DOCTORAL THESIS

Mechanistic study of Chinese herbal medicines on melanogenesis and anti-melanoma effects

Tsang, Ting Fung

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Mechanistic Study of Chinese Herbal Medicines on Melanogenesis and Anti-Melanoma Effects

TSANG Ting Fung

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Principal Supervisor: Prof. Wen Luan Wendy HSIAO

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Abstract:

Melanogenesis is a physiological process of melanin production in response to UV exposure, which is modulated through multi-signaling pathways including cAMP/PKA, Wnt/β-catenin and MAPK signaling cascades. However, deregulation of the melanogenic process might trigger the onset of skin cancer. In this study, we investigated both the melanogenesis and anti-melanoma effects of triterpenoid saponins of *Gynostemma pentaphyllum* (GpS), a medicinal herbal plant. In the investigation of the potential melanogenic activity of GpS, we found that non-toxic dosages of GpS markedly increased melanin formation. Western blot analysis showed that GpS treatment significantly up-regulated the expression levels of the key melanogenic proteins, including tyrosinase (TYR), microphthalmia-associated transcription factor (MITF), TRP-1 and TRP2 in a dose-dependent manner. The p-CREB, which is the down-stream target of PKA is also elevated upon GpS treatment. We further observed that H89, a PKA inhibitor, attenuated the GpS induced tyrosinase activity, melanin content, the expression of p-CREB. In addition to the cAMP/PKA signaling pathway, GpS treatment also up-regulates the nuclear β-catenin of the Wnt signaling pathway which is involved in the transcriptional activation of MITF in melanogensis. To identify the active saponins, GpS was fractionated in the MCI-CHP 20P column and eluted with methanol in a gradient of 50 to 100%. The resulting ten fractions were tested for their melanogensis activities.

We found that Fractions 5 to 9 showed the strongest effect in melanin synthesis in B16 cultures. Western blot analysis revealed that Fractions 5 to 9 significantly up-regulate the expression of TYR and MITF, while Fractions 7 to 9 are more effectively increased nucleus β-catenin expression.

Our early works showed that GpS exhibits strong anti-cancer effects in both cellular and animal models. We were wonder whether GpS would exert anti-cancer effect against melanoma. To address this question, we tested and showed that GpS inhibited B16 cells migration in a dose-dependent manner. Tumorigenisis assay demonstrated the GpS significantly suppresses the growth of B16-induced tumor in congenic mice. Our study seems to be in line with the recent reports that stimulation of melanogenesis might be associated with the anti-melanoma effect by decreasing proliferation and invasiveness of melanoma (Chien, *et al*.,2009). Based on the above results, we proposed that GpS might exert the anti-melanoma effect by stimulating the melanogenesis through the activation of cAMP/PKA and Wnt/β-catenin signaling pathways.

In addition to the work on GpS, we also investigated the potential anti-melanogenic effect of Qian-Wang-Hong-Bai-San (QW), which had been used traditionally in China as a skin whitening formula. QW inhibited melanin synthesis and decreased tyrosinase activity in B16 cells at no or mild cytotoxic dosages. Western blot analysis showed that

QW treatment down-regulated the levels of p-p38, p-CREB, MITF, TYR, TRP-1 and TRP-2 in a dose-dependent manner. At the same time, QW treatment for 48 h blocked the IBMX-induced elevation of cellular melanin content and tyrosinase activity. To conclude, the anti-melanogenic activity of QW in B16 melanoma cells can be attributed, at least in part, to the inhibition of the p38 MAPK and PKA signaling pathways. Our data provides, for the first time, the underlying mechanism of the skin whitening property of this ancient formula.

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