在體外及體內試驗評估嵌段式共聚合物微膠體的傳遞基因效力

Evaluation of the Block Copolymeric Micelle-Mediated Gene Transfer In Vitro And In Vivo

中文摘要

爲了評估嵌段式共聚合物用於基因穿透上的可行性，故選用一個帶有經巨細胞病毒啓動子啓動的大腸桿菌基因 LacZ 爲報告基因的質體 DNA。體外試驗乃利用在人類肝臟腫瘤 Hep G-2 細胞株或小鼠腎上腺皮質的癌細胞株 Y-1 上，加入經過不同比例混合的嵌段式共聚合物與 DNA，經過 X-gal 染色後，可比較出在 0.5-1% 的嵌段式共聚合物濃度範圍下可得到較佳的基因表現效果。除此之外，在 0.5-1% 濃度範圍下的細胞毒性經 MTT 分析後皆大於 IC50，且製劑的粒徑分布和界面電位分別在 160nm 和 —4.4mV。當額外給予細胞一些影響細胞內傳遞途徑的抑制劑時，發現報告基因表現量降低，因此推測傳遞途徑可能爲 endocytosis。在動物實驗方面，在兔子及裸鼠眼睛局部滴入 DNA 與嵌段式共聚合物的製劑後，可在兔子的虹膜及裸鼠眼球內部發現有來自報告基因的藍綠色表現，若投予可打開 tight junction 的試劑，在定量及定性實驗上，皆可發現就基因表現量和能出現報告基因表現的眼球組織面積皆呈增加的趨勢。故由這些結果，推測這種嵌段式共聚合物在體內及體外皆有可能成爲一頗具潛力的傳遞基因的製劑。

英文摘要

In order to evaluate the feasibility of using the block copolymer for gene transfer, a plasmid (pCMV-˜gal) carring the Escherichia coli ˜galactosidase gene (LacZ) driven by a CMV promoter was used as a reporter. Different ratios of copolymer and plasmid DNA were prepared and added to human hepatocarcinoma Hep-G2 and mouse adrenal tumor or Y-1 cell lines in vitro as well as by eye drops in ocular tissues of rabbits and mouse in vivo. In addition, the physical-chemical properties of polymeric micelles/plasmid polymeric micelles carrier, including Zeta potential, partical size, and critical micelle concentration (CMC) measurements were analyzed. The results have found that the block polymeric micelles were formed above 0.1% (W/V) of block copolymer with 160 nm size and − 4.4 mV potential. After staining with X-gal at 72 hours post-treatment, the most efficient reporter expression occurred when 0.5% -1% concentrations of the block copolymers were used as the carrier. In addition, the cytotoxicity for the Hep-G2 and Y-1 cells was determined by MTT assay and it was found that the IC50 of the block copolymer was 1% and 1.8% (IC50= 1, 1.8%), respectively. Furthermore, the reporter expression was detected around the iris of rabbits and the intraocular tissues of mice upon in vivo topically

applying the copolymer/DNA formulation for 48 hours. In the meantime, after some enhancer and endocytosis inhibotrs, the transport mechanisms of block copolymeric micelles was found probably through endocytosis in cultured cells and animals as well as enhancement through tight junction pathway. These in vitro and in vivo experiments postulate the possible potential usage of the block copolymers for DNA transfer.