WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 4, 756-768. Review Article ISSN 2277– 7105

A COMPREHENSIVE REVIEW ON GENE TRANSFER TECHNIQUES: AN OVERVIEW

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Article Received on
05 Feb. 2021,

Revised on 25 Feb. 2021,
Accepted on 17 March 2021

DOI: https://doi.org/10.17605/OSF.IO/CY6WS

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ABSTRACT

Biolistic are developed as a system for gene delivery into plant cells, but has recently been introduced for transfection in to mammalian tissue, including few attempts in neural cells. Basically, in this system the plasmid DNA of interest is coated on to small particles, that are accelerated by a particular driving force. The combination of several so-called 'ballistic' parameters and tissue parameters determines the transfection efficiency. In terms of transfection efficiency, biolistic seems favorable above conventional techniques, like calcium phosphate precipitation and lipofection. Compared to viral techniques biolistic may be less efficient, but is quicker and easier to handle and seems to produce fewer complications for in vivo gene delivery. Therefore, although the technique is only in a development al stage. Preliminary results seem promising, and optimization of the method may prove useful in scientific research and/or clinical use.

INTRODUCTION

Current neuroscience research is to understand the functions of the thousands of brain-specific genes involved in neural development, plasticity, physiology, and function. To accomplish this goal, we must have access to techniques in which gene expression can be monitored and manipulated in healthy cells, slices, embryos, and adult animals. Historically, transfection of postmitotic neurons has been labor-intensive, inefficient, unreliable, and/or cytotoxic. This inability to express foreign proteins in postmitotic neurons has, until the past few years, hampered neuroscience research. Fortunately, a large number of diverse techniques for transferring genes into postmitotic neurons have recently been developed and

optimized.[1] For basic research purposes, the ideal transfection method should: first, be capable of transfecting postmitotic neurons with high efficiency; second, allow transfection of constructs of varying sizes, including transfection with multiple constructs; third, have limited cellular toxicity; and fourth, be easy and safe to perform. Thus, the specific advantages and disadvantages of each technique must be considered in selecting a transfection method for any particular experiment.[2] Because one ultimate goal of gene transfer lies in therapeutic remedies, much of the research into DNA delivery to the nervous system is geared towards gene therapy. All the same, reviewing the large and rapidly growing field of gene therapy is outside the scope of this review for gene therapy issues, including information on the use of lentivirus in gene transfer, the reader is referred to several recent reviews and reports.[3,4]

Recombinant virus-based technologies

Gene transfer into postmitotic neurons is a young field. One of the first major breakthroughs in transfecting postmitotic neurons came in 1988 with the demonstration of the first high-efficiency, virally mediated transfer of a foreign gene into neurons.[5] The increasing use of viral vectors for the transfer of DNA to neurons is undoubtedly due to extremely high infection efficiencies (up to 95% of neurons) compared with non-viral methods. This superiority of virus-based systems comes as little surprise, because one is benefiting from what viruses have evolved to do-insert their DNA or RNA into host cells and express it. This basic predisposition for infection makes viruses relatively easy to use in both young and adult tissue and on such diverse preparations as dissociated cells, slices and in vivo. Because many recombinant viral vectors are replication incompetent, most are also relatively safe to use. All the same, these advantages are counterbalanced by some serious limitations-potential toxicity to neurons, the effort and time to construct recombinant viral vectors, limitations on size of the DNA expression cassette, and potential safety hazards to laboratory personnel.[6-8]

Herpes simplex virus

The first virus to be used for gene transfer was herpes simplex virus (HSV) Neurons are a natural host for HSV and expression of HSV-transduced genes can last for months to years. However, because of its cellular toxicity, its difficulty to construct, and its high potential risk to humans, HSV is not commonly used. Recent advances in amplicon-based HSV vectors decreases in toxicity, and increasing ease of use may allow these viruses to live up to their early promise in the near future.[9]

Adenovirus

Adenovirus has historically been the most commonly used viral vector, with applications ranging from gene transfer in vivo, to in vitro slices and dissociated neurons. The first reports of recombinant as an effective gene delivery system for postmitotic neurons in vivo were published in 1993. Expression begins a few days following infection and lasts for weeks to months. Although this vector can transduce postmitotic neurons in culture well, the success of recombinant Adv. in transducing postmitotic neurons in intact tissue can be variable.[10,11]

Adeno-associated virus

One of the most promising viral vectors is adeno-associated virus (AAV). In 1994, Kaplit et al. discovered that AAV vectors can selectively transfect neurons. AAV is the least toxic of all viral vectors, leads to high levels of gene expression and has the potential for site-specific integration, leading to long-lasting gene expression. The limitations of AAV vectors are two-fold: the recombinant protein starts to be expressed after a delay of about two weeks post-infection and the maximal insert size is only about 5000 nucleotides. Recently, AAV vectors have been used to transduce postmitotic neurons in vivo, in dissociated primary cultures, and in cultured brain slices.[12-14]

Vaccinia virus

Vaccinia virus was one of the first viral vectors to be used successfully in transducing hippocampal slice cultures at extremely high efficiencies.[15,16] Recombinant protein starts to be expressed from 6–16 h post-infection. In mammalian tissue, vaccinia quickly becomes highly toxic, causing 50% of transduced neurons to die within 18h following infection (R Malinow, personal communication). However, this toxicity is not seen in non-mammalian organisms. In Xenopus leave's, vaccinia vectors have been used successfully to transduce tectal neurons in dissociated cultures (H Cline, personal communication) and in vivo.[17,18]

Non-viral transfection methods

Non-viral transfection methods comprise an eclectic mix of chemical, physical and electrical methods for gene transfer. Non-viral methods are advantageous for gene transfer into postmitotic neurons because they are generally easier to use, less toxic, and not constrained to delivering plasmids below a relatively small size for comparison with viral techniques). However, transfection efficiencies resulting from non-viral transfection methods are generally considerably lower (except for electroporation) than efficiencies obtained with recombinant viral vectors.[1]

Chemical transfection methods

The first subgroup of non-viral technologies, the chemical transfection methods, includes calcium phosphate coprecipitation, liposomes, non-liposomal lipids such as Effecting (Qiagen), and high molecular weight cationic polymers. Calcium phosphate-mediated transfection is one of the oldest methods for gene transfer and is, along with lipofection, one of the most commonly used gene transfer methods for basic neuroscience applications. The physical basis for this method is unclear, although it is believed that the DNA-calcium phosphate coprecipitate enters the neuron through endocytosis.[1] to transfect dissociated neuronal cultures from the CNS and peripheral nervous system of many diverse species. co-transfection is also possible with calcium phosphate coprecipitation, leading to almost 100% co-transfection, although ratios of expression vary. The major drawback to this method is that transfection efficiencies are highly variable but consistently low, in the range of 1–3%.[19,20]

Physical transfection methods

The physical methods for transfection include microinjection\ and biolistic. Microinjection involves directly injecting plasmid DNA into the nucleus of a neuron.[21] or injecting cRNA into the cytoplasm.[22] Whereas this method is standard for transfecting oocytes, Xenopus blastomeres, and invertebrate neurons, it requires considerable skill with mammalian CNS neurons and has not become a routine approach. Microinjection is quite labor-intensive and can be used on only a small number of neurons at a time. However, for applications in which only one identified neuron needs to be transfected, this method can be used effectively and elegantly. Techniques for gene transfer into neurons Wash bourn and Cis and trans co-transfection of fluorescently tagged proteins into young dissociated primary cortical cultures using lipofection. This figure demonstrates co-transfection of the same neuron with two constructs, or neighboring neurons with distinct constructs. Neurons were dissociated and cultured as described and then transfected using Lipofectamine 2000 (Gibco) at four days in vitro. (a) Neurons were cis co-transfected with a postsynaptic scaffolding protein (postsynaptic density protein 95kDa PSD95]) linked to EGFP (PSD95–EGFP; in green) and an N-methyl-D-aspartate (NMDA) receptor subunit coupled to Ds Red (NR1–ds Red; in red). Both fusion proteins are expressed in dendrites but show distinct subcellular distributions in young cortical pyramidal neurons. (b) Neurons were trans co-transfected with growth-associated protein 43 (GAP43) — an abundant protein in growth cones — coupled to EGFP (GAP43–EGFP; in green) and NR1–Ds Red (in red). The NMDA receptor subunit is localized to dendrites, where it is highly expressed in the cell body and proximal apical

dendrite, but expressed at levels comparable to endogenous levels in puncta in basal and distal apical dendrites. GAP43, transfected into a neighboring neuron out of the illustrated field, is expressed in the axons contacting the neuron transfected with NR1–Ds Red. Biolistic, short for biological ballistics, involves bombarding neurons at high velocity with DNA-coated gold particles.[23,25]

Application of gene transfer

Over 200 clinical studies in gene transfer are currently in existence and have involved over 2000 patients worldwide. Approximately 25% of these studies utilize 'marker' genes to examine basic biological questions regarding the fate of transduced cells. A further 60% involve patients with cancer. The remaining studies examine the potential role of gene transfer· in single gene defects and acquired non-malignant disease. This review cannot cover all the possible applications of gene transfer and instead will focus on areas of hematopoietic gene transfer and potential gene therapy approaches for cancer.

Gene transfer into hematopoietic progenitors

As bone marrow cells are easily accessible for in vitro manipulation, there has been considerable focus on the transfer of genes into hematopoietic cells. However, the challenge of developing a successful strategy which results in long-term gene expression in such cells is immense. To be effective, the target cells must be transduced with high efficiency, must survive the process and must engraft into the marrow niches or lymph nodes (and survive any immune or inflammatory response). The gene must be transcribed and the product expressed in sufficient quantities. Most studies to date have involved gene marking of the autograft which is infused following high-dose conditioning chemotherapy. These studies have been critical to our understanding of the fate of gene-marked cells once they are re-infused into the host and they have answered important biological questions relating to the influence on relapse of reinfused tumor cells. Other areas where gene transfer into hematopoietic cells is being investigated include the human immunodeficiency virus (mV) infection, the transduction of the multi-drug resistance (MDR) gene and the transduction of genes that are abnormal or absent from the hematopoietic stem cell or its progeny, ie; hemoglobinopathies, hemophiliacs, chronic granulomatous disease (CGD) and severe combined immunodeficiency (SCID). Although these latter diseases are exceedingly rare, the importance of these studies cannot be over emphasized as they provide 'proof-in-principle' that gene-marked cells can survive and be expressed for extended periods of time once re-

introduced into the host. Hematopoietic stem cells (HSC) are, by definition, cells that are capable of long-term hematopoietic reconstitution and that possess the ability of self-renewal over the course of a lifetime.[26,27] The vast majority of studies of gene transfer into hematopoietic cells rely on recombinant retroviruses as the delivery system of choice, largely because they can integrate into the genome and have the potential to express their gene products permanently while being able to pass the gene onto their progeny.[28] As retroviral transduction into the most immature of these cells requires cellular division, one can predict that the most efficient transduction process will be one that has maximum retroviral exposure at the time of HSC division. Indeed, the optimal transduction protocol would be one that would actively stimulate HSCs into cycle. However, to date the exact mechanisms that trigger HSCs into division remain elusive. Indeed, none of the known hematopoietic growth factors or cytokines appears to have the ability to actually trigger a HSC into cycle. Consequently, the various current clinical protocols attempt to stimulate proliferation and incubate the target cells with a cell-free retroviral supernatant, in the presence or absence of bone marrow stroma, with serum or serum-free media and with or without growth factors.[29,30]

Gene marking studies

Genetic marking of cells is a powerful approach for addressing biological questions in clinical trials of experimental cancer treatment.[31] Such cell marking facilitates studies of the long-term distribution and survival of marked cells in vivo and assessment of their contribution to clinical outcome. In clinical trials involving only gene marking, cells to be marked are incubated ex vivo, with a replication-incompetent retrovirus bearing a reporter gene. A commonly used reporter gene is neo, encoding bacteria] neomycin phosphotransferase which, when expressed, confers resistance to the neomycin analogue G41S.[32] The stable and unique integration pattern of proviral DNA in the genome of marked cells can provide a permanent marker for individual hematopoietic cells and their clonal descendants. The fates of marked hematopoietic stem cells, infused into autologous recipients following marrow-ablative cancer therapy, can thus be readily determined using sensitive genetic-based detection systems (e.g.; PCR) or by clonogenic assays for progenitors resistant to toxic concentrations of a41S. The first evidence for gene transfer into HSCs was provided by Brenner et al. in a clinical gene marking trial. Ex vivo transduction with the reporter gene neo was performed with 20% of bone marrow colony forming units positive for provirus DNA at IS months. The marker genes continued to be detected and expressed for up to 3

years in the mature progeny of marrow precursor cells, including peripheral blood T- and B-cells and neutrophils. In peripheral blood cells, expression was five-fold and ten-fold lower and was variable between different lineages. The highest level of expression was seen in myeloid cells and the lowest level was seen in Lymphocytes. One critical aspect of these studies was that they were performed in the pediatric population, a population which consistently demonstrates higher transduction efficiencies than adults.[33]

Severe combined immunodeficiency (SCID)

Clinical gene transfer trials in adenosine deaminase (ADA) deficient patients were among the first undertaken. Peripheral blood lymphocytes were harvested and expanded in vitro, transduced using a retroviral vector, containing both the ADA gene and marker gene (neo phosphotransferase), and subsequently infused monthly into patients. Follow-up has demonstrated detectable vector DNA, mRNA and functional marker DNA beyond three years from first treatment (and 545 days following last infusion). In addition, improvements in immune function have been demonstrated. Although ADA levels have been 25% of normal, these patients have, for obvious ethical reasons, remained on enzyme replacement therapy, and thus the true clinical impact has not been tested.[34]

Multi-drug resistance gene (MDR)

The gene for MDR transcribes p-glycoprotein. This pglycoprotein pump exists on the cell surface and actively 'pumps out' toxic substances (i.e.; plant-derived chemotherapeutic agents such as the anthracyclines, vinca alkaloids and Texans) and is a major contributor to the resistance of tumor cells to chemotherapy. Investigators are examining the effect of transducing this gene (or alkylating agent resistance genes) into normal hematopoietic progenitors in an attempt to render them resistant to subsequent chemotherapy. This increased tolerance would allow for dose-intensification of chemotherapy. Such an approach may have an important role in protecting the marrow microenvironment (stromal and accessory cells) following high-dose therapy and transplantation.[35,36]

Coagulation factor Deficiencies and Hemoglobinopathies

In patients with less than 1 % factor VIII or IX activity, dramatic clinical improvements are seen if exogenous factor replacement increases plasma levels by only a few per cent. Hence, these diseases are conceptually attractive targets for gene therapy strategies, given that even an inefficient system may produce sufficient protein to have a clinically meaningful impact. However, issues such as large gene size, choice of appropriate target cell (liver, muscle,

stroma, hematopoietic cells), efficiency of transduction, duration of expression, transcriptional regulation.

A commonly used reporter gene is and post-transcriptional processing, have hampered development in this area.[37,38]

Human immunodeficiency virus (H/V)

A number of gene transfer approaches are being investigated as potential anti-HIV strategies and this area has been well reviewed elsewhere.[66-68] The vast majority of these protocols utilize viral vectors, and most early studies have focused on transducing mature lymphocytes. More recently the transfer of genes into the early hematopoietic progenitors (which would differentiate into myeloid, Band T-lineages) has been investigated. Briefly, the strategies used include: transducing the genes for dominant negative mutations (DNM) such as the rev DNM gene (these DNM genes produce proteins which compete for the binding sites on regulatory RNA); RNA decoys (the transduced genes produce RNA similar to trans-activating response (TAR) or rev response element (RRE) which compete for the binding of regulatory proteins such as Tat or Rev, respectively); anti sense RNA strands that bind and block transcription sites; ribozymes (catalytic antisense molecules) which bind and splice RNA; soluble CD4 genes to block HIV binding sites; and chimeric T-receptors formed by inserting the gp140 receptor gene. A particularly novel strategy is the use of intracellular 'intrabody' genes. This involves transducing CD4 T-Lymphocytes with the anti-gp120 gene, the anti-gp120 inactivates the gp120 receptor in these T-cells and thus blocks HIV virions entering the cell.[39]

Gene therapy for cancer

A failure of the host to mount an antigen-specific T–cell mediated immune response against malignant cells is critical for tumor proliferation. The mechanisms utilized by tumors to evade the immune response are numerous.[40,41] and will only be briefly discussed here. A brief review of T-cell responses to foreign antigen serves as a background to understanding potential therapeutic strategies for gene transfer. After exiting the thymus, T-cells respond to antigens in an antigen-specific and antigen-nonspecific manner. The former requires the interaction of the T-cell with specialized (professional) antigen-presenting cells (APCs). The generation of an immune response by T-cells following the presentation of antigen by an APC, requires three distinct stages of cell-to-cell interaction, i.e.; adhesion, recognition and co-stimulation Cellular adhesion results following the interaction of APCs and T -cells via

surface ligands and their receptors (adhesion molecules). The subsequent recognition stage involves the presentation of sufficient quantities of antigen by APCs in the context of the major histocompatibility complex (MHC). The MHC-T -cell receptor interaction initiates a number of complexes signaling events via the CD3 subunits.[42] This signaling (signal 1) is critical to T-cell activation but insufficient to complete antigen-specific T-cell activation. A further co-stimulatory signal (signal 2) is essential for T -cell activation. This second signal is initiated following the binding of co-stimulatory molecules to epitopes on the T -cell surface. Two of the most important co-stimulatory molecules are B7-1 (CD80) and B7-2 (CD86), which bind to their T-cell ligands, CD28 and CTLA4. Signal 2 ultimately results in initiation and enhancement of T-cell clonal expansion, lymphokine secretion and effector function. If signal 2 is not delivered, T-ceIls enter a state of anergy (long-term unresponsiveness to specific antigens).[43]

Tumor suppressor genes

A variety of tumors express abnormal tumor suppressor genes. Conceptually, if the wt (normal tumor suppressor activity) gene is replaced then tumor growth may be retarded. Indeed, Roth et al. recently demonstrated regression of non-small cell lung cancers in a small number of patients following transduction of their tumor with wt. p53 gene. However, the clinical relevance of these findings has been debated because of the lack of suitable control vectors. More importantly however, this strategy may have limited applicability because all tumor cells would need to be transduced with the wt. (normal) gene.[44]

Suicide genes

Brain tumors, T-cells and plasma cells have been retrovirally transduced with the 'suicide gene' herpes simplex thymidine kinase (HSTK). This gene produces the inert enzyme HSTK in the target cell. However, when the target cell is exposed to the otherwise nontoxic ganciclovir, HSTK converts it to its toxic metabolite. Furthermore, this metabolite can escape through gap junctions resulting in the so-called 'bystander effect.[45,46]

CONCLUSION

Numerous studies have demonstrated successful transduction into human target cells with a variety of vectors. Even, they have answered an important biological question, i.e., that tumor cells in the autograft do contribute to relapse. All the same, none of the studies examining the therapeutic benefit of gene therapy has definitively demonstrated a clinically meaningful benefit. Nonetheless, the results of studies involving gene transfer for SCID, COD,

melanoma, and lung cancer highlight the potential benefit of this strategy. Along the way, much has been learned about gene structure, transcription, and control, while the area of cancer immunotherapy is expanding exponentially. If gene therapy is to succeed, it will require improvements in vector design, transduction strategies, administration protocols, and, importantly, the design of clinical trials.

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