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Nonviral cancer gene therapy: Delivery cascade and vector nanoproperty integration☆

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ABSTRACT

Gene therapy represents a promising cancer treatment featuring high efficacy and limited side effects, but it is stymied by a lack of safe and efficient gene-delivery vectors. Cationic polymers and lipid-based nonviral gene vectors have many advantages and have been extensively explored for cancer gene delivery, but their low gene-expression efficiencies relative to viral vectors limit their clinical translations. Great efforts have thus been devoted to developing new carrier materials and fabricating functional vectors aimed at improving gene expression, but the overall efficiencies are still more or less at the same level. This review analyzes the cancer gene-delivery cascade and the barriers, the needed nanoproperties and the current strategies for overcoming these barriers, and outlines PEGylation, surface-charge, size, and stability dilemmas in vector nanoproperties to efficiently accomplish the cancer gene-delivery cascade. Stability, surface, and size transitions (3S Transitions) are proposed to resolve those dilemmas and strategies to realize these transitions are comprehensively summarized. The review concludes with a discussion of the future research directions to design high-performance nonviral gene vectors.

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1. Introduction

Advances in genetics and molecular biology have revealed that cancer development is associated with multiple genetic alterations and disorders. Gene therapy, delivering therapeutic nucleic acids into cells to correct or modify genetic information, has proven to be a promising approach for treating cancer at the genetic level. During the past 25 years, >2,000 gene therapy clinical trials have been performed, approximately two thirds of which are for treatment of various types of cancers [1]. Cancer gene therapy includes suicide gene therapy, silencing oncogene expression, mutation correction, tumor-suppressor enhancement, suppressing tumor angiogenesis and stimulation of immune response against tumor cells [2–4], and has become a promising cancer treatment featuring high selectivity and efficacy as demonstrated in experimental animals and clinical trials [5].

For *in vivo* gene therapy, the gene must be suitably delivered to the cells at the targeted site. Directly using free nucleic acids has produced efficient transfection levels when administrated intratumorly or intramuscularly, away from degrading plasma enzymes. However, naked nucleic acids do not achieve desired outcomes due to their rapid clearance [6], rapid enzymatic degradation [7], nonspecific biodistribution and low cellular uptake [8]. Therefore, the primary challenge for gene therapy is to develop safe and efficient carriers to protect nucleic acids and facilitate their transfer to targeted cells at the targeted site. For *in vivo* gene delivery, the carrier/nucleic acid systems, named vectors, are categorized into two classes: viral vectors and nonviral vectors. Viruses such as retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses have evolved various specialized molecular mechanisms for overcoming cellular obstacles to efficiently transport their genomes inside cells. Thus, engineered viruses carrying target genes, viral vectors, can harness these mechanisms and achieve very high gene expression efficiencies [9,10]. Most gene vectors in clinical trials (69%) are viral vectors (http://www.wiley.co.uk/genmed/clinical/). Some inherent shortcomings, including broad tropism and safety concerns, remain as barriers to wide applications [5,11,12]. Moreover, the cost for virus-based gene therapy are nearly unaffordable: For instance, Glybera cost around $1 million per treatment in 2015 [13]. Nonviral carriers are advantageous in their low immunogenicity as well as high packaging capacity and the potential for scale-up manufacture. However, compared to viruses that have evolved to precisely and smartly adapt their gene delivery process, nonviral vectors are much more simply functionalized and cannot efficiently overcome the gene delivery barriers, resulting in low gene transfection efficiency.

In the past decades, applying nanotechnology in design of nonviral vectors has greatly improved their gene transfection efficiency, specificity and biocompatibility. The number of nonviral vectors in clinical trials has also been increasing. These nonviral vectors are engineered with many favorable properties to overcome the biological barriers in their journey from the administration site to the action site – the cytoplasm or nucleus. In the blood, gene delivery vectors are designed to avoid rapid kidney filtration, escape the mononuclear phagocytic system (MPS) and the reticuloendothelial system (RES) to prolong their blood circulation for greater accumulation in tumor tissue via the enhanced permeability and retention (EPR) effect. They are also devised to respond to the tumor microenvironment and intracellular signals to deliver nucleic acids into the cytosol or nucleus for gene silcencing or expression. This review further analyzes the delivery process steps and biological barriers, as well as their nanoproperty requirements and summarizes the recent advances in vector design for overcoming the barriers. The aim is to distill the further research directions for creating efficient and low-toxicity nonviral gene vectors.

2. Nonviral vectors for cancer gene delivery

Nonviral gene vectors able to protect fragile nucleic acids from degradation and deliver them to tumor cells can be categorized as polyplex, lipoplex, lipid-polymer hybrid (lipopolyplex), and organic-inorganic hybrid vectors (Figs. 1 and 2A). Organic nanocarriers can be made biodegradable and biocompatible, and easily functionalizing. Inorganic nanocarriers have also been explored because they are chemically and thermally stable, easily controlled in particle size, shapes and structures, and useful for real-time *in vivo* tracking studies (Fig. 2A) [14].

2.1. Cationic polymers

Amine-based cationic polymers such as polylysine (PLL), poly(ethyleneimine) (PEI), polyamidoamine (PAMAM) and chitosan have been exploited as polymer gene carriers [15] (Fig. 1A). They can complex with the negatively charged nucleic acids to form nanosized polyplexes and thus protect them against enzymatic degradation and enhance their cellular entry. PLL is one of the first and most used polycations for gene delivery. A biodegradable peptide structure makes PLL attractive for *in vivo* use [16]. The gene transfection of PLL polyplexes is weak because they can become trapped in endosomes and lysosomes after cellular endocytosis [17]. Thus, PLL is usually modified with endosomolytic groups (e.g., histidyl [18] and imidazole [19]) to facilitate its lysosomal escape and thus improve transfection activity.

PEI, particularly with molecular weight of 25 kDa, shows great transfection efficiency in a broad range of cell types [20]. Its high gene transfection efficiency is, arguably, because it can rupture endosomes through the proton-sponge mechanism [21,22]. The transfection efficiency and toxicity of PEI are closely associated with its molecular weight. High-molecular-weight PEI usually has favorable transfection efficiency, but also high toxicity because of its induced cell membrane damage and apoptosis [23,24]. PEI with the molecular weight in the range of 20 to 30 kDa exhibits high transfection efficiency; especially, 25 kDa PEI is considered the gold standard in polymer gene transfection [25]. Though low-molecular weight (<2,000 Da) PEI has reduced toxicity, it is incapable of condensing DNA and so is ineffective for transfection [24,26].

Cationic dendrimers such as PAMAM, polypropylenimine (PPI) and PLL dendrimers are attractive nonviral gene vectors primarily due to their monodispersity, three-dimensional and precise structures, well-controlled sizes and abundant surface groups for functionalizations [27,28]. An optimal generation of cationic dendrimers is required for effective complexation with nucleic acids through electrostatic interaction [27]. High-generation dendrimers also have high toxicity [29]. PAMAM is the most studied dendrimer as a gene carrier for its high gene-transfection efficacy and commercial availability [17]. Sixth-generation of PAMAM is optimal for gene transfection efficiency and biocompatibility [30].