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Characterization of recombinant plasmid PEPCK-PIRES2-EGFP and evaluation of PEPCK promoter specificity in hepatocytes cells culture transfected with liposomes

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Gene therapy consists of introducing foreign genetic material, DNA or RNA, into cells with genetic disorder, in order to create beneficial biological effects. In order to achieve this effect, it is necessary to introduce the therapeutic genes through a genetic vehicle. Liposomes are gene delivery vectors with very low immunogenicity and toxicity, ease of manufacture, furthermore, they lack DNA insert size limitation. Cationic liposomes seem to be the most hopeful, due to his ability to interact with the DNA and the cell surface. Once material genetic gets inside cell, it can carry therapeutic genes whose expression is regulated by tissue and organ-specific promoters. PEPCK promoter is stably active and specific in hepatocytes, so it could be adapted to therapeutic interest genes and get into the body for selective expression in liver cells. In this work, the genetic construction pPEPCK-IRES2-EGFP was characterized by enzymatic restriction, PCR and nucleotide sequencing, confirming the presence of the organ specific promoter PEPCK. Once the identity of the genetic construct was confirmed, it was used to transfect the hepatocarcinoma cell line HepG2, the embryonic kidney cell line Hek293FT as a control of specificity and a primary culture of hepatocytes, using cationic liposomes from the DLO and DLE lipids as a delivery vector. The specificity of expression in liver cells was evidenced by the green fluorescent protein gene contained in the recombinant plasmid used. This is intended to create a powerful tool to address the expression of therapeutic genes in the treatment of liver diseases, without affecting other cell types.

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