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Review

Progress in developing cationic vectors for non-viral systemic gene therapy against cancer

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ABSTRACT

Initially, gene therapy was viewed as an approach for treating hereditary diseases, but its potential role in the treatment of acquired diseases such as cancer is now widely recognized. The understanding of the molecular mechanisms involved in cancer and the development of nucleic acid delivery systems are two concepts that have led to this development. Systemic gene delivery systems are needed for therapeutic application to cells inaccessible by percutaneous injection and for multi-located tumor sites, i.e. metastases. Non-viral vectors based on the use of cationic lipids or polymers appear to have promising potential, given the problems of safety encountered with viral vectors. Using these non-viral vectors, the current challenge is to obtain a similarly effective transfection to viral ones. Based on the advantages and disadvantages of existing vectors and on the hurdles encountered with these carriers, the aim of this review is to describe the "perfect vector" for systemic gene therapy against cancer.

1. Introduction

Cancer has become the first killer in developing countries and is on the verge of becoming the first cause of death in industrialized countries. Due to its invasive, aggressive growth profile as well as the complex mechanisms involved in cancer development and propagation, classical treatments such as surgery, chemotherapy and radiotherapy are still insufficiently effective in many cases, and are often up against resistant and infiltrating tumors. New anticancer strategies are thus urgently required.

A better understanding of the genes involved in the development and growth of cancer is leading to new approaches to treat this disease. Oncogenes and tumor suppressor genes, not working properly in most cancers, play a crucial role in the beginning and growth of the cancerous processes [1]. The approach of gene therapy provides a promising tool to eradicate this disease by treating it at its source. It can be particularly advantageous in that a relatively short expression of therapeutically active proteins may be sufficient to eradicate tumors, unlike genetic diseases such as cystic fibrosis in which there is a need for long-term expression [2]. Interestingly, between 1989 and 2004, cancer was the first candidate for gene therapy clinical trials (66% of all gene therapy trials) [3] (http://www.wiley.co.uk/genmed/clinical/). In the field of cancer gene therapy, four major targets have to be reached by vectors: turning off oncogene expression, enhancing tumor suppressor expression to induce apoptosis of cancer cells, inhibiting neoangiogenesis, and stimulating immune system against tumors cells.

Historically, there have been three different approaches applied to gene delivery. The first approach consists of the use of naked DNA. Direct injection of free DNA to the tumor site has been shown to produce high levels of gene expression and the simplicity of this approach led to its use in a number of experimental protocols [4,5]. This strategy appears to be limited to tissues that are easily accessible by direct injection such as the skin and muscles [6] and is unsuitable for systemic delivery due to the presence of serum nuclease. The second approach involves using genetically altered viruses. Viral vectors are biological systems derived from naturally evolved viruses capable of transferring their genetic materials into the host cells. Many viruses including the retrovirus, adenovirus, herpes simplex virus (HSV) and adeno-associated virus (AAV) have been modified to eliminate their toxicity and maintain their high capacity for gene transfer [7] hence presenting various advantages [8–10]. Viral vectors are very effective in achieving high efficiency for both gene delivery and expression. However, the limitations associated with viral vectors, in terms of safety, immunogenicity, low transgene size and high cost, have encouraged researchers to focus on alternative systems. The third approach for delivery systems concerns non-viral vectors, which are mainly of a cationic nature: cationic polymers and cationic lipids. They interact with negatively charged DNA through electrostatic interactions leading to polyplexes and lipoplexes, respectively. The advantages associated with these kinds of vectors include their large scale

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Table 1
Physicochemical characteristics of current cationic systems

Cationic systems[a]	Particles charge[b] (mV)	Particles size (nm)	Degradability	Ref.
PEI/DNA	+30	20–130	No	[20,21]
PLL/DNA	+40	60–140	Yes	[25–28]
Chitosan/DNA	+25 to +37	20–500	Yes	[34,37–42]
PAMAM dendrimer/DNA	+ 9 to +20	50–100 (generation 6–7)	Yes	[45–47]
DOPE/DOTAP	+ 30 to +50	60–120	Yes	[60]
DOPE/DC-Chol	+20 to +50	70–120	Yes	[60]
DOTAP/Chol	+50 to +60	100–126	Yes	[76]

[a] PEI: poly(ethyleneimine), PLL: poly(L-lysine), PAMAM: poly(amidoamine), DOPE: 1,2-dioleyl-*sn*-glycerol-3-phosphoethanolamine, DOTAP: 1,2-dioleyl-3-trimethylammonium-propane, DC-Chol [*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl]cholesterol, Chol = cholesterol.
[b] Depending on the ± charge ratio.

manufacture, their low immunogenic response, the possibility of selected modifications and the capacity to carry large inserts (52 kb) (Table 1) [11,12]. While the transfection efficiency of non-viral vectors is still lower than that for their viral counterparts, a number of adjustments (e.g. ligand attachment) could improve this category of carriers which are, thus far, believed to be the most promising of gene delivery systems. Nonetheless, this class of vectors has to be modified to make systemic delivery possible. To date, systemic administration has resulted in a toxic response (linked to their positive charge), incompatible with clinical applications.

Currently, the main objective in gene therapy via a systemic pathway now is the development of a stable and non-toxic gene vector that can encapsulate and deliver foreign genetic materials into specific cell types such as cancerous cells with the transfection efficiency of viral vectors.

In parallel to existing review in non-viral gene delivery against cancer ([13–16]), the aim of this work is to provide a non-exhaustive list of the cationic vectors currently developed for systemic delivery (for other administration pathways see reviews in Refs. [17–19]). The obstacles to their systemic injection and cell trafficking will be described. The possible strategies to overcome these problems will be argued thereafter.

2. Non-viral vectors: current cationic systems

2.1. Cationic polymers

2.1.1. Poly(ethyleneimine) (PEI)

PEI can be synthesized in different lengths, be branched or linear (Fig. 1), and undergo functionalized group substitution or addition. It is a versatile polymer which has a privileged place in the components of non-viral gene delivery, due to its superior transfection efficiency in a broad range of cell types compared to other systems described later. PEI polymers are able to successfully complex DNA molecules, leading to homogeneous spherical

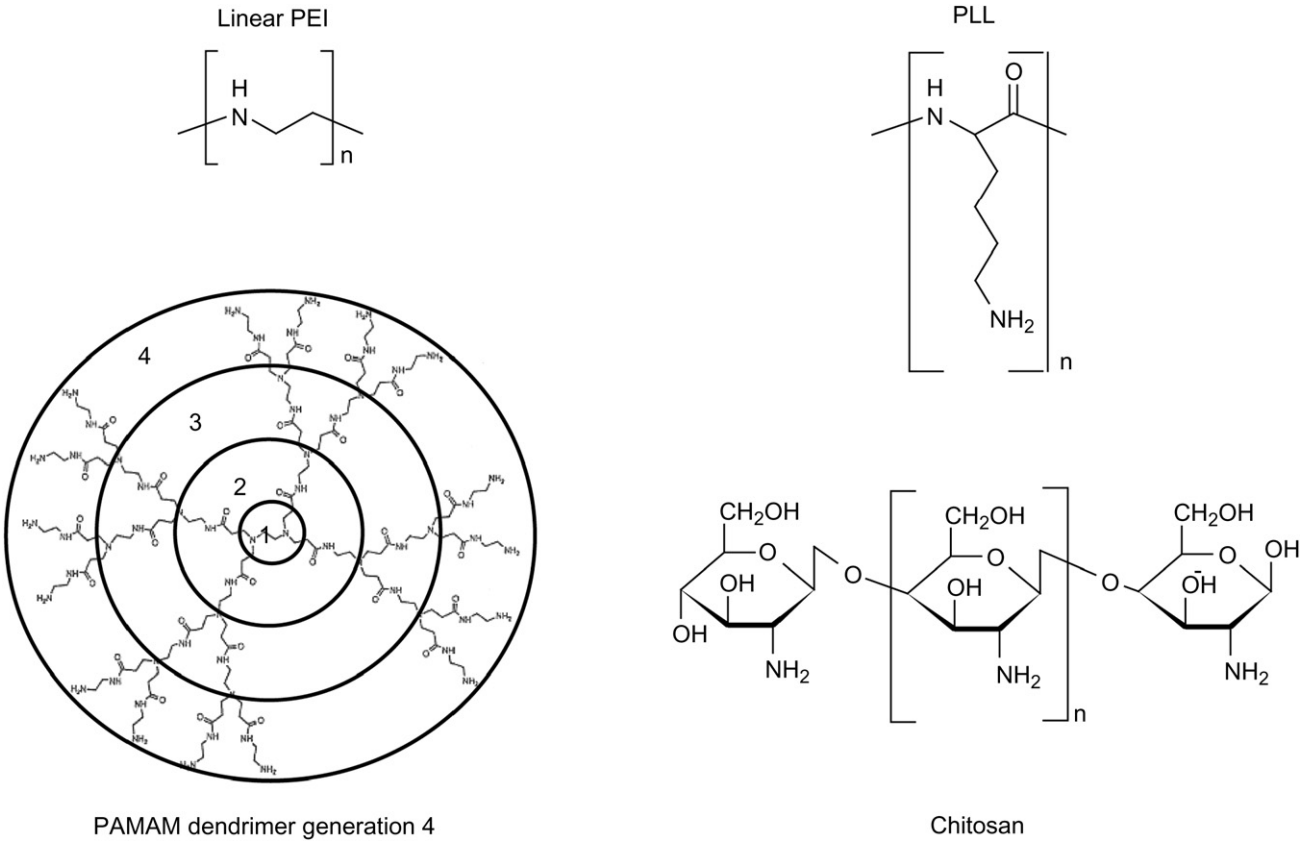

Fig. 1. Structures of current cationic polymers used in gene therapy. PEI = poly(ethyleneimine), PLL = poly(L-lysine), PAMAM = poly(amidoamine).