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论著

半乳糖化壳聚糖-低分子聚乙烯亚胺/DNA复合物的肝靶向性研究

谭平萍, 李曼, 王颖, 冯德云

中南大学基础医学院病理学系，长沙 410078)

摘要：

目的：研究半乳糖化壳聚糖-低分子聚乙烯亚胺(galactosylated chitosan-graft-polyethyleneimine, GC-PEI)/DNA复合物在体内外的肝靶向性。方法：GC-PEI与增强型绿色荧光蛋白(enhanced green fluorescent protein, EGFP) 质粒(pEGFP-Cl)在0.01 mol/L PBS, 150 mmol/L NaCl, 5%葡萄糖溶液中自组装成3种不同溶媒介导的GC-PEI/ DNA复合物，检测复合物粒径大小与形态，Zeta 电位以及结合和保护DNA 的能力；并进一步测定GC-PEI聚合物的毒性，研究复合物的肝靶向转染效率。结果：在GC-PEI与DNA质量比为1∶1～2.5∶1时，GC-PEI聚合物能有效地结合和保护所携带的DNA免受核酸酶和血清的降解。复合物粒子呈规则的球形，有明显的核壳结构。GC-PEI聚合物在检测细胞中未显示出明显毒性；动物体内急性毒性实验显示：通过尾静脉注射50～300 μg的GC-PEI聚合物入小鼠后，实验小鼠2周内无急性毒性反应和死亡发生。荧光显微镜和流式细胞仪检测证实GC-PEI/DNA复合物在肝细胞系(QSG7701/core，L02)中的绿色荧光蛋白（green fluorescent protein, GFP）表达明显高于非肝细胞系（SGC-7901，HBE)细胞。体内实验表明转染48 h后，小鼠肝组织在荧光显微镜下可以检测到明显的绿色荧光，而其他主要脏器未见明显荧光。结论：GC-PEI聚合物能够在体内外特异性将外源基因或DNA导入肝细胞，具有良好的肝靶向性。

关键词： 半乳糖化壳聚糖-低分子聚乙烯亚胺；肝靶向性；受体介导的基因转移

Hepatocyte-targeted gene transfection of galactosylated chitosan-graft low molecular polyethyleneimine/DNA complexes

TAN Pingping， LI Man， WANG Ying， FENG Deyun

Department of Pathology, Basic Medical College, Central South University, Changsha 410078, China

Abstract:

ObjectiveTo investigate the hepatocyte targeted specific property of galactosyla-ted chitosan-graft-polyethyleneimine (GC-PEI)/DNA complexes in vitro and in vivo. MethodsWith the plasmid expressing enhanced green fluorescent protein (pEGFP-C1) as the reporter gene, the formation of GC-PEI/DNA complexes was induced to self-assemble in 0.01 mol/L phosphate buffered saline(PBS), 150 mmol/L NaCl, or 5% glucose solution (GS).The complexes were cha-racterized by the particle size, Zeta potential, DNA binding and protection capacity, and further tested for cytotoxicity and hepatocyte targeted transfection activity. ResultsWith the GC-PEI/DNA mass ratio from 1∶1 to 2.5∶1, the GC-PEI/DNA complexes effectively bound and protected the DNA from degradation of DNase Ⅰ and the serum, which presented as a well-formed sphere or compacted nucleocapsid structure at a diameter of 50—200 nm. The GC-PEI copolymer showed no obvious toxicity in the tested cell lines. Acute toxicity assay revealed that the mice grew well in 2 weeks with GC-PEI dosage from 50 to 300 μg. The assay by flow cytometry and fluorescent microscope showed that the transfection efficiency in hepatocyte lines (L02, QSG7701/core) was higher than that in non-hepatocyte lines (SGC7901, HBE) in vitro. In vivo, the GFP was obviously expressed in the liver tissue and not expressed in other organs 48 h after the transfection. ConclusionGC-PEI copolymer may carry the exogenous gene specifically to hepatocytes in vitro and in vivo，which has very good liver targeted specific property.

Keywords: galactosylated chitosan-graft-polyethyleneimine; liver-targeting receptor-mediated gene transfer

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通讯作者:

作者简介:

作者Email: dyfeng743@yahoo.com.cn

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