

University of Macau

Abstract

**Nobiletin inhibits angiogenesis on human umbilical vein endothelial cells**

**(HUVECs) *in vitro* and zebrafish embryos *in vivo***

by Kai Heng Lam

Thesis Supervisor: Dr. Simon, Ming Yuen Lee

Chinese Medicinal Sciences

*Pericarpium Citri Reticulate*, known as “Chen Pi” in Chinese, the dried peel of mature fruit of *Citrus reticulata*, is commonly used in traditional Chinese medicine to treat respiratory diseases such as cough with sputum. Up to now, over ten different polymethoxylated flavones such as narirutin, nobiletin, and tangeretin have been isolated and characterized from *Pericarpium Citri Reticulatae*. Nobiletin (5,6,7,8,3',4'-hexamethoxyflavone), and its derivatives have been reported to have a broad range of biological effects including anti-inflammatory and anti-carcinogenic activities, and neurotrophic action. Recently, we are the first team to identify nobiletin having potent anti-angiogenic activity in human endothelial cells and zebrafish embryos.

Angiogenesis plays an important pathological role in a wide range of diseases, such as cancer and inflammation. Anti-angiogenesis is demonstrated to be effective therapeutic strategy on inhibitory of tumor growth by cutting down the delivery of nutrient and oxygen to tumor target. Recently, angiogenesis is found to be a prominent

feature of the structural tissue remodelling that occurs in various chronic airway diseases associated with regional vascular inflammation, such as asthma and smoking-related Chronic Obstructive Pulmonary Disease (COPD). *Pericarpium Citri Reticulate* are widely used to alleviate coughs and reduce phlegm in the respiratory tract while compounds isolated from *Pericarpium Citri Reticulatae* were well reported to have potent anti-carcinogenesis and anticancer effect in several recently published *in vitro* and *in vivo* studies. It would be very interesting to explore if underlying mechanism of action of *Pericarpium Citri Reticulatae* involves anti-angiogenesis.

Our results showed that water and ethanol extracts of *Pericarpium Citri Reticulate* could inhibit inter segmental vessel (ISV) and dorsal anastomotic vessel (DLAV) formation in live transgenic zebrafish embryo (Tg: *flil*-EGFP) that expresses GFP in the vasculature. We further identified nobiletin as an active constituent to exhibit anti-angiogenic effect, preferentially in 24 hpf zebrafish embryos but less effect on the phenotype of well established vasculature system in 56 hpf zebrafish larva.

Nobiletin induced G1/G0 cell cycle arrest in GFP positive cells which represented endothelial cell population in the zebrafish. As in *in vitro* study, nobiletin inhibited VEGF induced human endothelial cell proliferation by inducing G1/G0 cell cycle arrest in a dose dependent manner. The anti-angiogenic effect of nobiletin was found to involve specific upregulation of *VEGFa* mRNA expression in a dose dependent manner, without alternation of *flk-1*, *flt-1* and *flt-4* mRNAs expression.

Angiogenesis plays an important pathological role in a wide range of diseases, such as cancer and respiratory inflammatory diseases. In this study, we found that nobiletin exerted anti-angiogenic effect *in vitro* and *in vivo* through regulating cell cycle arrest

and VEGF pathway. These findings are very important to provide rationales for future development of *Pericarpium Citri Reticulate* and its constituent nobiletin, on treatment of tumour angiogenesis and various inflammatory diseases.