以斑馬魚卵產製新穎抗高血壓胜

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摘 要

高血壓（hypertension）疾病至今為國人十大死因之一，同時亦是罹患心血管疾病之風險因子。然隨消費者預防保健意識之提升，控制血壓相關機能性食品與類藥物營養劑之需求已日益增加，其中預防高血壓之血管緊縮素轉化?抑制胜?（angiotensin converting enzyme inhibiting peptide，ACEIP）更受矚目。目前國立中興大學生命科學系陳全木教授與大葉大學分子生物科技學系陳小玲教授已自 kefir grain 發酵乳原料中分離出一新穎之抗高血壓胜?（anti-hypertensive peptide No. 1，AP1），經定序解析後發現此關鍵胜?為 β-酪蛋白（β-casein）中一特定片段之胜?，其活性表現亦優於現有市售之調節血壓健康食品產品之關鍵主成分VPP 與 IPP。本論文先前以斑馬魚（zebrafish，Danio rerio）為模式動物產生抗菌蛋白（anti-microbial peptide）平台技術之經驗，針對上述之功能性蛋白生產進行試驗。本試驗將抗高血壓胜?AP1 基因構築於斑馬 VTG 之啟動子下游，續將之選殖入pAAV-IRES-hrGFP 及 pEGFP-N1 之表現載體，並以吳郭魚卵巢細胞株（tilapia ovary，TO-2 cells）分析其表現，並以顯微注射導入斑馬魚受精卵內，觀察並分析其表現位置。結果顯示於細胞轉染第 48 小時後可觀察到其綠螢光之信號，續以 RT-PCR 與西方墨點反應分析，進一步證實具綠螢光蛋白之表現。SDS PAGE 分析亦顯示 AP1 蛋白之可能表現。另將上述之載體以顯微注射之方式轉置至斑馬魚胚胎中之結果顯示，於注射後 24、72、120 及 168 小時以螢光顯微觀察於卵黃囊或肝臟皆具綠螢光之表現，此親代斑馬魚並可產製具螢光表現之第二子代斑馬魚。統計分析結果顯示，96 顆魚卵中，6 顆具螢光表現並持續表現至受精後 2 個月，可達 6.2%之成功率。因此，本試驗已將 AP1 基因成功轉置於斑馬魚基因組中，後續將可藉斑馬魚為生物反應器產製抗高血壓胜?（AP1）並進行功能性分析。

關鍵詞 : 高血壓、 β-酪蛋白、抗高血壓胜?、吳郭魚卵巢細胞株、斑馬魚

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