Iranian J Publ Health, Vol. 43, No.6, June 2014, pp.847-848

Letter to the Editor

Icariin Induced B16 Melanoma Tumor Cells Apoptosis, Suppressed Tumor Growth and Metastasis

**** Xiuying LI [1], Jinfan SUN [2], Shunqin HU [3], Jiaxin LIU [1]***

1. *Medical Faculty, Kunming University of Science and Technology, Kunming, China*
2. *LaoBoYunTang Medicine Co., Ltd, Chuxiong, Kunming, China*
3. *Dept. of Gynecology and Obstetrics, the First Affiliated Hospital of Kunming Medical University, Kunming, China*

***Corresponding Author:** Email: lixying98@163.com

(Received 10 Mar 2014; accepted 24 Mar 2014)

Dear Editor-in-Chief

"(deleted) Melanoma is often considered as one of the most aggressive and treatment-resistant human cancers" (1). The need for more effective treatments is pressing. Plants are valuable sources of bioactive compounds and used for medicinal purposes in Asia including China. Icariin (C33H40O15, MW: 676.65) is a major component isolated from *Epimedium brevicornum*. As a highly interesting natural flavonoid compound for drug development, icariin has a broad spectrum of pharmacological functions, such as inhibiting tumor growth (2), inhibiting tumor cells to invade and migrate (3). Apoptosis is a cell defense mechanism to eliminate malignant cells and plays an important role in preventing tumor development. In fact, many anti-cancer drugs can induce apoptosis through regulating apoptosis-associated signaling (4, 5). Caspases play a pivotal role in apoptosis (6). Based on previous reports, we hypothesized whether icariin could inhibit B16 melanoma tumor cells' growth by apoptosis.

The B16 tumor cells (ATCC) were incubated in complete medium containing different concentrations of icariin (20-200μg/ml). After 72h of incubation, the viability of the cells was determined by the MTT colorimetric assay. Our study demonstrated that icarrin inhibited the proliferation of B16 tumor cells in a dose dependent manner, with IC50 values (the concentration of drug inhibiting 50% of cells) at 72h around 84.3ug/ml. The exponentially growing B16 tumor cells were cultured with 84.3ug/ml icariin for 72h; and their apoptosis were analysis by flow cytometry and DAPI nuclear staining. We observed cytoplasmic shrinkage, nuclear condensation and the formation of apoptotic bodies in B16 melanoma tumor cells treated with icariin by Dapi staining; and icarrin can induce B16 melanoma tumor cells apoptosis by flow cytometry analysis. The caspase-9 was identified by western blot analysis; we found that icarrin can decrease the expression of procaspase-9 while increasing cleaved caspase-9 expression at protein level. To investigate the ability that icariin can inhibit the tumor growth in vivo, C57 mice, 6 to 8 weeks old were randomly divided into three groups (n=6), they were prevention and therapy group, therapy group and vehicle control group. Prevention and therapy group was given icariin 65ug/kg every day by oral gavage for 15 days. Then tumor xenografts were established by 5×10^5 B16 melanoma cells injected s.c. into the right flank of the mice. Treatments were initiated on the day of inoculation. Mice were given icariin (65ug/kg) or vehicle every day by oral gavage for 20 days. Our study indicated that icariin can apparent inhibit tumor growth in

xenografted B16 melanoma mice model, and the lifespan of mice given icariin was apparent prolonged than control mice. To investigate the inhibitory effects of icariin on tumor cells metastasis, B16 melanoma cells metastasis model was established by i.v. injecting $1x10^5$ B16 melanoma cells into C57 mice, and the administration was same. Mice were sacrificed when the mice in the control group began to die and lung was harvested to measure the metastasis node number. We found that icariin can apparent inhibit the metastasis of B16 melanoma cells. Furthermore, our study indicated that prophylactic and therapeutic with icariin simultaneously was more effect; and indicated that icariin has some immunomodulatory functions; this is compatible with the observation that icariin had the immunoregulatory effect (7).

Taken together, our findings demonstrate that icariin could induce B16 melanoma tumor cells apoptosis in vitro and inhibit tumor growth and metastasis in vivo.

Acknowledgements

The authors declare that there is no conflict of interest.

References

1. Tsao H, Chin L, Garraway LA, Fisher DE (2012). Melanoma: from mutations to medicine. *Genes Dev*, 26 (11): 1131-55.
2. Li S, Dong P, Wang J, Zhang J, Gu J, Wu X, Wu W, Fei X, Zhang Z, Wang Y, Quan Z, Liu Y (2010). Icariin, a natural flavonol glycoside, induces apoptosis in human hepatoma SMMC-7721 cells via a ROS/JNK-dependent mitochondrial pathway. *Cancer Lett*, 298 (2): 222-30.
3. Wang Y, Dong H, Zhu M, Ou Y, Zhang J, Luo H, Luo R, Wu J, Mao M, Liu X, Zhang J, Wei L (2010). Icariin exterts negative effects on human gastric cancer cell invasion and migration by vasodilator-stimulated phosphoprotein via Rac1 pathway. *Eur J Pharmacol*, 635: 40–48.
4. Lowe SW, Lin AW (2000). Apoptosis in cancer. Carcinogenesis, 21: 485-495.
5. Fesik SW (2005). Promoting apoptosis as a strategy for cancer drug discovery. *Nat Rev Cancer,* 5: 876-85.
6. Ghobrial IM, Witzig TE, Adjei AA (2005). Targeting apoptosis pathways in cancer therapy. *CA Cancer J Clin*, 55: 178–194.
7. Liang HR, Vuorela P, Vuorela H, Hiltunen R (1997). Isolation and immunomodulatory effect of flavonol glycosides from Epimedium hunanense. *Planta Med*, 63: 316–9.