REVIEWS

Different strategies of gene delivery for treatment of cancer and other disorders

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ABSTRACT

Gene therapy is the gene transfer into host cells for treatment of acquired and genetic disorders. For this purpose, there are a wide variety of gene delivery methods with special properties including viral and non-viral vectors. The non-viral methods use physical forces or chemical compounds (natural or synthetic) to transfer DNA into a cell. The efficiency of the non-viral gene therapy depends on conquering four different intra- and extra-cellular barriers such as cellular uptake, endosomal escape, nuclear entry, and gene expression. Among various gene carriers, some viral vectors such as Adenovirus, Lentivirus, Vaccinia as well as gene gun and lipofection achieved to clinical trials. In this mini-review, we briefly describe different approaches for gene delivery and their applications in various phases of clinical trials.

Key Words: Gene therapy, Delivery system, Chemical vector, Physical vector, Viral vector, Clinical trial

1. INTRODUCTION

Gene therapy has been used for treatment of different diseases (*e.g.*, genetic disorders, cancer and neurodegenerative disease) by transfer of the genetic materials such as small DNA or RNA fragments into the cells.[1–3] Therapeutic genes could be transferred into the somatic cells or integrated into the germ cells (*e.g.*, sperm or egg).[4] Generally, various gene delivery procedures can be divided in three groups including chemical, viral, and physical methods.[5] Briefly, gene therapies contain several advantages, for example, the ability to replace a defective gene; the prevention of the toxic effects caused by other therapies in the body; the decrease of the cost of various therapies;[6] silence a gene and subsequently silence the disease before its onset; and the potential to prevent or eliminate hereditary diseases (*e.g.*, cystic fibrosis).[7] The most important side effects following gene therapy contain flu, fever, leukocytopenia, lymphopenia, and mild transient anemia. Moreover, there are some problems such as mutagenicity, toxicity, and immunogenicity for treatment of diseases with viral vectors.[8] Other concern of gene therapy is its short-term effect for effective treatment of multigenic or multi-factorial disorders such as high blood pressure, Alzheimer's disease, heart disease, diabetes and arthritis.[4,9] In this review, we discuss different ways of gene delivery along with their use in clinical trials.

1.1 *Ex vivo*, *in vivo*, and in situ gene therapy

Various approaches of gene transfer include *ex vivo* and *in vivo* gene therapy.[3] *Ex vivo* gene therapy contains the cell harvest from a patient, in vitro genetically modifica-

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tion through viral or non-viral gene delivery vectors and then, transfer of the transduced cells to the patient. *Ex vivo* gene therapy is a novel therapeutic approach for targeting a specific organ in comparison with treatment of a whole organism.[10] Its advantages include specificity, safety, the lack of immune response, and prevention of graft-versus-host disease in hematopoietic stem cell transplantation between two individuals.[9,10] However, *ex vivo* gene therapy was not a successful strategy for treatment of heart, brain, or lung disorders. Thus, the researchers investigated *in vivo* gene therapy as a therapeutic goal for hereditary disorders, and acquired diseases. In this strategy, the genetic material (*e.g.*, DNA) was used to modify the genetic repertoire of target cells.[10] In this line, different vectors could effectively enter target cells to induce short-term or sustained gene expression.[3] On the other hand, in situ gene therapy includes the direct injection of the genetic material into the target tissue. This gene delivery method was used to target a specific site in the respiratory tract using lipid and adenoviral vectors as well as cancer treatment. However, the major problem of this system is low efficiency of transduction. For instance, the tumor cell can be re-established in cancer therapy.[11]

1.2 Different fields of gene therapy

Gene therapy was used to generate the recombinant cancer vaccines. The cancer cells were engineered to be further recognizable to the immune system by the insertion of genes producing pro-inflammatory immune stimulating molecules and highly antigenic protein. These modified cells were utilized as a killed vaccine. In addition, immunotherapy was performed using the delivery of immuno-stimulatory genes such as cytokines into the tumor.[12] The oncolytic viruses have been genetically engineered to target and destroy cancer cells through expression of cytotoxic proteins and cell lysis while the normal cells remained intact.[12,13] On the other hand, delivery of the therapeutic gene to the target cell elicited an effective response such as down-regulation, silencing, modification, and repair of the target cell genes.[8,12] For instance, gene silencing includes specific delivery of a small interfering double-stranded RNA (siRNA) into target cells, which interferences with RNA functions and protein synthesis. Several delivery systems have been developed to protect siRNA from enzymatic degradation, and facilitate their effect in silencing specific genes. The proper design of siRNA in gene silencing could provide a major therapeutic effect in treatment of cancer, viral diseases such as HBV & HPV, liver cirrhosis and hypercholesterolemia.[8,14] On the other hand, gene repair was performed using zinc finger nuclease linked to the lentiviral vector. Also, gene therapy could be successfully directed to cytoplasmic organelles including mitochondria responsible for metabolic functions.[8]

2. GENE DELIVERY BY NON-VIRAL VECTORS

The non-viral gene delivery methods use physical forces or chemical compounds (natural or synthetic) to transfer DNA into a cell.[15–17] Generally, the success of the non-viral gene delivery systems depends on overcoming different intra- and extra-cellular barriers such as cellular uptake, endosomal escape, nuclear entry, and gene expression.[13] Herein, we describe various non-viral delivery systems in gene therapy.

2.1 Non-viral physical methods

Physical delivery systems create transient membrane pores for facilitating the gene transfer from extracellular to nucleus using physical forces including light, electric or magnetic field, electric pulse, particle impact, ultrasound, hydrodynamic pressure, local or rapid systemic injection, and laser irradiation as follows.[18,19]

Microinjection: In microinjection, cell membrane or nuclear membrane is penetrated by simple mechanical force using a needle of 0.5 μm-5 μm diameter.[5,20] This gene delivery system is mainly used to inject DNA constructs *in vivo*.[21]

Jet injection: In jet injection method, mechanical compression is utilized to move fluid containing DNA without using any needle for injection of particles.[18]

Balistic DNA injection or gene gun: The particle bombardment or gene gun uses heavy metal microparticles (*e.g.*, gold or tungsten, 1 μm-5 μm in diameter) to penetrate the target cells.[20–22] This method has two major advantages such as safety, and high efficiency against parenteral injection. The advantages of this approach are included: 1) total amount of DNA required for delivery is low, 2) no receptor is required, 3) size of DNA is not a problem, and 4) production of DNA-coated metal particles is easy to generate. Major disadvantage is that it induces greater immune responses than microinjection.[18,20] Figure 1A shows the use of gene gun for gene therapy against cancer in clinical trials.

Electroporation: Electroporation uses electrical pulse to generate transient pores in the plasma membrane allowing efficient transfer DNA into cells.[22,23] This approach has been effectively applied in humans in order to enhance gene transfer and tested in several clinical trials such as leukemia, brain carcinomas, prostate cancer, colorectal cancer, malignant melanoma, Alzheimer, Parkinson, and depression.[8,18] Major disadvantage is that it often results in a high incidence of cell death.[5,11]

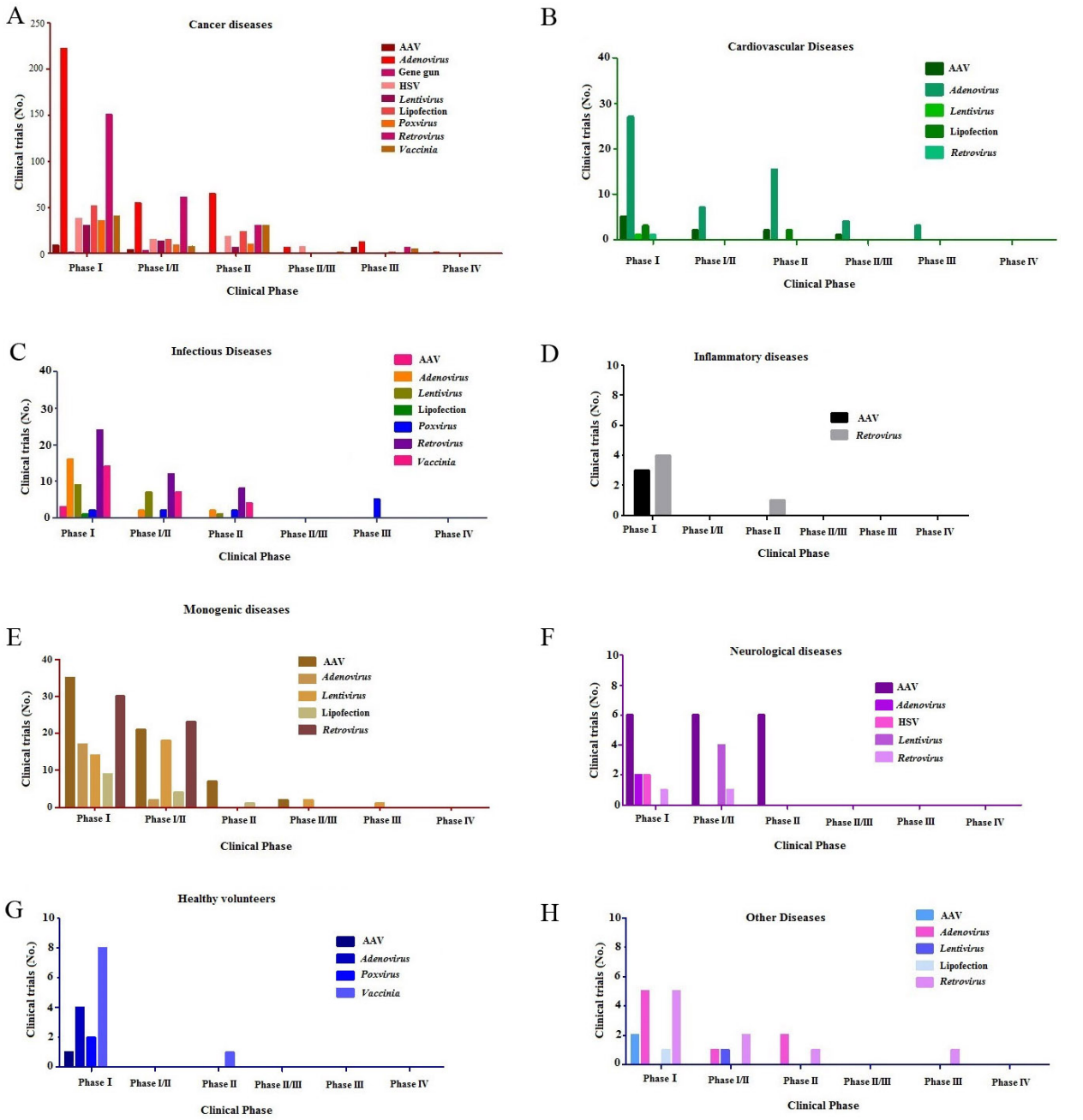

Figure 1. The applications of different gene delivery methods in clinical trials (Clinical Trial Wiley Database). A) Cancer diseases; B) Cardiovascular diseases; C) Infectious diseases; D) Inflammatory diseases; E) Monogenic diseases; F) Neurological diseases; G) Healthy Volunteers; H) Other diseases.

Sonoporation or ultrasound: Ultrasound, as a non-invasive and site-specific approach, can deliver DNA into the cells by making nanomeric pores in cell membrane and subsequently destroy tumor cells after systemic delivery. Indeed, the efficiency of this system depends on concentration and size of plasmid DNA, the intensity of the pulses, frequency and duration.[11,20,21]

Photoporation: Photoporation method uses a single laser pulse using generation of transient pores to transfer plasmid DNA into the cells. The data showed that the level of transgene expression is similar to that of electroporation.[21]

Magnetofection: In this approach, DNA is transferred into the cells by forming the complex with magnetic nanoparticles made of iron oxide and coated with cationic polymers or lipids through electrostatic interaction. This simple delivery system has similar efficiency with the non-viral chemical

(*e.g.*, cationic lipids or polymers) and physical (*e.g.*, electroporation or gene gun) transfection methods.[1, 12]

Hydrodynamic or hydroporation: Hydrodynamic is a simple and potent approach for direct delivery of water-soluble particles (*e.g.*, large volume of DNA) into internal organs.[11, 21] The efficiency of gene delivery depends on some factors including the anatomic structure of the organ, the injection volume, and the speed of injection.[18]

Naked DNA injection: Direct injection of plasmid DNA is a simple method to express gene (2 Kb-19 Kb) *in vivo* and to treat certain genetic diseases with low immunotoxicity.[5, 9, 18] The injection sites of the naked DNA include thymus, skin, cardiac muscle, liver cells, and skeletal muscle.[11] There is a hypothesis that mechanical massage of liver produces transient defects in cell membrane facilitating the delivery of plasmid DNA into hepatic cells by diffusion.[24] Its limitations are included low levels of transfection and transgene expression due to the rapid degradation by nucleases in the serum, and the clearance by the mononuclear phagocyte system.[25]

2.2 Non-viral chemical methods

Chemical non-viral methods have been known as an important delivery system designed as natural or synthetic compounds such as polymers, lipids, peptides, and inorganic methods as mentioned in next sections.

2.2.1 *Polymers*

Most polymers applied for gene therapy contain positive charge groups (*e.g.*, amines) which interact with the negative charge groups of DNA (*i.e.*, phosphates) to form compact structures named as polyplexes. These structures can be endocytosed by cells similar to lipoplexes.[26, 27] There are four main properties for successful delivery by polymers including: 1) enhancement of extracellular and intracellular stability of DNA by its packaging in small sizes; 2) cellular uptake of particles by endocytosis; and 3) DNA transportation, and 4) its release within the nucleus. The researchers concentrated on three strategies for polymeric gene transporters such as: encapsulation, electrostatic interaction, and adsorption.[28] Cationic polymer-based gene carriers indicated good biodegradability, low toxicity, and relatively higher transfection efficiency than liposomes. The structural diversity in polymers for gene delivery contains cationic residues, endosomal escape units, and degradable fragments.[29, 30] Some examples of polymers such as Chitosan, Gelatin, Polyethylene imine (PEI), Poly lactic-co-glycolic acid (PLGA) and poly lactic acid (PLA), Dendrimers were described as following.

Chitosan: Chitosans [β (1-4) 2-amino-2-deoxy-D-glucose] are natural and biodegradable polysaccharides with positive charges which differ in the degree of N-acetylation (40%-98%) and molecular weight (50 kDa-2,000 kDa). Its cationic polyelectrolyte nature protects the DNA from nuclease degradation.[26] In vitro studies showed that plasmid DNA-loaded chitosan nanocarriers capable of achieving high transfection levels in most cell lines. In addition, siRNA-loaded chitosan nanocarriers suppressed the gene expression similar to siRNA delivered by lipofectamine reagent. In this line, chitosan with low molecular weight (LMW) was more efficient than chitosan with high molecular weight (HMW) for transfection.[26] The effect of deacetylation degree in cell transfection is obscure. For example, chitosan with high degree of deacetylation showed the best transfection in cell lines. In contrast, intramuscular (*i.m.*) injection of chitosan complexes with a low degree of deacetylation indicated high transfection, *in vivo*. It was shown that the transfection efficiency of chitosan is slightly lower than liposomes for DNA delivery, but however, it is significantly less toxic and easy to work with.[26] Recently, various chemical modifications of chitosan have been followed to increase its gene transfection efficiency. For example, cell penetrating peptide (CPPs) conjugated chitosan could enhance DNA delivery into cells.[31]

Gelatin: Gelatin, a natural polymer, is formed after hydrolysis of collagen and can be used to release DNA. The stronger cross-links of gelatin by glutaraldehyde prevent fast degradation in vitro and *in vivo*.[26]

Polyethylene imine: PEI generated by polymerization of aziridine as the linear or the branched forms, has been used to deliver genes into different cell types. The studies showed that the branched structure of PEI is more efficient in DNA packaging than the linear form. Indeed, the ability of PEI as a gene carrier is due to its endosomal escape and also the formation of stable complexes with DNA.[26] When PEI was covalently linked to hyaluronic acid, transfection efficiency achieved to comparable levels with lipofectamine (up to 34%, 26). PEI has been suggested as a suitable non-viral delivery system for gene therapy of airway disease.[32] Regarding the studies, transfection efficiency of PEI depends on several factors including molecular weight, degree of branching, N/P ratio (nitrogen to phosphate ratio), and complex size. However, the use of high molecular weight PEI for *in vivo* gene delivery was restricted due to the relatively low transfection efficiency, short duration of gene expression, and high toxicity.[26] Our research group indicated that a novel delivery system including low molecular weight PEI (600 Da) conjugated with HIV Tat peptide (PEI600-Tat) has a high transfection efficiency for DNA vaccine expressing HPV16 E7 protein as a model antigen.[33]

PLGA and PLA: PLGA and PLA contain units of lactic acid and glycolic acid linked through ester group. PLGA particles (< 10 μm) showed a significant potential for immunization, because they could be efficiently phagocytosed by professional antigen presenting cells (APCs). Gene delivery depends on the copolymer structure of the PLGA, molecular weight, particle size and morphology. The advantages of this polymer are low cytotoxicity and long-term transgene expression as a result of the gradual release of DNA.[26]

Dendrimers: Dendrimers contain a central core, repeating units, and terminal function groups.[21,28] Despite other polymers, dendrimers can be made by modification of the surface functional groups with hydrophilic groups (or positive charge). The repeated functional groups and symmetrical structure of dendrimers lead to effectively encapsulate the genes and high delivery to targeted sites.[34] Their transfection efficiency depends on the size and diameter of dendrimers. The studies showed that the transfection efficiency by high generation dendrimers is higher than low generation dendrimers.[35] Polyamidoamine (PAMAM) dendrimers, the hydrophilic branched spherical polymers, have produced very stable and highly soluble DNA complexes.[5,29] The efficiency of this gene delivery could be enhanced by dendrimer flexibility using a triethanolamine core.[29] However, there are some cytotoxicity issues for application of dendrimers.[36]

2.2.2 *Lipid-based non-viral vectors*

The lipid-based non-viral vectors are mainly included cationic lipids and liposomes or combination of two or more techniques as described in below:

Cationic lipids: The combination of cationic lipids with DNA generates the lipoplex structures which are more complex than simple liposomes.[37] Cationic lipids are typically composed of a positively charged head group, a flexible linker group (*e.g.*, an ester or ether), and two or more hydrophobic tail groups.[5] For improving transfection efficiency, cationic lipids are often mixed with helper lipids such as cholesterol or DOPE (1, 2-dioleyl-sn-glycerol-3-phosphoethanolamine) potentially promoting conversion of the lamellar lipoplex phase into a hexagonal structure.[35] The lipoplexes enter cells via the endocytotic pathway and effectively protect the foreign DNA from the degradation within cells. Targeted transfection can be increased by the addition of tissue-specific target ligand and the applications of proteoglycans in this process. The transfection efficiency of the lipoplexes was determined by the differences in the structures of different cationic lipids (*e.g.*, the hydrophobic anchor).[18,38] Due to the lipid nature of the carrier, the transfection ability of lipoplexes is generally high in a wide range of cell types.[18,39] Lipoplexes are effective carriers for delivering plasmids into target cells. However, plasma proteins and other extracellular proteins capable of binding non-specifically to the hydrophobic and positively charged surface of lipoplexes and can inactivate them.[19] Various cationic lipids such as lipofection or cytofectin reagents were used as a delivery system in cell culture, animals, and clinical trials (phases I and II).[9,35] The intravenous (*i.v.*) administration of cationic lipids indicated some toxicity issues in clinical trials. Therefore, they have been applied to transfer gene as intraperitoneal injection (*e.g.*, in metastatic ovarian cancer).[40] It has been shown that cationic solid lipid nanoparticles (SLNs) can successfully link to DNA, protect them from degradation by DNase I, and deliver them into cells. The properties of the complexes are dependent on the ratio between particles and nucleic acids.[21] SLNs are biodegradable, stable for a long time and easy to scale up as compared to other colloidal systems.[41] On the other hand, lipid emulsions were shown to be better than liposomes due to stability, biocompatiblity, and high solubilizing capacity.[42] The stability of lipid emulsion is a critical factor for patient safety because larger particles may cause oil embolism when administered systemically.[43] In addition, physical properties and serum stability of the DNA/ nanoemulsion complexes represent that cationic lipid nanoemulsions can be more potent carriers for *in vivo* or in vitro gene (plasmid DNA) delivery than liposomes.[21,43]

Liposomes: Liposomes are divided according to the number of lipid layers and also their charges (*e.g.*, cationic, anionic, or neutral). Thus, liposomes can be different in size and surface charge.[21,28] The studies showed that viral vectors and then polymeric vectors are more effective than liposomes for *in vivo* gene delivery. The disadvantages of liposomes include short time gene expression, low level of transgene expression, and poor physiological stability.[28] In this line, PEGylation (poly (ethylene) glycol) could improve liposomal circulation half-life *in vivo* through reduction of clearance and recognition by immune system as well as the restriction of non-specific absorption of serum proteins.[44] Several liposome-based vectors (*e.g.*, Allovectin-7®, USA) have been used in clinical trials for cancer therapy.[21] Figure 1 shows the use of lipofection for gene therapy against different diseases in clinical trials.

Hybrid methods: In order to increase transfection efficiency and remove limitations in gene delivery, several hybrid methods were improved using combination of two or more approaches. For example, virosomes were generated by the combination of liposomes with an inactivated influenza virus or HIV. This structure could transfer gene in respiratory epithelial cells more efficient than either viral or liposomal methods alone.[7]

2.2.3 *Peptide-based non-viral vectors*

Many types of cationic peptides are able to interact with plasmid DNA as a safe option for gene therapy. Furthermore, the studies showed that the linkage of a peptide to a lipoplex or polyplex allows targeting to specific cell types.[21] In another example, complexation of the polycation peptide (protamine sulphate) with DNA followed by addition of cationic lipids enhanced transgene expression in cultured cells as compared to delivery with lipid alone.[5] The structural studies indicated that peptide sequence derived from protein transduction domains (PTDs) can selectively lyse the endosomal membrane in its acidic environment leading to cytoplasmic release of the particle. Also, nuclear localization signals (NLS) for DNA transfer to the nucleus could be provided from short peptide sequences of viral proteins.[21] Our research group recently showed that MPG peptide is an efficient delivery system for expression of HPV16 E7 gene in vitro and enhancement of the potency of DNA vaccine expressing E7 in tumor mice model.[45] Generally, there are several peptide-based non-viral vectors known as cell penetrating peptides. Herein, two peptides of Poly-L-lysine (PLL) and GALA are briefly mentioned in below:

PLL: PLL is a biodegradable peptide capable of forming complexes with nucleic acids as nanoparticles due to the presence of amine groups on the lysine residues.[21] The length of the PLL could affect the particle size and stability of the DNA complex and subsequently the efficiency of gene transfer. Indeed, cytotoxicity was observed when the length of the PLL increased.[46] On the other hand, a low molecular weight PLL (MW < 3kDa) could not form stable complexes.[47] However, high molecular weight PLL was more suitable for gene delivery through systemic injection. For example, PLL (211 kDa) / DNA complexes were found in the blood after 30 minutes up to 20-fold higher than PLL (20 kDa) / DNA complexes.[35] In addition, PLL represented modest transfection and required to add an endosomolytic agent such as chloroquine or a fusogenic peptide to release DNA into the cytoplasm. Another useful modification was the linkage of PEG to the polymer for prevention of plasma protein binding and enhancement of half-life for circulation of the complex.[46]

GALA: GALA is a glutamic acid-alanine-leucine-alanine repeat (30 amino acids) containing a histidine and tryptophan residue as spectroscopic probes.[48] At neutral pH, GALA is a water-soluble peptide and when the pH is acidic (less than 5.0), it interacts with bilayers and shows high affinity of binding to neutral and negatively charged membranes.[49] In fact, GALA can bind to bilayer membranes at endosomal pH and induce leakage of phosphatidylcholine vesicles.[49] However, GALA could not directly bind to DNA due to the strong negative charges of GALA and DNA backbone, thus KALA (Lys-Ala-Leu-Ala), as a synthetic cationic peptide, was applied to attach directly to DNA.[48,49]

2.2.4 *Inorganic nanoparticles*

Some inorganic materials such as gold, silica, calcium phosphate or magnetic nanoparticles (described as below) can bind to the plasmid DNA and deliver it through endocytosis into the cells.[26,40] Transfection efficiency is very moderate for these methods, but they have several advantages, such as low toxicity, good shape control and easy storage ability for further concentration on improving these types of delivery agents.[26]

Calcium phosphate: Calcium phosphate particles were already used for gene therapy.[50,51] The interaction between calcium phosphate and nucleic acid occurs due to the affinity of calcium to the phosphate backbone in DNA. Therefore, the nucleotide sequence is not important, but its length is critical issue.[50] In addition, the concentrations of calcium, phosphate, and DNA were found as the main parameters in gene delivery. For example, high concentrations of calcium and phosphate resulted in spontaneous precipitation of nanoparticles. Also, the formation of precipitates was blocked by high concentrations of DNA (> 50 μg/ml); while low concentrations of DNA led to a rapid particle formation within 1 min.[50]

Silica: The non-metal oxide, silica (SiO_2) nanoparticles has been used in DNA delivery, drug delivery, cancer therapy, and enzyme immobilization. An *in vivo* study indicated that silica nanoparticles are not toxic in mice, but further studies should focus on safety issues in human.[52] In addition, mesoporous silica nanoparticles have demonstrated the efficiency of in vitro gene transfection in glial cells. On the other hand, silica nanoparticles combined with PEI were used to transfect human mesenchymal stem cells (MSCs). This combination of plasmid DNA delivery agents is more successful at inducing transfection than silica or PEI alone.[26] Silica nanotubes have been also evaluated as an efficient gene delivery system and imaging agent.[21]

Gold: Gold nanoparticles (AuNPs) have several advantages such as 1) easy preparation, 2) low toxicity, and 3) surface modification using different chemical methods.[21] Size and shape of gold nanoparticles offers great promise as an intracellular therapeutic delivery vector. High reduction potential of Au keeps nanoparticles intact in the blood, especially when PEG molecules were used to extend retention and make NPs as non-opsogenic.[34] Gold nanoparticles are excellent candidates for gene delivery, as they can be synthesized to carry inherent positive charge without any surface molecule coating. Studies have shown the main clinical potential of

gold nanoparticles for *in vivo* gene therapy, and bioimaging.[34,53]

Magnetofection: Another approach for binding DNA to the surface of particles includes the electrostatic interactions between the negative charge of DNA (*i.e.*, phosphate backbone) and positive charge molecules linked to the particle surface.[54,55] A study suggested chitosan coated Fe_2O_3 magnetic nanoparticles (MNPs) for gene therapy in specific sites of the body. Two points should be considered to achieve high transfection/transduction efficiency by in vitro magnetofection: 1) low vector dose, and 2) the reduced incubation time.[55]

3. VIRAL METHODS

The innate ability of viruses for gene therapy is an important field to correct or overcome a genetic deficiency.[3,8,56] Viral vectors are new genetically engineered viruses widely used for gene delivery to human cells.[3] Viruses can be modified genetically to be non-infectious.[8] Herein, the term transduction comprises the entry of a vector into a cell, and the next expression of the therapeutic protein. Thus, transduction efficiency indicates the success of gene transfer.[3,7,8] Many mammalian viruses have been considered as gene delivery vectors classified as two main categories: integrating vectors and non-integrating vectors.[9,56] The most widely used viral vectors in gene therapy include Retrovirus, lentivirus (LV), adenovirus (AdV) (25), adeno-associated virus (AAV), herpes simplex virus (HSV) and Poxvirus.[5,8,9,11,57] For instance, modified adenoviral vectors have been designed in gene therapy that can easily be generated in large scales, and are able to transfer pro-drug genes.[8] Also, the recombinant vaccinia virus vectors were used for expression of HPV16/18 E6 and E7 genes in cervical cancer patients to suppress tumor growth.[11] Figure 1 shows the use of viral vectors for human gene therapy in different diseases.

4. BACTERIAL METHODS

Bacterial vectors (*e.g.*, *Escherichia coli*, *Salmonella typhimurium*, *Clostridium*, and *Listeria*) can transfer pro-drug converting enzymes and cytotoxic agents into tumor cells, and also stimulate the host immune response. The genetically engineered bacterial vectors for RNA interference (RNAi) are relatively safe and potent, and cheaper to construct in comparison with viral vectors. They selectively colonize, grow within the tumor and can be injected orally for prevention of gastrointestinal disorders.[8]

5. BACTERIOPHAGE-GUIDED GENE THERAPY

Bacteriophages are safe and can be engineered to deliver genes into mammalian cells. A novel generation of hybrid vector was reported as a chimera between eukaryotic AAV and the filamentous M13 bacteriophage, both contain single stranded DNA genome, known as AAVP (AAV/phage). This vector expresses three to five copies of the cyclic RGD4C (CDCRGDCFC) ligand on the phage pIII minor coat protein for specific targeting to the $\alpha v\beta 3$-integrin receptor expressed mainly on tumor cells.[25] The National Cancer Institute of the USA (NCI) has utilized the ligand-targeting properties of the RGD4C-AAVP to deliver tumor necrosis factor alpha (TNF-α) to the angiogenic vasculature of human melanoma xenografts in nude mice. The systemic injection of the phage particles showed that the TNF-α expression leads to induce apoptosis in tumor blood vessels and significant inhibition of tumor growth.[25]

6. GENE THERAPY AND OTHER THERAPEUTIC MODALITIES FOR TREATMENT OF CANCER

The most important challenge in cancer treatment is to attain the highest levels of efficacy and specificity. Recently, the linkage of an imaging reporter gene to the therapeutic gene could determine whether the gene is expressed at a sufficient level and the correct location. Indeed, molecular imaging could play an important role in cancer detection and diagnosis.[58] However, molecular imaging needs high resolution and sensitive tools and also specific imaging agents that connect the imaging signal with molecular events. In this line, nanoparticles modified with small molecules such as peptides, and aptamers were broadly used for preclinical studies. Therapeutic genes could be incorporated into nanoparticles to construct multi-functional imaging agents which allow for therapeutic applications.[59] Sometimes, the combination of gene therapy with other modalities could increase its potency *in vivo*. For example, gene therapy is a suitable approach to treat glioblastoma (GBM), in combination with surgery and chemo-radiotherapy. Four types of gene therapy have been already used to treat glioblastoma such as suicide gene therapy, immuno-modulatory gene therapy, tumor-suppressor gene therapy, and oncolytic virotherapy. The data showed that the delivery of genes to the tumor site was performed by viral or non-viral vectors[60] as above described. Generally, it is required to combine some methods with gene therapy for efficient tumor treatment.

7. SUMMARY

Gene therapy is a successful method in patients with primary stages of malignancies. The use of tumor genomic analysis and the evaluation of humoral and cellular immune responses will facilitate the selection of the most effective gene therapy method for patients. Furthermore, this approach is fast, ef-

fective, low toxic and inexpensive with high cure ratios to the majority of patients in the world. Thus, the selection of suitable gene delivery system is a critical issue for treatment of diseases especially cancer therapy in future.

AUTHORS CONTRIBUTION

Elnaz Agi and Zahra Mosaferi are both first author.

CONFLICTS OF INTEREST DISCLOSURE

The authors declare that there is no conflict of interest statement.

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