

## **Pinocembrin 抑制酒精誘發之 tissue transglutaminase 表現於人類**

### **肝癌細胞之分子機制研究**

## **The Molecular Mechanisms of Pinocembrin Inhibited Ethanol-induced Tissue Transglutaminase Activity in Human Hepatocellular Carcinoma Cells**

### **中文摘要**

tissue transglutaminase (tTG)是一個 Ca<sup>2+</sup>-dependent 的酵素，藉由催化 protein-protein 間 cross-linking，而穩定細胞外基質蛋白或造成纖維化的發生。酒精被認為是導致肝纖維化的主因，我們首先研究酒精在人類肝癌細胞株 Hep3B、HepG2 中調控 tTG mRNA 表現的機制。我們發現在酒精刺激下只有 Hep3B 細胞被誘發 tTG mRNA 的表現。文獻指出，轉錄因子 NF- $\kappa$ B 參與 tTG 的調控表現。接著我們利用 NF- $\kappa$ B 的抑制劑--TLCK 來研究 tTG 和 NF- $\kappa$ B 間的相關性，研究發現，酒精誘發的 tTG 表現會被 TLCK 所抑制，所以我們認為 NF- $\kappa$ B 參與 tTG 表現的調控。另外，在 Hep3B 細胞中也發現酒精誘發 ERK 磷酸化的情形，並且 NF- $\kappa$ B 的活化被 MEK 的抑制劑--PD98059 所抑制。因此，我們推測酒精是透過 ERK 及 NF- $\kappa$ B 的訊息傳遞路徑來調控 tTG 的表現。Pinocembrin 是富存於蜂膠及蜂蜜中之 flavanone，可作為抗氧化劑。我們發現 pinocembrin 可抑制由酒精所誘導的 tTG 表現及活性，並抑制 NF- $\kappa$ B p65 subunit 轉位到核裡的能力。另外 pinocembrin 也抑制酒精所誘發之 ERK 的磷酸化現象。綜合上述結果，我們證實 pinocembrin 透過抑制 ERK 與 NF- $\kappa$ B 的活化來調控 tTG 的作用，而具有預防肝臟纖維化的潛力。

### **英文摘要**

Tissue transglutaminase (tTG) is a Ca<sup>2+</sup>-dependent enzyme which plays an important role in the stabilization of the extracellular matrix (ECM) and formation of fibrotic lesions by catalysing protein-protein cross-linkage. Ethanol is considered as a significant inducing mediator of the liver fibrosis, we firstly investigated the mechanism of how ethanol regulates tTG mRNA expression in the Hep3B and HepG2 cell. We found that only Hep3B cell synthesize tTG mRNA after addition of ethanol. Recent works have shown that transcription factor NF- $\kappa$ B was required to regulate tTG expression. In this study, we used the NF- $\kappa$ B-specific inhibitor, TLCK, to investigate the correlation between tTG and NF- $\kappa$ B and demonstrated that ethanol-induced tTG expression can be suppressed after addition of TLCK, providing evidence that NF- $\kappa$ B may participate the regulation in tTG expression. In addition, phosphorylation of ERK was also induced by ethanol in the Hep3B cell, and

activation of NF- $\kappa$ B can be suppressed by adding MEK inhibitor, PD98059. Therefore, ERK and NF- $\kappa$ B are the possible signaling molecules to mediate the tTG activation. Pinocembrin, the most abundant flavanone which was isolated from the various types of propolis and honey as an antioxidant. We found that the pinocembrin suppressed ethanol-induced tTG expression and its activity, along with the inhibition of nuclear translocation of NF- $\kappa$ B subunit p65. Furthermore, pinocembrin also suppressed ethanol-induced activation of ERK. Overall, these data demonstrated that the pinocembrin inhibits the activation of ERK-and NF- $\kappa$ B-mediated tTG expression, which shows that pinocembrin has a potential to prevent liver fibrosis.