *Lonicera japonica*; Melanoma; STAT3; miR-205-5p; Luteolin;

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