Nanomedicine & Biotherapeutic Discovery

Extended Abstract

Light-triggerable liposomes for enhanced endo/lysosomal escape and gene silencing in PC12 cells

Wei Deng

Abstract

Liposomes are an effective gene/drug delivery system, widely used in biomedical applications including gene therapy and chemotherapy. Here we designed a photo-responsive liposome (lipVP) loaded with a photosensitizer verteporfin (VP). This photosensitizer is clinically approved for photodynamic therapy (PDT). LipVP was employed as a DNA carrier for pituitary adenylyl cyclase-activating polypeptide (PACAP) receptor 1 (PAC1R) gene knockdown in PC12 cells. This has been done by incorporating PAC1R antisense oligonucleotides inside the lipVP cavity. Cells which have taken up the lipVP were exposed to light from a UV light source. As a result of this exposure, reactive oxygen species (ROS) were generated from VP, destabilizing the endo/lysosomal membranes and enhancing the liposomal release of antisense DNA into the cytoplasm. Endo/lysosomal escape of DNA was documented at different time points based on quantitative analysis of colocalization between fluorescently labeled DNA and endo/lysosomes. The released antisense oligonucleotides were found to silence PAC1R mRNA. The efficiency of this photo-induced gene silencing was demonstrated by a 74±5% decrease in PAC1R fluorescence intensity. Following the light-induced DNA transfer into cells, cell differentiation with exposure to two kinds of PACAP peptides was observed to determine the cell phenotypic change after PAC1R gene knockdown.

Gene delivery and cistron medical aid suppose effective exogenous nucleic acids transfer into cells. Thanks to the high transfection potency, infectious agent carriers are a ordinarily used technique of cistron delivery. However, the event and application of infectious agent carriers is hindered by a spread of limitations as well as poison production, restricted size of transgenic DNA, packaging difficulties, and therefore the risk of recombination. to beat these limitations, artificial non-viral cistron delivery systems, specifically, nanomaterial-based systems, are extensively studied and developed. Among these nanomaterials, liposomes, particularly as well as cationic macromolecule elements, have attracted important interests as a drug and/or cistron delivery vehicle since the Eighties. Up-to-date, varied varieties of liposomes are clinically wont to improve the effectiveness and biodistribution of medicine, as well as cancer medicine. In recent years, variety of studies reported the appliance of liposomal carriers to varied cistron-targeting ways in cancer gene medical aid. for instance, Mendonça et al. applied siderophilin receptor-targeted liposomes encapsulating antisense oligodeoxynucleotides (asODNs) and tiny interference RNA (siRNA) into the treatment of chronic chronic myelocytic leukemia. Wu et al. incontestable liposome-based cooperative treatment of internal secretion promoter-thymidine enzyme cistron medical aid followed by ganciclovir pharmacotherapy, leading to economical ablation of the neoplasm size in mice. Therefore, liposomes will function associate economical technique for targeted cistron transfer in cancer cistron medical aid.

Passive liposomal delivery is difficult thanks to biological extracellular and animate thing barriers like protein degradation, pH change, and endolysosomal lysis. so as to beat these barriers and enhance the effectiveness of liposome-mediated cistron and/or drug delivery, varied ways are used to develop active liposomes whose bilayer may be destabilized by exploitation external stimuli, as well as temperature, pH, ultrasound, specific enzymes, force field and ikon irradiation as well as {uv|ultraviolet|ultraviolet radiation|ultraviolet light-weight|ultraviolet illumination|UV|actinic radiation|actinic ray} light. light-weight is particularly engaging as a triggering modality as a result of it may be applied remotely with high spatiotemporal exactitude, whereas light-weight parameters like wavelength, power density, and illumination time may be adjusted to manage the discharge platform. In recent years, increased cytoplasmatic delivery of molecule compounds by

Wei Deng
Macquarie University, Australia, E-mail: wei.deng@mq.edu.au

chemistry disruption of the endolysosomal membrane, said as chemistry learning (PCI), has been actively investigated within the context of cistron delivery, as well as siRNA, amide nucleic acids (PNAs), and cellular inclusion DNA (pDNA), and pharmacotherapy. for instance, Park et al. incontestable endolysosomal escape of the therapeuticgene carried by polymer-gene complicated once illumination with a 671 nm optical device. Here, we tend to use an analogous strategy to deliver a cistron to silence one in every of the pituitary adenylyl cyclase-activating peptide (PACAP) receptors.

PACAP may be a member of the vasoactive enteral peptide (VIP)-glucagon-growth internal secretion emotional factor-secretin taxonomic category, and its 2 amidated forms: PACAP-38 and PACAP-27. Loosely expressed in nerve cells, PACAP may be a pleiotropic protein, moving differentiation, proliferation, and maturation of most neural and non-neural cell varieties. PACAP conjointly plays a task in neoplastic cell proliferation. It induces cell proliferation in little carcinoma cells and malignant neoplasm cells, however it inhibits cell growth of carcinoma and carcinoma. To boot, PACAP is a vital neuropeptide that plays a significant role within the regulation of high blood pressure. The PACAP-specific cell wall receptors embody the PAC1, vasoactive enteral amide receptor (VPAC)1, and VPAC2. Among these receptors, PACAP receptor one (PAC1R) has the very best affinity for PACAP at physiological concentrations. as a result of PC12 cells solely specific PAC1R, this cell line was an honest model to analyze the impact of PAC1R knockdown. PC12 cells, a being cell line derived from a phaeochromocytoma of the rat ductless gland, were used because the in vitro model for assessing differentiation and neurite growth as a result of they'll be stirred for neurite outgrowth by the nerve protein (NGF). PACAP, acting as a neurochemical, conjointly induces PC12 cell differentiation, via a unique communication pathway from nerve growth factor.

In this study, we tend to incontestable PAC1R cistron knockdown by light-triggerable liposomes and therefore the result of PACAP on PC12 cell differentiation following PACAP cistron silencing. To arrange the liposomes, we chose 1, 2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) as a neutral lipid56 and one, 2-di-(9Z-octadecenoyl)-3-trimethylammonium-propane (DOTAP) as a cationic macromolecule. The latter will enhance cistron delivery as a result of it's able to freely labor under charged cell membranes.14 Verteporfin (VP), an extremely potent photodynamic medical aid (PDT) photosensitizer,57 was loaded within a liposomal bilayer, said as lipVP. As ODN molecules of PAC1R were incorporated within a liposomal cavity. These liposomes were endocytosed by cells, wherever they became entrapped within the endosomes and lysosomes. VP was wont to generate an ample quantity of reactive gas species (ROS) for destabilization of the liposomal and endolysosomal membranes below light-weight illumination. The mechanism on asODN unharness from the endosomes and lysosomes by ROS. Specifically, we tend to quantitatively make a case for the endolysosomal escape method through subcellular colocalization analysis supported the free profiles of DNA molecules and endosomes and lysosomes. As ODN molecules were then free from the endolysosomal compartments into the protoplasm and silenced the PAC1R ribonucleic acid.

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Wei Deng
Macquarie University, Australia, E-mail: wei.deng@mq.edu.au