

## EDITORIAL



# Non-viral vectors for gene therapy

Nejat Düzgüneş

“Even without a targeting ligand... non-viral vectors have the ability to inherently distinguish between cancer and normal cells.”

Of the 2210 gene therapy clinical trials reported until July 2015, only 115 were carried out by the use of cationic lipid-mediated transfection or “lipofection” [1]. Some trials (387) employed plain plasmid DNA. Most of the trials utilized viral vectors, including adenovirus, adeno-associated virus, vaccinia virus, retroviruses and lentivirus, since they are considered to be highly efficacious compared to non-viral vectors in gene delivery. The immunogenicity of viral vectors, however, is a serious concern. The death of an 18-year-old patient with partial ornithine transcarbamylase deficiency who received  $6 \times 10^{11}$  particles/kg of adenovirus type 5 encoding human

ornithine transcarbamylase led to reconsideration of the facile use of viral vectors [2,3]. Another consideration beyond the safety issue is the immunity developed against viral vectors that may preclude their repeated use.

Cationic lipids were first employed in 1987 by Phil Felgner and colleagues at Syntex (California, USA) [4]. They utilized small unilamellar liposomes containing the synthetic cationic lipid, *N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium chloride (DOTMA), which formed complexes with DNA and mediated both transient and stable expression of the DNA in cultured cells. The

levels of expression were 5-fold to >100-fold higher than that obtained with calcium phosphate or DE-AE-dextran. Wu and Wu utilized an asialoorosomucoid-poly-L-lysine conjugate complexed to plasmid DNA to target the complexes to hepatoma cell lines that expressed the asialoglycoprotein receptor to achieve receptor-mediated endocytosis of the DNA [5]. Complexes of cationic polymers and DNA are termed “polyplexes.”

Although non-viral vectors are used in gene therapy for delivering therapeutic genes, their transfection efficiencies (which we define as the percentage of cells that express measurably the delivered transgenes) are

generally considered to be inferior to that of viral vectors. Our recent results with a novel, lipidic transfection reagent, however, appear to negate this notion, at least with cultured cells [6].

No serious adverse effects of cationic lipid-DNA complexes (“lipoplexes”) in humans have been reported. Intravenous injection of mice with lipoplexes, however, can lead to leukopenia, thrombocytopenia, high serum transaminase levels, and complement activation, together with the production of interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , interleukin-6 and interleukin-12 [7,8]. The inflammatory response induced by lipoplexes can be reduced when CpG motifs are removed from plasmid DNA [8]. Additional advantages of lipoplexes over viral vectors include the accommodation of large expression cassettes, absence of self-replication, and the possibility of rendering the carried DNA recombination-defective [9]. In addition, lipoplexes are non-infectious, cost-effective, and easy to administer. The intracellular barriers to the nuclear delivery of DNA by lipoplexes include escape from endosomes and entry into the nucleus [10].

Cationic polymers such as polyethylenimine (PEI), polyamidoamine (PAMAM), poly(amino-co-ester)s (PAEs) and chitosan have been used for gene delivery [11,12]. Nevertheless, cytotoxicity is an important consideration in the use of polymeric carriers [13]. The different cationic lipids used as vectors have been outlined in previous reviews from our laboratory [14,15].

One aspect of non-viral vectors that confers an automatic advantage in the delivery of therapeutic genes, such as suicide genes, to cancer cells

is the observation that the breakdown of the nuclear membrane during cell division is essential for efficient gene expression. Thus, normal, non-dividing cells are expected to be naturally immune to the activity of a therapeutic gene that will mediate cytotoxicity in rapidly dividing cancer cells. Even without a targeting ligand, which adenoviruses may require for efficient gene delivery, non-viral vectors have the ability to inherently distinguish between cancer and normal cells.

Injection of highly cationic lipoplexes into tumors may result in their entrapment at the site of injection and inability to diffuse into the entirety of the tumor. The non-covalent binding of transferrin with cationic liposomes before complexation with DNA reduces the overall charge and electrophoretic mobility of the ternary complexes, thus enabling the complexes to diffuse further into the tumor mass. Transferrin-lipoplexes have been used in the treatment of colon cancer tumors and orthotopic oral squamous cell carcinoma tumors [16,17].

One final note on the use of lipoplexes and polyplexes: investigators should consider that not all non-viral vectors can be applied to the treatment of all types of tumors. It may be very difficult to develop a lipoplex or polyplex system that can be delivered intravenously and that can be expected to be targeted to tumor tissue. Readily accessible cancers such as early lesions of oral squamous cell carcinoma may be ideal targets for non-viral gene therapy.



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## AFFILIATION

## Nejat Düzgüneş

Department of Biomedical Sciences,  
University of the Pacific, Arthur A.  
Dugoni School of Dentistry, 155 Fifth  
Street, San Francisco, CA 94103, USA  
[nduzgunes@pacific.edu](mailto:nduzgunes@pacific.edu);  
+1 415 929 6565