

## DOCTORAL THESIS

### A study on the involvement of TLR4/STAT3 signaling in the antimelanoma effects of atractylenolide II

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*Date of Award:*  
2017

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## ABSTRACT

Melanoma is the leading cause of skin cancer-related death. The STAT3 (signal transducer and activator of transcription 3) and TLR4 (toll-like receptor 4) signaling pathways have been shown to be activated in melanoma. Activation of each of the two pathways can promote melanoma growth, angiogenesis and metastasis. Suppressing TLR4 signaling or STAT3 signaling has been proposed as an approach for melanoma management although the TLR4/STAT3 pathway has not yet been established in melanoma. *Atractylodis Macrocephalae Rhizoma* (*Baizhu* in Chinese), a *Qi*-tonifying Chinese medicinal herb, is commonly prescribed by Chinese medicine doctors for treating melanoma. Our previous studies demonstrated that atractylenolide II (AT-II), isolated from *Atractylodis Macrocephalae Rhizoma*, could induce apoptosis, and inhibit proliferation and migration in B16 melanoma cells. However, the antimelanoma properties of AT-II and the underlying molecular mechanisms have not been fully understood. In this study, we further investigated the antimelanoma effects of AT-II *in vivo* and *in vitro*, and explored the TLR4/STAT3 signaling-related mechanism of action of AT-II.

Results showed that AT-II induced apoptosis, and inhibited proliferation, migration and invasion in multiple melanoma cells, and significantly inhibited melanoma growth, angiogenesis and metastasis in mice. AT-II suppressed the activation of STAT3 and Src (a STAT3 upstream tyrosine kinase) in mouse melanoma tissues and inhibited the EGFR/Src/STAT3 signaling in cultured melanoma cells. The free binding energy of AT-II with EGFR (an upstream receptor tyrosine kinase of STAT3) was relatively low in molecular docking assays, suggesting that AT-II might inhibit EGFR activation *via* other molecules. We found that activation of TLR4 enhanced EGFR/Src/STAT3 signaling in melanoma cells, and activation of the TLR4/STAT3 pathway contributed to melanoma progression *in vivo* and *in vitro*. These observations suggested that the TLR4/STAT3 pathway was established in melanoma. Molecular docking showed that AT-II could bind to the TLR4/MD-2 receptor complex. AT-II reduced the binding of LPS (a TLR4 ligand) to TLR4, and inhibited LPS-triggered activation of EGFR/Src/STAT3 signaling as well as LPS or MPLAs (synthetic monophosphoryl lipid A, a TLR4 agonist) induced invasion

in melanoma cells. Overexpression of a constitutively active variant of STAT3 (STAT3C) in A375 cells diminished the anti-proliferative, apoptotic and anti-invasive effects of AT-II; and overexpression of an active form of TLR4 in A375 cells diminished AT-II-exerted anti-invasive effects in cultured cells, and attenuated the inhibitory effects of AT-II on tumor growth and angiogenesis in mice. These suggested that suppression of TLR4/STAT3 signaling contributed to the antimelanoma effects of AT-II.

In conclusion, we established the TLR4/STAT3 pathway in melanoma, which provides novel insight into melanoma pathophysiology. We demonstrated that AT-II exerted antimelanoma effects *in vivo* and *in vitro*, and inhibition of TLR4/STAT3 signaling contributed to these effects. These findings advanced our understanding of the antimelanoma properties and the underlying mechanism of action of AT-II, and provided a chemical and pharmacological justification for the clinical application of *Atractylodis Macrocephalae Rhizoma* in melanoma management. This contribution is significant because it is one step in a continuum of research that is expected to lead to future clinical trials of AT-II as a novel antimelanoma agent.

**Key words:** *Atractylodis Macrocephalae Rhizoma*; Atractylenolide II; Melanoma; STAT3; TLR4; Chinese medicine

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