University of Macau

Abstract

CALYCOSIN PROMOTES ANGIOGENESIS INVOLVING ESTROGEN RECEPTOR AND MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNALING PATHWAY

by Tang JingYan

Thesis Supervisor: Dr. Ming Yuen LEE

Chinese Medicinal Science

Angiogenesis, the formation of new blood vessels, is essential for normal growth and homeostasis in human body. However, certain diseases can be exacerbated by the loss of balance in angiogenesis, which results in either excessive or insufficient blood vessel formation. Diseases such as cancer, diabetic retinopathy and rheumatoid arthritis are characterized by excessive blood vessel formation while peripheral and coronary ischemia and infarction, chronic wound healing failure and ulcers are characterized by insufficient blood vessel formation. *Radix Astragali*, named huangqi in Chinese, is commonly used in the prescriptions of traditional Chinese medicine. In traditional systems, it is used to replenish the vital energy for the treatment of lacking strength, anorexia and loose stools, prolapse of uterus and anus, spontaneous sweating, and chronic nephritis with edema and proteinuria, and to dispel pus and accelerate the healing of chronic ulcers. These suggest that *Radix Astragli* is a potential candidate for treating diseases associated with dysregulation of angiogenesis.

The angigogenic effect of *Radix Astragali* Extract (RAE) and its underlying

mechanisms of action are yet to be fully elucidated by real-time imaging-based screening in live cell and zebrafish bioassays. In this study, calycosin, a major constituents isolated from RAE, was identified to have potent angiogenic activity using multiple bioassays. Calycosin specifically induced human endothelial cells (HUVECs) proliferation and the number of branching points during endothelial cell capillary formation on Matrigel but inhibited breast cancer cell proliferation. Calycosin-induced angiogenesis involved activation of ERK1/2 and p38 MAPK, and specific blockers of ERK1/2 and p38 MAPK inhibited the calycosin-induced HUVEC proliferation. Meanwhile, calycosin competitively bound with estrogen receptor (ER) in a cell free competitive binding assay, and modulated ERα and ERβ transcriptional reporters in cell based assays. The agonistic activity of ERα and ERβ induced by calycosin was slight, while the antagonistic activity was significant. Since calycosin selectively modulated ER transcriptional activation activities, the effect of ER inhibitor on calycosin-induced HUVEC proliferation and the expression of phospho-ERK1/2 were examined. These data suggest that calycosin acts as a novel selective estrogen receptor modulator (SERM), and promotes angiogenesis through activation of ER and MAPK signaling pathway. The results provide rationales for further development of *Radix Astragali* and its active constituent, calycosin as therapeutic agent on treatment of estrogen deficient problems such as cardiovascular diseases in post-menopausal women.