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| • 系統編號 | RN9406-0093 | | |
| • 計畫中文名稱 | 評估眼角膜專一啓動子之超微粒載體傳送表現 | | |
| • 計畫英文名稱 | Evaluation of Sspecific-Cornea Promoters with Polymeric Mmicelles Delivery | | |
| • 主管機關 | 行政院國家科學委員會 | • 計畫編號 | NSC91-2320-B038-036 |
| • 執行機構 | 臺北醫學大學藥學系 | | |
| • 本期期間 | 9108 ~ 9207 | | |
| • 報告頁數 | 10 頁 | • 使用語言 | 英文 |
| • 研究人員 | 廖嘉鴻 Liaw, Jia-Horng | | |
| • 中文關鍵字 | 眼角膜; 角蛋白; 圓椎形角膜素; 啓動子; 巨細胞病毒; lacZ 基因; 聚氧化乙烯膠微粒 | | |
| • 英文關鍵字 | Cornea; Keratin; Katakana; Promoter; Cytomegalovirus; lacZ gene; Polyethylene oxide micelle | | |


• 中文摘要

一般維持脊椎動物眼角膜 (Cornea) 之完整性時，角質素 (Keratin) 與圓椎形角膜素 (Katakana)，這二種分別位在眼角膜上皮(Epithelium)、與實質 (Stoma)細胞組織中，爲其主要成分；而其功能在維持眼角膜的組織與細胞內之架構，並影響眼角膜之明晰、彈性、脆硬度功能，例如 Merman's 先天性 Keratin K12 基因缺失失養症，或實質失養症。然而這些疾病，大多屬於先天性基因不完整或缺失之疾病。雖然，先前我們曾利用 PEO 聚合載體與非專一性之啓動子 (Cytomegalovirus, CMV)基因，並結合 Lack (Reporter gene)報告基因，進行兔子與老鼠的體內眼藥水投與給藥方式，發現在角膜、鞏膜、虹彩、及肌肉層中，均可以發現有此基因之蛋白質表現 (Gene There 2001, 8, 999)，但是，發現此基因，卻缺乏眼角膜組織之特異與專一性。因此，本研究目的，結合最近二個被發現具有眼角膜上皮與實質組織之專一性啓動子 (Promoters)：Keratin (K12)與 Katakana (Ketch, K3.2)基因質體與 PEO 聚合載體製備，進行動物眼藥水體內給藥方式，評估其基因質體在角膜組織部位上之表現與影響穿透機制。結果發現製備三個不同大小基因質體 pCMV-LacZ (7.2 Kb)， pK12-LacZ (8.6 kb)， pK3.2-LacZ (10.9 kb)與 PEO 聚合載體後，粒徑呈現隨著基因質體大小，而呈現製備後有變大特性 (142, 182, and 187 nm)；同時，測量其擴散係數時，亦呈現下降趨勢 (2.7, 2.3, 1.9 x 10/sup -8/ cm2/s)。在 DNA 酵素中，則發現 PEO 聚合載體之保護這三種基因質體能力時，至少均可以在維持在 45 分鐘內；但隨著質體大小越小而其保護能力可以增加至 105 分鐘。而評估這三種專一啓動子之基因質體，在體內眼角膜組織部位中之專一性分佈時，發現除了 pCMV-LacZ 基因質體表現與先前實驗中，在角膜、鞏膜、虹彩、及肌肉層中，均可發現有其半乳糖基因表現之無專一性外， pK12-LacZ， pK3.2-LacZ 均可以在眼角膜上皮、與實質細胞組織中，分別有半乳糖基因專一表現與分佈。同時，利用先前投與 EDTA 後，發現只有實質專一啓動子之基因質體 (pK3.2-LacZ)，

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| | 可以利用此添加方法而增強基因表現量。再利用反轉錄聚合連鎖反應 (RT-PCR)方式，萃取出角膜中之半乳糖 mRNA，亦發現與其基因表現量有相同印證。 |
| • 英文摘要 | Keratin (K12) and keratocan (Ktcn, K3.2) are major components of extracellular keratocyte-specific keratin sulfate proteoglycan (KSPG) in vertebrate cornea epithelium and stroma. It has been suggested that cornea KSPGs modulate intermediate filament that form cytoskeletal networks in epithelial cells and stroma collagen fibrilogenesis. These two gene, thus, contribute to the corneal function and transparency. Although, previous our studied have found that efficient and stable transfer of the functional LacZ reporter gene with non-specific early promoter cytomegalovirus (CMV), could be achieved with PEO polymeric micelles as a carrier for eye drop gene delivery of plasmid DNA in vivo (Gene Ther 2001, 8, 999), it was lack of specific-tissues localization of gene expression. Therefore, these studies are to provide two specific-tissue promoters of keratin K12, and keratocan (Ktcn) with topical eye drop delivery into corneal epithelium and stroma areas. Our results found that three different promoters with LacZ gene: pCMV-LacZ (7.2 kb), pK12-LacZ (8.6 kb), and pK3.2-LacZ(10.9 kb) were able to formulate with PEO polymeric micelles. The formulated particle size of three plasmids with PEO polymeric micelles was increased with increasing plasmids size, 142, 182, 187 nm, respectively. In the meantime, the diffusion coefficients of three plasmids with PEO polymeric micelles were all consistently decreased with increasing particle size (2.7, 2.3, and 1.9 x10/ usp -8/ cm2/s). Under DNase treatment, the PEO polymeric micelles were able to protect three plasmids from degradation within 45 min. However, the protective ability of pCMV-LacZ was found to increase up to 105 min. Using eye drops delivery of three plasmids all containing beta-gal cDNA driven by pCMV, K12 and K3.2 promoters with PEO polymeric micelles in nude mice, we found that the specific LacZ gene expression of K12 and K3.2 were specific localized in the epithelium and stroma of cornea, though the gene expression with pCMV promoter still observed randomly distribution in whole eye. Furthermore, prior EDTA delivery on the cornea, the enhancement of LacZ gene expression could only found in the K3.2 promoter of plasmid. In the meantime, the mRNA of LacZ gene was extracted from each eye and able to correlate with quantitative LacZ gene expression of plasmid delivery as well as prior EDTA treatment. |