在酒精長期刺激人類肝癌細胞株 Hep 3B 的模式中，探討酒精誘發 Tissue transglutaminase 表達的分子機制

Study on the ethanol long-term stimulating Hep 3B model for examining the molecular mechanism of ethanol- induced tissue transglutaminase expression.

中文摘要

本研究主要探討在酒精長期處理的 Hep 3B 細胞株中，酒精誘發 tissue transglutaminase（TGM2）表達的分子機制。TGM2 具有多種功能，包括 Ca2+-dependent transamidation，以催化蛋白質之間的 cross- linkage，穩定細胞外基質蛋白或造成纖維化的發生；另外一個功能爲 GTPase protein，作爲訊息傳遞分子。在本研究中，比較不處理酒精的 Hep 3B 細胞株和酒精（0.06％）長期處理的 Hep 3B 細胞株時，可以發現在酒精長期處理的 Hep 3B 細胞株，其 TGM2 的 mRNA 和蛋白質皆有穩定的表現。在酒精長期處理下，欲知影響 TGM2 轉錄的轉錄因子，而文獻指出，NF-kappaB 參與調控 TGM2 的表現，此外亦有文獻指出，受到酒精刺激，AP1、SP1 會被活化並轉位到細胞核內，進而調控目標基因的表現。因此利用 chromatin immunoprecipitation 和核質分離，證明在酒精長期處理的 Hep 3B 細胞株中，調控 TGM2 mRNA 表現的轉錄因子爲 AP1、SP1，並非 NF-kappaB。文獻指出，酒精會透過 MAPK 作爲訊息傳遞路徑，特別是 ERK pathway，在酒精長期處理的 Hep 3B 細胞株中，本研究證實酒精不是透過 ERK 的訊息傳遞路徑來誘導 TGM2 表現。

英文摘要

The purpose of this investigation is to study the molecular mechanism of ethanol-induced tissue transglutaminase expression in ethanol long term- treated Hep 3B cells. TGM2 is a multifunctional enzyme. TGM2 is a Ca2+- dependent enzyme which plays an important role in the stabilization of the extracellular matrix (ECM) proteins and formation of fibrosis by catalyzing protein-protein cross-linkage. TGM2 also is a GTPase protein. In this investigation, mRNA and protein levels of TGM2 can express stablely in ethanol long term-treated Hep 3B cells. Recent works have shown that transcription factor NF-kappaB was required to regulating TGM2 expression. Another recent works have shown that transcription factors AP1 and SP1 are activated and translocated into nucleus for regulating gene expression when exposing ethanol. Using chromatin immuno- precipitation and isolation of nucleus and cytosolic extracts, we found that transcription factors regulating TGM2 expression are AP1 and SP1, not NF-kappaB in ethanol long term-treated Hep 3B cells. Recent works have shown the

ethanol-induced signal transduction go through MAPK pathway, especially ERK pathway. We found the signal transduction pathway of regulating TGM2 expression isn''t go through ERK pathway in ethanol long term-treated Hep 3B cells.