Future of Gene Transfer in Oral Biology

John A. Chiorini

Gene Therapy and Therapeutics Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, U. S. Department of Health and Human Services, Bethesda, USA

Summary: The ability to manufacture and administer purified recombinant proteins has dramatically enhanced the therapeutic options for countless patients. For example, patients with hemophilia now receive recombinant coagulation factors or patients with pituitary disease receive recombinant growth hormone. However, this treatment is not a permanent 'cure'. Limitations to this therapeutic approach include the systemic delivery of non-physiological boluses of protein through repetitive injections, thereby making it difficult to maintain a constant therapeutic dosage level. Gene therapy offers an alternative possibility. In combination with the crossroads location of the oral cavity, both systemic and local conditions can be treated via gene therapy, thus overcoming some of the limitations of protein replacement therapy. This review discusses the use of gene therapy and the salivary gland as a target for such treatment. It also focuses on different methods of gene delivery and the host's immune response.

Conclusion: Recent advances have expanded the efficiency and number of vectors available for gene transfer, but a greater understanding of the host's response to vector infusion is necessary prior to wide utilization of gene therapy.

Key words: adeno-associated virus, AAV, gene therapy, salivary gland

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INTRODUCTION

Gene therapy is an experimental process that involves altering the genetic composition of cells for the treatment or prevention of disease. One example of gene therapy's practical application would be adding a gene to a cell that encodes for the production of a specific missing protein. Water channels such as AQP5 or AQP1 could be added to cells in the salivary gland to restore saliva flow as a result of genetic disease or following irradiation – induced damage to the gland, respectively (Braddon et al, 1998). Gene therapy can also be used to augment cells' protein production, for example the addition of sequences encoding antimicrobial or fungal proteins to salivary gland cells to assist in managing immune-deficient patients (O'Connell et al, 1996; Huang et al, 2002). Genes specifying genetic information for immunomodulatory proteins can be used to treat autoimmune or inflammatory diseases (Yamano et al, 2002). Genes also can be added to cells to regulate the degree of expression of other host proteins such as up regulation of water channels by adding specific transcription factors instead of broad chemical inducers (Motegi et al, 2005). In addition to adding nucleic acids encoding a therapeutic protein, gene therapy can deliver regulatory RNA molecules such as siRNA (Ryther et al, 2005). Inhibitory RNA (siRNA) molecules can be developed to prevent viral replication for controlling hepititis B virus infections or to silence expression of a dominant negative gene associated with spinal ataxia or Huntington's disease.

GENE TRANSFER AND SALIVARY GLANDS

In oral biology, vector–mediated gene transfer is a promising approach for the treatment of autoimmune disease- and irradiation-induced damage to the salivary glands, loss of bone as a result of trauma or osteoradionecrosis, or for the treatment of chronic pain (for review see Finegold et al, 1999; Baum, 2004; Sobajima et al, 2004). Because of the salivary glands' unique location and activity as both an endocrine and exocrine organ, gene transfer to this organ possesses the potential to treat several genetic or acquired disease conditions. While it is often

desirable to produce a protein in its normal physiological tissue, this site may not be accessible either because of disease or surgical limitations. Therefore the use of a strategically located secretory depot organ would be necessary. The salivary glands offer a number of advantages as a depot organ for therapeutic protein production following gene transfer.

Gene transfer to the salivary gland can lead to therapeutic protein expression in the saliva to treat or prevent diseases in the oral, pharyngeal, esophageal and gastric tissues; to the blood to treat systemic or local disorders; or remain within the salivary gland cells to address specific intracellular defects. The salivary glands are well encapsulated, limiting the undesirable spread of vector while ensuring the infused vector is not diluted, thus enabling a high concentration of vector to be delivered at a low vector dose. Salivary epithelial cells are a slow-dividing population that constitutes a not-critical-for-life tissue that is a natural secretory organ capable of producing large amounts of a wide range of proteins (Arneberg, 1971; Dodds et al, 2005). Thus, the salivary glands provide a relatively stable cell population capable of long-term protein production. Finally, the development of intraductal cannulation methods allows for easy delivery of vectors to the luminal membranes of almost every parenchymal cell in the salivary glands.

METHODS OF GENE DELIVERY

While our understanding of the genetics of disease has greatly expanded, in order for gene therapy to become an effective therapy several technical hurdles must be addressed. For the therapeutic protein to be expressed in the cell, the vector DNA introduced into target cells must remain functional, mandating that the cell containing it must also remain operative. For vectors designed to persist in cells by integrating into the host's genome, one concern is that the introduced gene will integrate into the genome and disrupt gene function, resulting in mutation or disease. For many gene therapy applications, the current levels of protein expression possible are below the therapeutic level needed and new delivery vectors will be required to be efficacious. Other applications will require regulated expression of the genes, however there are currently no clinically approved systems for the control of transgene product expression. Not all diseases are the result of mutations in a single gene, thereby making multigene or multifactorial disorders more difficult to treat effectively using gene therapy. The most amenable diseases to gene therapy are those that are caused by a mutation in a single gene or that can be treated by protein replacement therapy. While the therapeutic gene is known for many diseases as noted above, it might not be possible for it to be expressed in the natural cell type. Also, appropriate choices of alternative depot organs are not always clear. Just as in transplantation therapy, the host's immune response to current vectors and the therapeutic transgenes remain a concern. Many current vectors are associated with toxicity or immune and inflammatory responses to the vector. The host response to the vector varies depending on the vector dose and route of administration, yet the host response to the vector always remains a critical concern. Furthermore, some viral vectors may be complemented by host cell factors or endogenous viruses, triggering the replication and spread of the gene transfer vector, which raises long term safety concerns. Thus a general limitation for the advancement of gene therapy is not the appropriate genes to deliver, but rather the method of delivery, along with the host's response to both the vector and the therapeutic protein.

The ultimate success of any gene therapy approach is dependent upon the efficient delivery of the therapeutic nucleic acid. In the oral cavity a number of approaches have been tried, ranging from injection of naked DNA, to engineered viruses and DNA lipid carrier complexes. While naked DNA has been used as part of a vaccination approach, viral vectors have the ability to direct both long-term and transient expression with greater efficiency than naked DNA alone. Two classes of viral vectors that have shown promise in the salivary gland are recombinant adenoviral (serotype 5; rAd5) and adeno-associated viral (serotype 2; rAAV2) vectors. Adenoviruses serotypes 2 and 5 are non-enveloped viruses containing linear double-stranded DNA genomes. Adenoviruses normally replicate episomally and do not integrate as part of their lifecycle. Thus integration into the host genome and the risk of insertional mutagenesis is low with this class of vector.

In vivo salivary gland gene transfer using rAd5 vectors results in a transient but robust expression (Baum, 2002). rAd5 vectors (10^{10} vector genomes (vg) per gland) will transduce approximately 35% of rodent salivary parenchymal cells and can deliver genes to almost all cell types in the gland (Delporte et al, 1997). However, rAd5 vector-mediated gene expression is transient. Using low dose of first generation rAd5 vectors (containing a deletion of the Ad5 E1, E3 genes but retaining 28kb of the Ad5 coding sequence), expression typically peaks after one to three days and returns to background levels within two weeks (Adesanya et al, 1996; Delporte et al, 1997). Lack of persistent expression is the result of an immune response directed against rAd5-vector-transduced cells, which leads to the elimination of the transduced cells and the therapeutic gene. Extensive studies of the safety and biodistribution of rAd5 vectors after their administration to rodent salivary glands indicate some toxicity and a transient, local dose-related inflammatory response (O'Connell et al,

2003). Infusion of high doses of the rAd5 vector (> 10^{11} vector genomes (vg) per gland) can result in widespread distribution of vector (O'Connell et al, 2003) in rodents. Thus, rAd5 vectors appear to be particularly useful at low doses for local gene therapeutic applications, such as antibiotic-resistant infections, tissue repair, or as an adjuvant in a genetic vaccination protocol (O'Connell et al, 1996; Wang et al, 2004; Vanniasinkam and Ertl, 2005). Elimination of the remaining Ad5 coding sequence or incorporation of DNA elements flanking the therapeutic gene would allow for stable integration of the therapeutic gene and may increase the persistence of rAd5 vectors (Zheng et al, 2000; Ehrhardt and Kay, 2002).

By contrast, rAAV2 vectors can direct long-lived, stable transgene expression in salivary glands and many other tissues with minimal immune response (Voutetakis et al, 2004). The AAVs were originally classified according to size, structure, and dependence upon a helper virus for replication. Because the majority of AAV isolates were first identified as contaminants of laboratory stocks of adenovirus, little is known about their natural tissue tropism. Currently over a dozen isolates have been cloned and their initial characterization indicates that each serotype possesses unique binding/cell tropism characteristics. Most preclinical studies and current Phase I clinical trials use vectors derived from adeno-associated virus serotype 2 (AAV2). This serotype has a broad tropism in vitro and can transduce both dividing and post-mitotic cells.

Experiments with rAAV2 vectors in mice have shown long-term transgene expression from transduced salivary glands (Voutetakis et al, 2004). Furthermore, the immune response to rAAV2 vectors is primarily antibody compared with the innate, cellular, and humoral response to rAd5 vectors (Kok et al, 2004). However at a comparable particle dose, rAAV2 vectors transduce only 10–15% of salivary parenchymal cells and exclusively target striated ductal cells (Yamano et al, 2002). Safety studies with rAAV2 vectors suggest the vector is well tolerated with no adverse effects on salivary gland function but a humoral response to the vector can block repeat vector administration. Biodistribution studies with AAV2 demonstrate that vector spread is limited and vector is restricted to the salivary gland tissue (Kok et al, 2003; Katano et al, unpublished). The limited vector spread indicates a fundamental difference in the interaction between AAV and Ad5 vectors with the salivary gland. The restricted dispersal suggests that in contrast to AAV2, Ad5 infusion or the immune response to the vector may disrupt cellular junctions within the gland thus allowing vector access to the circulation.

While rAAV2 vectors appear to be useful for salivary gland gene transfer, several reports show that different serotypes of rAAV can have distinct patterns of cell tropism and distribution in a tissue. These unique qualities may allow other serotypes to overcome some of the limitations noted with AAV2 vectors (Chao et al, 2000; Davidson et al, 2000; Zabner et al, 2000; Gao et al, 2002; Rabinowitz et al, 2002). The differences in tropism and transduction are primarily due to dissimilarities in the interactions with the target cells but also related to anatomical barriers that exist within the tissue that may limit optimal virus spread. Efficient AAV2 transduction requires expression of heparan sulfate proteoglycan (HSPG) on the target cell surface. In lung epithelia, HSPGs are primarily found on the basolateral surface and may account for the limited transduction following AAV2 vector delivery to the lumen (Duan et al, 1998). Other serotypes such as AAV type 5 (AAV5) utilize the platelet derived growth factor receptors (PDGFRα or PDGFRβ) for entry, which are expressed on the apical surface (Chiorini and Zabner, unpublished). Initial rodent studies with AAV5 and another isolate, AAV4, demonstrate improved transduction activity in the mouse salivary gland and the vectors also appear to be well tolerated (Katano et al, unpublished).

THE HOST'S IMMUNE RESPONSE

Introduction of any vector into a cell can potentially stimulate an immune response. One of the greatest challenges for advancing gene therapy is controlling the host's immune response to the vector and transgenes. In order to ensure the safety of the therapy it is important to understand the immune response to the vector and the therapeutic protein. Studies with AAV2 vectors suggest that AAV2 does not appear to efficiently transduce macrophages but they can induce transient expression of chemokines and cytokines in epithelial cells (Zaiss et al, 2002). While transduction of epithelial cells does not result in a sustained activation of the innate immune response, gene transfer with AAV vectors does elicit an antibody response against the vector capsid (for review see Zaiss and Muruve, 2005).These anti-AAV antibodies can have a neutralizing effect on subsequent rounds of vector administration, thereby inhibiting AAVs transduction activity. Furthermore epidemiological studies suggest that as much as 80% of the population has antibodies against AAV2 (Chirmule et al, 1999; Erles et al, 1999; Moskalenko et al, 2000), which presents a major obstacle to use of this vector.

Recent studies of vector infused salivary glands suggest that it is possible to induce an immune response that is neutralizing to a subsequent infusion of the same vector four weeks later (Kok et al, 2004). This antibody response appears to be both time and dose dependent suggesting that readministration may be possible. The use of alternative serotypes of AAV such as AAV5 maybe advantageous since the prevalence of neutraliz-

ing antibodies against AAV5 is substantially lower in the population than AAV2, and AAV5 transduction activity in the salivary gland is higher allowing for the infusion of a lower dose of vector that should result in the same level of gene expression. The use of transient immune suppression may also be useful in preventing activation of the gene therapy-induced humoral immune response.

CONCLUSIONS

The field of gene therapy holds great promise for treating genetic disease by addressing its source rather than by masking the symptoms. Furthermore, gene therapy offers the possibility to direct the expression of a therapeutic protein either locally or systemically. Salivary glands appear to be an excellent depot organ to deliver therapeutic proteins to both the upper gastrointestinal tract as well as the systemic circulation. However, a major hurdle to achieving this goal is to develop approaches for delivering nucleic acid to the appropriate cells in a safe and efficient manner. While current AAV vectors appear to be safe vehicles for long term gene transfer, further work will be required to develop vectors with improved transduction activity in both ductal and acinar cells and to limit the immune response to the vector and the therapeutic protein. Other safety considerations that must be evaluated in future vectors are the use of regulated expression systems and the addition of tissue specific promoters. Thus, a greater understanding of oral biology and the physiology of the salivary gland will greatly impact the advancement of the field of gene therapy.

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Reprint requests:
John A. Chiorini PhD
Gene Therapy and Therapeutics Branch
National Institute of Dental and Craniofacial Research
National Institutes of Health
U. S. Department of Health and Human Services
Bethesda, MD 20892
USA
E-mail: jchiorini@dir.nidcr.nih.gov