Transferring Genes to Salivary Glands

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Abstract: This review provides a brief description of gene transfer studies using salivary glands as the target tissue. The aggregate results demonstrate the potential clinical value of this methodological approach for managing several conditions lacking fully satisfactory conventional treatments. Routine clinical applications are still seven to ten years away, primarily because of the need for improved gene transfer vectors. Overall, this body of work provides the dental educator with a substantive example of how biotechnological progress will significantly affect the treatment of oral problems in the near future.

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Gene transfer has emerged as a potentially valuable tool for managing some clinical disorders. Originally, clinical gene transfer was conceived for use as therapy for single gene-deficiency disorders. However, it is now clear that the possible applications of in vivo gene transfer are much broader than initially thought. In particular, interest has grown in applying gene transfer methods to numerous acquired and non-life threatening conditions for which no conventional therapies exist. Additionally, investigators have begun to transfer genes to a wide variety of tissues not originally considered for this molecular intervention. Gene transfer results in a change in cellular phenotype due to the expression of the transgene (foreign gene). Thus, investigators are able to use gene transfer methods not only to address significant clinical problems but also to study difficult biological questions.

Salivary Glands as a Gene Transfer Site

Salivary glands are an example of one tissue type that was not initially considered for applications of gene transfer technology. Major salivary glands, however, have many features that make them attractive targets for gene transfer. First, they are very accessible, since the ductal orifices of all major salivary glands open directly into the oral cavity. Second, essentially all epithelial cells (acinar and ductal) in these glands have their plasma membranes (apical surfaces) directly in contact with the ductal tree, and thus with the mouth. Third, human salivary glands are well encapsulated, a circumstance that should eliminate or at least minimize concern about vector spread beyond the glandular tissue. Finally, salivary gland epithelial cells are capable of producing considerable amounts of protein especially for export, albeit mostly in an exocrine direction.

For our studies, genes are delivered to cells after direct cannulation of the glands through ductal orifices, followed by the slow infusion of the vector via a syringe. There are two general methods for gene transfer in vivo: viral and non-viral. Viruses have evolved as natural gene transfer vectors and have developed highly efficient means of transferring exogenous DNA to cells. However, they can pose a potentially high safety risk to the host. Conversely, non-viral methods present a much lower safety risk to the host, but show less efficiency in transferring foreign DNA. Published studies of gene transfer to salivary glands have used both general methods, but most frequently viral, especially adenovirus. Other viral vectors also have been used, along with several non-viral methods. However, most published reports of salivary gene transfer studies employ recombinant adenoviral vectors, the focus of this brief review.

Adenoviruses

Adenoviruses are double-stranded DNA viruses, ~36 kb in size. There are advantages and dis-
advantages to using recombinant adenoviruses for transferring genes to the salivary glands. On the positive side, recombinant adenoviral vectors are able to infect many different cell types, both dividing and nondividing. This is important because salivary cells divide very slowly. Also, adenoviral vectors are relatively easy to handle and manipulate. Additionally, they can be grown to very high titers suitable for \textit{in vivo} gene transfer use. On the negative side, recombinant adenoviruses are unable to integrate into a target cell’s genome, making expression of the transgene unstable. Further, adenoviruses can elicit a potent host immune response involving all three arms of the immune system (innate, humoral, and cellular). Although adenoviruses are not ideal gene transfer vectors, they are very useful for determining proof of biological concept. For the interested reader, there are many valuable reviews describing adenoviral biology as well as their use in gene transfer.\textsuperscript{6-9}

Adenoviruses enter a target cell by using a cell-surface receptor protein known as CAR (Coxsackie and Adenovirus Receptor).\textsuperscript{10} Pastan et al.\textsuperscript{11} showed that after an adenovirus binds to a target cell membrane, it is rapidly internalized in endosomal vesicles and transported to the nucleus. This internalization step is mediated by integrins (cell membrane “adhesive” proteins) of the $\alpha_v\beta_3$ subclass.\textsuperscript{12} The distribution of these integrin subunits, but not of CAR, has been reported in the apical membranes of salivary epithelial cells from several species.\textsuperscript{13} In general, the integrin subunits are widely distributed in different salivary cell types.

### Potential Clinical Applications of Adenoviral-Mediated Salivary Gland Gene Transfer

Our laboratory has focused on three applications of gene transfer to salivary glands. The first is to repair hypofunctional salivary glands, damaged as a result of either exposure to therapeutic irradiation or autoimmune pathology. The second is to augment the composition of saliva to help manage upper gastrointestinal tract diseases. The third is for systemic gene therapeutics in treating single gene deficiency disorders, that is, secreting proteins for general use directly into the bloodstream. For each of these clinical goals, proof of concept has been achieved in rat models.

#### The Repair of Severely Damaged Salivary Glands

There are two main conditions leading to severe, irreversible salivary hypofunction in the United States: therapeutic irradiation received during the course of treatment for head and neck cancers, and Sjogren’s syndrome, an autoimmune exocrinopathy. The resultant salivary hypofunction reflects a permanent damage to salivary parenchyma. Our initial focus was on the repair of salivary glands damaged by irradiation because it was easier to study than autoimmune pathology. In the United States, there are about 40,000 new cases of head and neck cancer diagnosed each year, and the majority of these patients are treated with ionizing irradiation. If the major salivary glands are located in the field of treatment, the glands are rendered hypofunctional.\textsuperscript{14,15} The most significant cellular damage occurs with acinar cells, the gland cell type responsible for fluid movement. Acinar cells are lost, and the remaining ductal cells are relatively water impermeable. There is no useful conventional treatment for the loss of saliva experienced by patients after radiation treatment. In 1997, our laboratory reported that it was possible to attain a type of correction for irradiation-induced salivary hypofunction by transferring the gene for a water channel protein (aquaporin-1, AQP1) into irradiated rat submandibular glands with a recombinant adenoviral vector.\textsuperscript{16} Irradiated rats administered a control adenovirus exhibited salivary flow rates ~65 percent lower than sham-irradiated animals. Conversely, when irradiated animals were administered the AQP1-encoding adenovirus, salivary flow rates were indistinguishable from control levels at three days post-administration. This study suggests that AQP1 gene transfer may be useful in treating post-irradiation salivary gland damage. However, caution is warranted because, thus far, primate studies have not shown AQP1 gene transfer to be as effective as observed in rats.\textsuperscript{17}

#### Enhancement of Salivary Secretions

The primary physiological role of saliva is to protect and nurture all tissues of the oral cavity and
the upper gastrointestinal tract. Despite the presence of normal salivary secretions, the oral cavity can be the site of significant morbidities, for example, dental caries, periodontal diseases, mucosal ulcerations, and candidiasis. To test the notion that gene transfer can be used to augment salivary secretions to help manage upper gastrointestinal tract pathology, we constructed a recombinant adenovirus (AdCMVH3) encoding the potent anti-candidal polypeptide histatin 3. The clinical condition we targeted with this vector is azole-resistant mucosal candidiasis, a potentially life-threatening condition often affecting immunosuppressed patients. AdCMVH3 was used to infect rat parotid and submandibular glands. Although humans secrete considerable histatin 3 in their saliva, rodents secrete none. After infection with AdCMVH3, histatin 3 was readily detected in rat saliva, at levels up to tenfold those found normally in human saliva. Importantly, the histatin 3 produced from this vector was capable of efficiently killing azole-resistant candida species.

Salivary Glands for Systemic Gene Therapeutics

For years, it has been suggested but not unequivocally proven that salivary and other exocrine glands could secrete proteins in an endocrine manner directly into the bloodstream. By using a recombinant adenovirus encoding human α1-antitrypsin, we were able to test this directly. We showed that, following gene transfer, rat salivary glands were able to secrete this protein into the bloodstream. Furthermore, we also showed that a transgenic protein secreted into the bloodstream from salivary glands could be systemically active. To do this, we constructed a recombinant adenovirus encoding the human growth hormone gene, termed AdCMVhGH. When administered to rat salivary glands, AdCMVhGH directed the production of growth hormone resulting in “supra-therapeutic levels” (~15-20 ng/ml) in rat sera. Most importantly, this elevation in growth hormone led to an increase in serum insulin-like growth factor-1, a recognized sequela of growth hormone receptor activation, and elevated general serum chemical indicators of anabolic activity (triglyceride levels; BUN: creatinine ratio).

Summary

These studies clearly show that gene transfer is readily accomplished in vivo with salivary glands, and is potentially of considerable clinical value. However, significant problems still exist, and routine clinical applications are unlikely for at least seven to ten years. Adenoviral vectors, while useful for the proof of concept studies described here, as noted earlier elicit a potent host immune response and can be directly cytotoxic. Better vectors are clearly needed. However, the greatest handicap to successful application is the absence of a critical mass of investigators studying issues relevant to salivary gland gene transfer. We not only have too little experience with different vector types in salivary glands, but we simply do not understand salivary biology adequately (for example, cell turnover, gland development, pathogenic mechanisms in irradiation damage and with Sjogren’s syndrome). These circumstances retard the progress into the clinic.

REFERENCES