Chemical Modification of Chitosan as Gene Carriers In Vitro and In Vivo

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Introduction

Gene therapy is now seen as a promising approach to the treatment of a wide range of diseases, both congenital and acquired diseases, by producing bioactive agents or stopping abnormal functions of the cells such as genetic disorder or uncontrollable proliferation of cells[1]. Viral vectors have been commonly employed due to the high transfection efficiency compared with non-viral vectors, however, their application to the human body is often frustrated by immunogenicity, potential infectivity, complicated production, and inflammation[2]. Non-viral vectors have been widely proposed as safer alternatives to viral vectors, and cationic polymers have gained increasing attention because they can form self-assembly with DNA. Chitosan is also considered to be a good candidate as a gene carrier, since it is already known as a biocompatible, biodegradable, and low toxic material with high cationic potential[3]. However, low solubility, cell specificity and especially, low transfection efficiency need to be overcome prior to clinical trial. In this study, we focused on the chemical modification of chitosan for enhancement of cell specificity and transfection efficiency and we investigated the potential of clinical applications.

Experimental

Galactosylated Chitosan (GC). Water-soluble chitosan (WSC) was coupled with lactobionic acid via an active ester intermediate using 1-ethly-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) in 0.1M MES buffer (pH 6.0). After stirred for 24hr at room temperature, the resulting GC was dialyzed for 4 days using Spectra/Por membrane (MWCO=12,000-14,000) against distilled water.

Mannosylated Chitosan (MC). WSC was dissolved in 1ml of water and mixed with mannopyranosylphenylisothiocyanate in 1ml of DMSO. And the solution was stirred for 24h at room temperature. The polymer was precipitated by adding 10 volumes of isopropanol and spun down by centrifugation at 10,000 rpm. After washing with isopropanol, the pellets were dried in the vacuum oven.

Urocanic Acid-Modified Chitosan (UAC). WSC was coupled with urocanic acid (mol %: 20, 50, and 70) via EDC/NHS. After stirring for 24hr at room temperature, the resulting UAC was dialyzed using Spectra/Por® membrane (MWCO=12,000-14,000) against distilled water for 4 days.

Preparation of Polymer/DNA complex. Complexes were induced to self-assemble by mixing plasmid DNA with appropriate polymer solution at the desired charge ratio. The complexes were allowed to stand at room temperature for 30 min.

Results and Discussion

Galactose Ligand Modification. The liver is an attractive target tissue for gene therapy due to its large size, metabolic capacity and rich blood supply that can be useful for the delivery of genes to the liver as well as for the distribution of gene products from the liver to the systemic circulation. Above all, mammalian hepatocytes are the only cells that possess large numbers of high affinity cell-surface receptors that can bind asialoglycoproteins (ASGP). For hepatocyte targeting, lactobionic acid bearing galactose group was coupled with WSC. GC/DNA complex showed much higher transfection efficiency compared with WSC/DNA complex on HepG2 whereas the luciferase activity of WSC and GC/DNA complex did not show any difference on HeLa cells that have no ASGP-R, indicating that galactose ligand attached on GC played a great role to recognize the ASGP-R.

Mannose Ligand Modification. For the possibility of therapeutic manipulation of a wide spectrum of immune functions, the regulation of immunity is a major goal in treatment and prevention of viral infections, cancer and autoimmune disease. It is well known that the antigen presenting cells (APCs) such as macrophages and immature dendritic cells (DCs) express high levels of mannose receptor that are used for endocytosis and phagocytosis of a variety of antigens that expose mannose. We prepared MC to induce the receptor-mediated endocytosis for targeting into APCs. MC/DNA complex showed higher transfection efficiency compared with WSC/DNA complex on Raw264.7 macrophage cell line and the transfection efficiency of the MC/DNA was decreased in the presence of mannose, indication of receptor-mediated endocytosis mechanism.

pH-Sensitive Modification. For efficient gene transfection, multiple steps are required including DNA complexation, cellular uptake of the complexes, release of DNA or complexes from endosomes, release of DNA from the carriers, and transfer into the nucleus. Inefficient release of the polymer/DNA complex from endocytic vesicles into the cytoplasm is one of the primary causes of poor gene delivery. We coupled urocanic acid (UA) bearing imidazole ring which can play the crucial role in endosomal rupture through proton sponge mechanism to chitosan. Transfection efficiency against 293T cells increased with increasing the charge ratio and substitution value of UA because of higher buffering capacity in the endosomal compartment, which leads to positively charged ions being trapped by amines in imidazole rings, and enhances osmolarity, subsequent endosomal rupture and escape into the cytoplasm.

In Vivo Application. We performed cytokine gene delivery after intratumoral injection of MC/pmIL-12 complex into BALB/c mice bearing tumor at the injected sites. As shown in Figure 1, intratumoral delivery of MC/pmIL-12 complex into BALB/c mice bearing tumor clearly suppressed tumor growth compared with control and vector itself due to the higher production of IL-12 p70 and INF-γ compared to control. As shown in Figure 2, apoptosis was detected in Programmed cell death 4 (PDCD4)-delivered K-*ras* null lung cancer model mice through aerosol gene delivery using UAC by terminal deoxynucleotidyltransferase-mediated nick end labeling (TUNEL) assay.

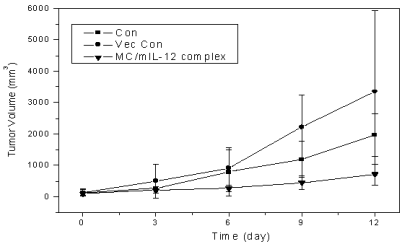

Figure 1. Suppression of tumor growth by MC/pmIL-12. Source: From Ref. [4]

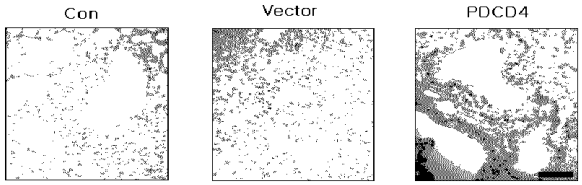

Figure 2. TUNEL assay. Source: From Ref. [5]

Conclusions

Chitosan is obviously versatile material for gene delivery and it is expected that relatively non-toxic chitosan is suitable for repeated administration to maintain sustained gene expression, thereby opening the possibility for human gene therapy.

References

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