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Minireview

Molecular Conjugates: Components of Synthetic Targeting Vectors for Cancer Gene Therapy

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Introduction

The ability to treat cancer with gene therapy is now becoming a reality. Cancer gene therapy is based on the concept of delivering and expressing a therapeutic gene in a tumor cell to either correct or alter tumor cell function. Strategies based on this approach range from vaccine therapy to those involving pro-apoptotic genes where the goal is to irradicate the tumor cell. The possible choices of therapeutic genes is not a current limitation and continues to increase as we understand more about the various pathways that control both tumor and normal cell growth. In addition, this will be further facilitated by the recent completion of sequencing the human genome[62]. However, one of the most important barriers that remains in obtaining successful gene therapy is the ability to efficiently and specifically deliver the therapeutic gene to tumor cells. While research continues on using viruses as delivery vectors, limitations specific to viruses such as toxicity and immunogenicity may never be overcome. These limitations were recently demonstrated when a patient with Ornithine Transcarbamylase deficiency died from an intravenous dose of recombinant adenovirus[42]. In contrast, non-viral based vectors have grown in popularity due to a lack of these limitations. Unlike viruses, these synthetic, self-assembling vectors have the ability to mediate delivery to a large number of cells irrelevant of cell division status, no current limitation on the size or type of nucleic acid that can be delivered, no intact viral component and therefore safe for the recipient, and the potential to target delivery to specific cells. One promising non-viral vector demonstrating these characteristics is referred to as a Protein/DNA complex or polyplex (poly = polycations). A Protein/DNA polyplex is based on using different versions of a "molecular conjugate", which is defined as a delivery specific vector component to which a nucleic acid or DNA-binding component has been attached. The polyplex forms by combining the conjugate (s) with DNA which can then produce general or specific delivery depending upon the components used. Since the initial use of a molecular conjugate for gene delivery over 14 years ago[71], we have learned that efficient, targeted gene delivery by the Protein/DNA polyplex requires components capable of; 1) ***targeting*** for cell-specific delivery of vector, 2) ***DNA-binding and compaction*** to ensure that vector is sufficiently small for cellular internalization, 3) ***endosome lysis*** to ensure the entry of functional DNA into the cell, 4) ***nuclear translocation*** to ensure efficient gene expression, and 5) ***DNA persistence or integration*** to obtain appropriate levels and duration of gene expression (Fig. 1). In this review, the components that have been used in these roles, the methods used to produce and analyze molecular conjugates and Protein/DNA polyplexes, as well as the applications of this vector will be discussed.

Targeting

The concept of using a molecular conjugate for gene delivery was originally based on the idea of attaching a ligand to DNA through the use of a DNA-binding component. Critical to this approach is that the ligand must be capable of both specific binding and internalization after conjugation. After combining the conjugate with DNA, the resulting vector would then be capable of targeted delivery

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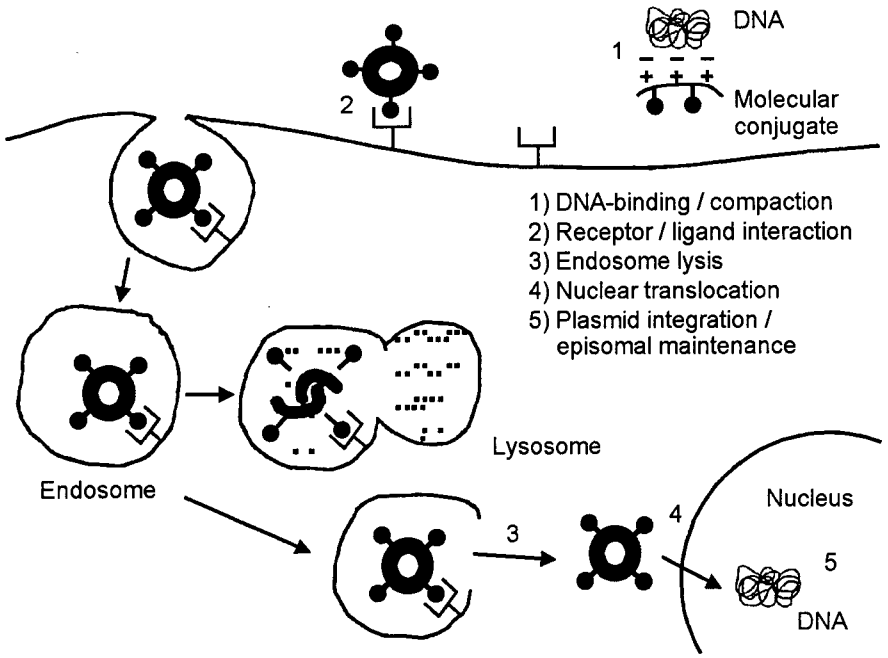

Fig. 1. The general functions of a molecular conjugate and a protein/DNA polyplex that are required for efficient gene delivery and expression. 1) DNA-binding and compaction, 2) targeting, 3) endosome lysis, 4) nuclear translocation, and 5) DNA persistence or integration.

to a specific population of cells depending upon the ligand used. One of the first examples of targeting with a molecular conjugate involved the use of asialoorosomucoid (ASOR)[71]. The ASOR receptor, which is expressed almost exclusively by liver parenchymal cells, binds ASOR via terminal galactose groups[59]. The first use of the ASOR conjugate demonstrated that a plasmid expressing the chloramphenicol acetyl-transferase (CAT) gene could be delivered specifically into HepG2 cells (liver cell line)[71]. Transient, low level CAT expression resulted that could be competed when delivery was done in the presence of free ASOR, indicating cell-specific targeting. Further analysis demonstrated liver specific CAT expression following a tail vein administration of the ASOR/DNA polyplex[72].

Since these initial experiments, many other ligands have been used as molecular conjugates for targeting. Transferrin, which has receptors that are expressed by many different cell types, has been used as a molecular conjugate to deliver DNA to erythroleukemic, lung, and liver cancer cell lines[10,67]. In addition, delivery has also been obtained in vivo to tumors as well as to the liver[35,36]. The malaria circumsporozite (CS) protein has been used to target the liver in situations where ASOR receptor expression is low, such as in cirrhosis, diabetes, and hepatocellular carcinoma[21]. Molecular conjugates have also been used in receptor identification. The Human Papilloma virus (HPV) capsid was used to partially identify the HPV receptor on cervical cancer cells by using the capsid as a ligand for the attachment and delivery of a reporter gene[46]. In contrast to these high molecular weight ligands, vitamins and peptides have also been used. The vitamin folate has been used to promote delivery of DNA into ovarian carcinoma cells that over-express the folate receptor[31]. More recently, small molecular weight growth factors have been used to promote selective or specific uptake of polyplex vectors by tumor cells[15,24]. The over-expression of the EGF receptor on cancer cell lines has allowed for specific uptake of an EGF/DNA polyplex by both non-small and small cell lung cancer cells[15,26].

It is now possible to replace proteins with synthetic ligands that decrease complexity, cost, and immunogenicity of conjugate based vectors. The protein ASOR has been replaced by a galactose conjugate for liver specific delivery via the ASOR receptor[56]. More recently, technological advances in other areas of cell biology are providing new sources for synthetic ligands. Phage display, which utilizes phage libraries that express a wide range of peptide sequences, is being used to identify peptides that

target receptors on a wide range of tumor and endothelial cells[53]. The rapid identification and testing of these and future ligands should be facilitated by the simplicity of producing conjugates and the self-assembling basis for Protein/DNA polyplex formation.

DNA-binding and Compaction

Central to the performance of molecular conjugate based vectors is the DNA-binding and compaction component. In addition, this component functions to provide a point of attachment to the vector for other components. Typically, polycations have been used in this role based on cost and availability. Poly-L-lysine (PLL) (a synthetic, linear molecule of repeating lysines) was first used in this role based on this cost-effective molecules efficient ability to bind and compact DNA. The resulting PLL/DNA polyplex interacts non-specifically with charged glycoproteins expressed at the cell surface[45]. However, PLL has been most commonly used in molecular conjugate formation with a wide range of ligands to target vector delivery through many different receptor mediated endocytotic pathway's[10,13~18,21,27,31,46,56,66,67,69,71,73].

Unfortunately, either alone or as part of a molecular conjugate, gene delivery is limited using PLL due to an inability to perform endosome lysis, which is required to ensure passage of the nucleic acid from the endosome compartment into the cytoplasm in a functional form (Fig. 1). As a result, research is now focusing on developing synthetic components that can perform endosome lysis and DNA-binding/compaction without increasing vector complexity. This has resulted in branched, synthetic polycations like polyethylenimine (PEI) and dendrimers receiving greater attention based on the ability of these molecules to mediate endosome lysis in addition to these other functions. These molecules are available in a wide range of molecular weights, but can vary in cost. Polyethylenimine has been used more because of it's low cost, easy availability, and high endosome lysis capabilities. Based on this last point, vector formulations involving PEI have now been shown to rival adenovirus in overall gene delivery efficiency at least in vitro, using many different types of cultured cells[68]. Unfortunately, in vivo delivery has been limited and is usually accompanied by toxicity, especially when PEI in molecular weights greater than 25,000 and/or high PEI/DNA ratio's (>5/1) are used[30]. Attempts have been made to reduce toxicity through galactosylation or glucosylation of the amine groups, but reduced gene delivery can result[43]. In contrast, the naturally occurring DNA-binding components, spermine and spermidine have been used for DNA compaction, but unfortunately, these components lack endosomolytic activity and tend to have a lower binding affinity for DNA based on their small size[63]. Additionally, naturally occurring compounds such as histone proteins have been used based on lower toxicity. In one study, histone proteins were galactosylated and used to target attached DNA to the hepatocellular carcinoma cell line HepG2[6]. An alternative approach has been to develop new synthetic molecules that have reduced toxicity, such as a linear version of PEI. The reduced toxicity of this molecule is due to a change in amine content and structure[68]. While capable of efficient gene delivery to the lung and to tumor cells in vivo (with little or no toxicity), the high cost of synthesis is a limitation that reduces enthusiasm for using this form in the clinic[9,32]. As a result, reducing toxicity and increasing transduction (without increasing cost) are still important limitations that must be overcome if polycations such as PEI are going to be used in future gene therapy applications.

Although the properties of DNA-binding components vary, the process behind DNA-binding and compaction is typically based on simple self-assembly through ionic interactions between the positive charge of the polycation amine groups and the negative charge of the nucleic acid phosphate backbone. The non-damaging, charge neutralization and compaction of the DNA molecule can be analyzed by agarose gel electrophoresis (charge neutralization of DNA), electron microscopy, atomic force microscopy, or instruments utilizing light scattering (particle size and charge analyzer)[13,37,71,73]. Since charge neutralization is the basis for Protein/DNA polyplex formation, theoretically any nucleic acid of any size can be compacted and delivered. This is in sharp contrast to viruses that have packaging constraints on the size and type of nucleic acid that can be compacted and delivered. As a result, molecular conjugates have been used to deliver nucleic acids as small as oligonucleotides to as large as artificial chromosomes[2,58]. The resulting polyplex vector can also range in size from as small as 10 nm to greater than >500 nm depending upon the type and amount of polycation or DNA used[13,63,73]. This size range is also reflected in a range in structure from

doughnut shaped toroids to ball shaped structures[13,37,63]. Most importantly, it is critical that the polyplex is sufficiently small for cellular internalization and passage through liver fenestrations or between endothelial cells to gain access to target cells. This size constraint is usually in the range of 100~300 nm, which is typically the size of an endosome compartment or a liver fenestration[52].

In addition to DNA binding/compaction, and endosome lysis, the polycation also serves as an attachment point for vector components. Critical to attaching proteins or peptides to the DNA-binding component is the correct coupling of the two molecules to ensure that the functions of the component i.e., receptor binding, endosomal lysis, etc. and the polycation are maintained. A wide range of linkages can be generated using several different chemicals. The water-soluble carbodiimide 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), has been used to link ASOR to PLL, by forming a covalent bond between the carboxyl groups of the protein and the amine groups of the polycation[13,71]. Unfortunately, this "zero-length cross-linker" results in little or no space between the components and polycation, which can affect component function based on steric hindrance or other physical interactions[15]. The chemical 3-(2-pyridyldithio) propionic acid N-hydroxysuccinimide ester (SPDP), has been used to link transferrin to PLL by the formation of a disulfide bond between sulfhydryl-modified amine groups[67]. However, this bond is very reactive with other sulfhydryl groups, which can potentially result in instability of the disulfide bond and difficulty in conjugate formation. A third attachment is based on the interaction between biotin and streptavidin with its four biotin binding sites. Biotinylation of proteins is a very mild modification, however; the inclusion of streptavidin into the vector formulation for biotin/streptavidin bridge formation can increase vector size and immunogenicity. The binding between these molecules is one of the strongest in nature and has been used for coupling epidermal growth factor or adenovirus to PLL[15]. In contrast, it is possible to directly link components to DNA through the use of agents such as an ethidium homodimer[64]. More recently, peptide-nucleic acids or PNA's have been developed that bind a specific plasmid sequence (based on the nucleic acid portion) and at the same time, introduce a peptide sequence that allows for the attachment of biotin, fluorescent labels, and other components to the DNA[39,49]. This provides the ability to easily attach vector components directly to the vector. As an example, a PNA has been used to easily attach transferrin to plasmid DNA for targeting myogenic cells[39]. Most importantly, a DNA compacting component is still required when direct coupling is used, which limits the utility of these approaches. Overall, as long as the function of the vector components is maintained, then any coupling method can be used.

Endosomal Lysis

Once a suitable DNA-binding component and targeting ligand have been identified and a molecular conjugate synthesized, the resulting vector must then be tested for gene delivery efficiency. The incubation of the PEI/DNA polyplex with cells results in non-specific binding through interaction with cell surface glycoproteins, which is followed by non-specific internalization[45]. However, in the context of the targeted polyplex, the binding between ligand and receptor results in ligand/receptor internalization or receptor-mediated endocytosis (Fig. 1). In the case of the folate receptor, the bound ligand is internalized through a slightly different process termed potocytosis, where the receptor binds the ligand, the surrounding membrane closes off from the cell surface, and the internalized material then passes through the vesicular membrane into the cytoplasm. As a result, the folate conjugate and attached DNA are not degraded but essentially remain trapped inside larger vesicles in the cell as the Folate/DNA polyplex is unable to pass through the membrane[31]. As a result, one of the more critical steps to overcome in each of these pathway's is endosome or vesicle entrapment of vector, which in the receptor mediated pathway can lead to fusion with the lysosome and degradation by lysosomal enzymes (Fig. 1). As a result, the DNA that is attached to either the polycation or the molecular conjugate will become degraded resulting in little or no gene expression[13,16].

Initial conjugate based vector formulations did not contain components to overcome vector entrapment. The success of these vector formulations was based on the "leakiness" of the uptake process in that some molecules of DNA escaped degradation during internalization. However, it became quickly apparent (based on the low level of gene expression) that some type of agent must be included in the vector formulation to enhance the efficiency of bypassing degradation. Several agents have been identified that either

disrupt the endosome or reduce DNA degradation. Chloroquine, which raises the pH of the endosome, has been used to decrease the degradation of endocytosed material by inhibiting lysosomal hydrolytic enzymes[10]. Physical procedures, such as a partial hepatectomy, when performed along with DNA delivery by an ASOR/DNA polyplex, has resulted in increased persistence and expression of delivered DNA, from days to months[69]. However, these procedures have had limited use because of low efficiency and a lack of utility for enhancing endosomal release in other tissues. In contrast, human replication-defective adenovirus (i.e. dl312, serotype 5 adenovirus, deleted E1a gene) has been identified to be one of the best agents at performing endosome lysis[13,16]. The receptor-mediated internalization of the viral particle leads to fusion of the penton base (located in the viral capsid) with the endosomal membrane, which leads to pore formation and endosome lysis[33]. When replication defective adenovirus is co-incubated with a Protein/DNA polyplex, both are internalized into the same endosome. The adenovirus then mediates endosomal lysis and the vector is released into the cytoplasm. The use of adenovirus in this role has resulted in as much as a 1000-fold increase in gene expression in many Protein/DNA polyplex targeting approaches as compared to vector alone[13,16]. Unfortunately, viral titers of 10^3 to 10^4 viral particles/cell must be used, which usually produces toxicity. The specificity of delivery is still maintained by the ligand in the Protein/DNA polyplex, as delivery can be competed with free ligand[13]. While this enhancement can also be increased further by directly coupling the virus to the Protein/DNA polyplex (resulting in at least a one-order of magnitude drop in viral titer), this polyplex has shown limited use in vivo, due mainly to problems with toxicity [14,17,27,66]. Attempts have been made to decrease this toxicity by using ultraviolet light to inactivate the viral genome[12]. Also, other viruses such as chicken adenovirus (CELO Virus) have demonstrated similar levels of enhancement, but without the associated toxicity[11].

In contrast, other approaches have focused on developing completely non-viral endosome lysis components. Peptides based on the membrane lysis portion of the influenza virus hemagglutinin HA2, have been incorporated into a transferrin/DNA polyplex[65]. These short, 20 amino acid peptides mediate endosomal lysis by inserting into the endosomal membrane upon acidification of the endosome, causing pores to form, which leads to lysis[57]. A comparison of endosome lysis efficiency between these peptides and adenovirus has shown that while gene delivery is increased, these molecules are still less efficient[65]. More recently, branched chain polycations such as PEI have been used in this role. This synthetic polymer has a highly branched ratio of primary : secondary : tertiary (1 : 2 : 1) amines[5]. This allows for DNA binding, endosome release, and ligand attachment all in one molecule. Endosome release is based on PEI's structure (terminal amines ionizable at pH 6.9, internal amines ionizable at pH 3.9) which allows the molecule to act as a "proton sponge" that buffers a pH change during endosome acidification, leading to vesicle swelling and lysis[5]. As a result, this multifunctional component is capable of efficient gene delivery even in the absence of a targeting component. However, as with fusogenic peptides, the delivery efficiency of vectors containing this agent are still limited, especially in vivo. Some groups have hypothesized that this is due to the lack of a nuclear translocation component in the vector. As a result, to increase overall transduction, greater focus is now being directed at including nuclear translocation agents and components that can increase the persistence and level of gene expression into conjugate based vectors.

Nuclear Translocation, Persistence, and Integration of DNA

Up to this point in time, much focus has been placed on developing potent non-viral delivery vectors, while less time has been spent on understanding issues related to the cellular fate of these vectors. For example, while recent studies have utilized the high endosomolytic activity mediated by PEI for gene delivery, little is known about the intracellular fate of the molecule and the attached DNA. Some studies have suggested that PEI travels to the nucleus by interacting with the cytoskeleton, but this is still unclear[29]. Some groups have made attempts to enhance nuclear translocation by using peptides based on viral protein sequences to achieve this goal. Small peptides based on the nuclear translocation sequences of SV40 T-antigen have demonstrated increased nuclear delivery in various applications[40]. However, a more critical aspect of nuclear delivery may be at the entry site to the nucleus, or the "nuclear pore complex"[61]. This structure is as small as 20 nm in size and raises the question as to how a vector

greater than 10 times in size can pass through this opening and deliver the nucleic acid to the nucleus. Studies are now being directed towards this question and should reveal the mechanism involved as well as how nuclear translocation peptides affect this process.

Once the DNA gains entry into the nucleus, the delivered plasmid remains episomal and does not integrate into the host genome. Initial studies indicated that a certain portion of the delivered DNA may integrate into the host genome, but the lack of sequences in current plasmids does not support this outcome. To increase the duration of gene expression, studies using partial hepatectomy following Protein/DNA polyplex delivery resulted in an increase in the length of gene expression from days to months[8,69]. Unfortunately, this technique is not a viable approach for delivery in other tissues. Further analysis of the affect of this procedure on the delivered DNA has identified that a greater amount of DNA is protected in vesicles in liver cells during the repair process and that the protected DNA is slowly released with time. This was determined through the use of peroxisome stimulating agents that reproduced this affect in liver cells[8,69]. Overall, while gene expression does occur, the plasmid DNA does not persist and the resulting duration and level of expression is transient requiring repeated administration of vector. The limited immunogenicity of the vector may facilitate this type of administration, but it would be advantageous to develop plasmids that either promote plasmid replication/maintenance or integration to allow for persistent, high-level expression of the therapeutic gene. Some studies have already demonstrated that plasmids modified to contain the EBV origin of replication and the EBNA-1 gene, persist for a longer period of time in replicating cells[50]. In contrast, some approaches are now focusing on integration of the delivered DNA. Recent studies using transposon technology, produced insertion of DNA into the mouse genome in 5~6% of transfected mouse liver cells[74]. These enhancements will be critical to the further use of non-viral vectors in clinical applications, particularly in cancer treatments where persistent (for the life of the tumor cell), high-level gene expression is needed. Since Protein/DNA polyplexes can be easily produced, testing of these enhancements will be greatly accelerated and simplified.

Polyplex Vector Formation and Delivery Applications

The simplicity of this vector is based in part on the self-assembling nature of the components. In addition, studies have been further facilitated by the fact that a plasmid can be used to express the "gene of interest" in the target cell. Plasmid DNA is easy to manipulate and can be cost-effectively produced in large quantities. Studies using plasmids have focused in part on developing conditions that allow for efficient formation of the Protein/DNA polyplex so that effective administration can be achieved. This has ranged from Protein/DNA polyplex formation carried out under concentrated conditions ([DNA] = 87 nm or 0.35 μg/μl) to polyplex formation at dilute DNA concentrations ([DNA] = 3 nm or 0.012 μg/μl)[67,71]. While the former produces vector capable of transduction in vivo (but with precipitation of the polyplex and a lack of reproducibility), the latter produces vector that has shown limited in vivo application (due to the low concentration of DNA), but is suitable for reproducible in vitro studies.

Most importantly, the simple self-assembling process by which Protein/DNA polyplexes can be generated, has demonstrated that there is no current limit as to the type and size of the nucleic acid that can be delivered. As mentioned, the primary type of nucleic acid delivered thus far is a DNA plasmid. Plasmids ranging in size from several kilobases (kb) to as large as a bacterial artificial chromosome have been used[2,71,73]. In one study, a 48 kb plasmid was delivered by a transferrin/DNA polyplex without a loss of delivery efficiency, when compared to polyplexes made with a smaller plasmid carrying the same gene[12]. In contrast, small oligonucleotides have also been used in the context of a liver targeted polyplex vector[41].

As a result of being able to use a plasmid of any size, anything from a reporter gene to a therapeutic gene can be delivered by this vector. The E.coli β-galactosidase (β-gal) and luciferase genes have been used the most as reporter genes[13,67]. Use of the β-gal gene allows for histochemical staining of cells to determine the number or percentage of cells transduced as well as quantitation of gene expression. In contrast, the luciferase gene allows for viewing of transduced cells by fluorescence analysis. While the expression can also be quantitated, this protein allows for continued viewing of the cells without fixation, which simplifies

time-course studies. In either situation, depending upon the ligand used, the resulting Protein/DNA polyplex produces variable levels of transduction and expression in vitro. Transduction efficiencies as high as 80~99% have been achieved using an ASOR/DNA polyplex or EGF/DNA polyplex to deliver the β-gal gene to primary hepatocytes or tumor cells respectively[13,15]. In addition, a wide range of therapeutic genes have also been used in various vector formulations. An ASOR/DNA polyplex has been used to deliver the phenylalanine hydroxylase and factor IX genes to develop therapies for Phenylketonuria and Hemophilia[13,14]. In relation to cancer therapy, a transferrin/DNA polyplex has been incorporated into a clinical protocol for the ex vivo transduction of melanoma cells with cytokine genes for the immunological rejection of melanoma cells[75]. More recently, an adenovirus/PLL polyplex has demonstrated efficient delivery of the tumor suppressor *p53* gene in p53 deficient tumor cells[48].

Unfortunately, the in vivo applications of Protein/DNA polyplexes have been somewhat limited thus far. Delivery has been demonstrated to the lung, liver, brain, kidney, and more recently to solid tumors[4,5,9,32,48,68]. A transferrin/DNA polyplex coupled to adenovirus resulted in only 1% of lung epithelial cells being transduced following an intratracheal administration[27]. More recently, an adenovirus/PLL conjugate was used to deliver the tumor suppressor *p53* gene by intratumoral administration to subcutaneous non-small cell lung cancer tumor in mice[48]. This resulted in high-level p53 expression that induced apoptosis and produced at least a 50% reduction in tumor size. Unfortunately though, each of these polyplex vectors required adenovirus as an endosome lysis agent. In contrast, much more recent formulations based on using PEI have now shown that gene delivery to the lung, brain, kidney and tumors can be obtained with this polycation[4,5,9,32,68]. Perhaps the most impressive result was obtained when a linear form of PEI was used to deliver the β-gal gene to the lung following intravenous administration[32]. This resulted in the vast majority of the lung staining positive for reporter gene expression.

In addition to limited in vivo delivery efficiency, there are also other limitations. The intratumoral administration of a PEI/DNA polyplex into a subcutaneous tumor produced little or no gene delivery[9]. While delivery could be increased by slowly infusing vector, procedures such as this increase the complexity of both vector administration and future clinical uses. In addition, it has been identified that polycation based vectors elicit interaction with blood proteins such as complement[55]. Further analysis has identified that the charge of the vector contributes to this interaction. More recent studies have now identified that agents such as polyethylene glycol must be included in the formulation to shield the vector from interaction with blood proteins[23]. Overall, while these obstacles have been encountered, progress continues towards overcoming these limitations. Most importantly, it is clear that the in vivo delivery efficiency of polycation and conjugate based vectors that has been limiting for such a long time, is now on the way to becoming a thing of the past.

Future Directions

The development of molecular conjugates as components of Protein/DNA polyplexes has resulted in the creation of a simple, self-assembling, non-viral vector for the targeted delivery of nucleic acids into specific cells. The continued development of this vector has resulted in the creation of a "synthetic virus", that has the capability of targeted delivery without the negative attributes of viruses. The simplicity of this vector allows for quick analysis of nucleic acids, expression vectors, and therapeutic genes in vitro and potentially in vivo. Just as important, the time that would be involved in the generation of recombinant viral vectors is not present. Future work will continue to utilize these advantages to address other problems, such as transient gene expression by developing integration and episomal maintenance plasmids based on viral systems. Peptides based on viral nuclear translocation signals will be developed to enhance nuclear delivery and increased gene expression. Crucial to the further development and use of this delivery vector will be the identification of new components that can improve efficiency with a limited increase in complexity. This could be possible by combining many of these components into one chimeric protein or peptide. In addition, further manipulation of the expression plasmid should lead to tissue specific and regulatable expression systems, adding another level of specificity to the vector. At this point in time, it is clear that any protein, peptide, or nucleic acid that can increase the utility of this vector, can be easily incorporated resulting in a much greater use of molecular conjugates and Protein/DNA polyplexes in can-

cer gene therapy applications.

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