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Gene Therapy

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Abstract

Applications of gene therapy have been evaluated in virtually every oral tissue, and many of these have proved successful at least in animal models. While gene therapy will not be used routinely in the next decade, practitioners of oral medicine should be aware of the potential of this novel type of treatment that doubtless will benefit many patients with oral diseases.

Keywords

gene therapy; mouth; oral diseases

As with every type of “drug treatment”, therapy involving gene transfer was first imagined and designed for patients with certain very specific ailments, in this case genetic disorders caused by a single gene defect, such as inborn errors of metabolism. To that list was soon added cancers refractory to conventional therapy (Anderson, 1984). Indeed, the earliest substantive reports on human gene therapy included clinical studies of gene transfer for adenosine deaminase deficiency (Blaese et al, 1995) and malignant melanomas that had failed standard treatment (Rosenberg et al, 1990).

However, it did not take long for this narrow, albeit important, list of applications to be expanded for much broader uses, in the general manner that new applications are commonly found for conventional therapeutics. However, unlike the latter, off-label usages often seen with pharmaceuticals, developing an expanded use of gene transfer clinically involves a considerable effort. Each new application of a gene therapy to humans undergoes extensive and multi-step approvals that are required to demonstrate both ample proof of efficacy and safety in animal models. Despite the appropriate rigors of this process, currently applications of gene therapy have been evaluated in virtually every major organ system, including the mouth.

Given this general situation, it is not surprising that almost every oral tissue, and a wide spectrum of oral pathological mechanisms, have been targeted for gene therapy. Thus, there are reported gene therapy applications, which have been at least successful in animal models, for the teeth, bone, mucosa and salivary glands, and are relevant to the sequelae of caries (e.g., Nakashima et al, 2004; Jiang et al, 2012), periodontal diseases (e.g., Cirelli et al, 2009), other oral infections (O'Connell et al, 1996), mucositis (Zheng et al, 2009), malocclusion (e.g., Iglesias-Linares et al, 2011), salivary dysfunction (e.g., Delporte et al, 1997; Lee et al, 2012), pain (e.g., Fink et al, 2011; Yu et al, 2013) and malignancy (e.g., O'Malley et al, 1996; Khuri et al, 2000). The focus of this commentary will be on two applications that have achieved human clinical trial status as of this writing (mid-November, 2013) - malignancy and salivary dysfunction. While neither of these oral applications is

ready for routine use, their possible utility has been demonstrated clinically and, coupled with successful animal studies in other clinically-relevant conditions, practitioners of oral medicine should be aware of the potential of this novel type of treatment. Gene therapy will not be used routinely in the next decade, but beyond that time it doubtless will provide an important part of an expanding armamentarium of biological therapies that will benefit many patients with oral diseases.

The term gene transfer refers to the delivery of a gene, a cDNA, a small RNA, i.e., any type of oligonucleotide that might have some therapeutic benefit, to a pre-determined target cell. The most common type of agent utilized for such gene transfer is a defective or modified virus, i.e., a viral vector, although non-viral gene transfer, e.g., use of a simple plasmid containing the oligonucleotide of interest, is also frequently employed. The former type is much more efficient, at least currently, while the latter poses less risk to the host, at least theoretically. It seems reasonable to think that in the future non-viral methods of gene transfer will be considerably improved and eventually will lead to the elimination of any need for viral vectors. Note that for the present purpose only the transfer of actual genes and cDNAs have been considered. Genes can be delivered *in vivo*, directly to a living organism, or *ex vivo*, to cells which, following the modification and usually cell amplification, can be returned back to the host. Gene transfer can be considered permanent, e.g., with the transferred gene integrating into a chromosome of the targeted cells, or transient, i.e., the transferred gene being located extra-chromosomally. Gene transfer can be an isolated therapy, used alone, or it can be adjunctive, used in combination with other, more conventional therapies, most notably in cancer treatments.

Initially, the clear and major focus of gene therapy in the oral region was for treating squamous cell carcinomas (e.g., O'Malley et al, 1996; Khuri et al, 2000), with the first human clinical trials occurring ~20 years ago (for overview see Chisholm et al, 2007, and Shillitoe, 2009). Several different strategies were attempted, but the most frequently used involved replacement of a defective gene, e.g., p53 tumor suppressor gene, and the use of an oncolytic adenovirus or other oncolytic virus type that would lead to lysis of all transduced (i.e., infected) malignant cells. The use of oncolytic viruses, in particular, resulted in multiple clinical studies that overall showed modest efficacy. Eventually, these led to the clinical approval, in 2003, for use of an oncolytic adenovirus (termed Gendicine) for treating head and neck cancer in China (Pearson et al, 2004). Generally, while progress in this application of gene transfer to the oral cavity has been real, it also appears to have reached a plateau, as there is no published evidence of Gendicine's continued use in the last five years (PubMed search performed on October 10, 2013). Importantly, gene therapy approaches to treat head and neck cancers are not in routine use in the United States and Europe, i.e., it still has experimental status (Chisholm et al, 2007). Indeed, some have argued that for clinically meaningful future progress to occur using gene therapy in treating head and neck cancers, significant and novel advances, especially in gene delivery methods, are required (Shillitoe, 2009).

Currently, and doubtless with considerable personal bias, I think the area of orally relevant gene therapy showing the most promise targets salivary glands. Salivary glands have been the focus of gene therapy research for more than 20 years, primarily examining either the (i) repair or prevention of gland damage related to therapeutic radiation for head and neck cancers or Sjögren's syndrome, and (ii) use of the gland to make therapeutic proteins for both systemic and oral benefit. Thus far, the area of greatest progress has been in radiation damage repair, with the first clinical trial reported relatively recently (Baum et al, 2012).

In brief, the strategy for this latter application was to re-engineer radiation-surviving salivary duct epithelial cells to secrete fluid by transferring the cDNA for the archetypal water

channel, human aquaporin-1 (hAQP1) via a defective adenoviral vector (AdhAQP1; Vitolo and Baum, 2002). After initial *in vitro* studies, *in vivo* proof of concept for this strategy was demonstrated in irradiated rats (Delporte et al, 1997). In that study, three days following delivery of AdhAQP1 to the submandibular glands, near-normal levels of salivary flow were observed in animals that had received a single 21 Gy dose four months previously. Importantly, use of a control adenoviral vector was without any significant effect, with irradiated rats exhibiting an ~64% reduction in salivary flow.

In general, for gene transfer as well as many other types of biological therapies, it is essential to demonstrate the ability of the proposed therapy to scale to a large animal model, e.g., to work in a dog or a non-human primate, as this would suggest that the therapeutic strategy could also be effective in humans. For AdhAQP1, this was demonstrated in an irradiated miniature pig parotid gland model (Shan et al, 2005). In that study, animals received a single dose of 20 Gy to one parotid gland and four months later exhibited an ~80% reduction in salivary flow rate from those glands. Subsequently, AdhAQP1 was delivered to the irradiated glands and three days following vector delivery parotid flow rates were near those measured prior to irradiation.

The next critical step, prior to seeking approval for a clinical gene therapy trial, was to conduct an extensive toxicology and bio-distribution study, i.e., asking if the vector was safe. We performed such a study in 200 rats (100 of each gender), with three dosage-groups receiving AdhAQP1 and one control group (n=25 rats/group). This study showed that delivery of the vector did not result in any significant, systematic adverse effects in the animals (Zheng et al, 2006).

Based on the results from the above animal studies, we submitted a protocol for the proposed clinical trial in late 2005 to five separate and required committees. The first two-committee approvals were sought simultaneously (National Institute of Dental and Craniofacial Research Institutional Review Board and the US Recombinant DNA Advisory Committee). Following approval from those two committees, the protocol was sent to the NIH Biosafety Committee, the US Food and Drug Administration, and to a study-specific Data Safety and Monitoring Board. Those approvals were received in that order, with the last obtained by February 2007. All infrastructure for the proposed clinical trial, e.g., database, electronic case report forms, monitoring policies, standard operating procedures, was in place about a year later and the first of eleven study subjects, each of whom was at least 5 years removed from completion of radiotherapy for a head and neck squamous cell carcinoma, and was without evidence of disease recurrence, was treated in July 2008.

The results from this first in human study demonstrated that the AdhAQP1 improved salivary flow rates in the targeted parotid glands of six subjects and led to a reduction in subjective complaints in five of those individuals (Baum et al, 2012). The most common reason for a lack of efficacy in the other subjects appeared to be the occurrence of a significant inflammatory response at higher vector doses. Thus, transferring the hAQP1 cDNA to some patients can result in significant objective and subjective improvements of their salivary hypofunction. Additionally, few adverse effects were seen in all study subjects, and these were considered either mild or moderate. Furthermore, no consistent changes were found in any of the clinical chemistry and hematology parameters examined over the course of the 42-days post-vector period reported. Currently, a long-term follow-up of all eleven subjects is underway to determine the maximum duration of the improved parotid function and to gather additional safety information. Additionally, a second clinical trial, employing a less immunogenic vector based on a serotype 2 adeno-associated virus (AAV) to transfer the hAQP1 cDNA, is planned to begin in 2014.

As personally satisfying as the above studies have been, it is always better to prevent a clinical disorder than treat one. One novel strategy to prevent salivary gland radiation damage has been developed by Sunavala-Dossabhoy and her colleagues and, in my view, shows particular promise. They have shown that transfer of the gene encoding tousel-like kinase 1B (TLK1B) to rat submandibular glands can prevent radiation-induced salivary hypofunction (Palaniyandi et al, 2011; Timiri Shanmugam et al, 2013). TLK1B is normally involved in chromatin remodeling at DNA repair sites and leads to increased cell survival following radiation (Sunavala-Dossabhoy et al, 2012). Of particular note, their most recent work shows that after use of an AAV vector (2/9 pseudotype) to mediate TLK1B gene transfer, there was no reduction in salivary flow in rats 8 weeks following a fractionated irradiation regimen (2.5 Gy $\times$ 8 fractions). Conversely, untreated rats or rats treated with a control AAV vector experienced a >90% reduction in salivary flow (Timiri Shanmugam et al, 2013). While this approach must be shown to scale in a large animal model, as well as to be safe in toxicology studies, it is encouraging and shows future promise for preventing radiation damage during head and neck cancer treatment.

The second major therapeutic focus of gene transfer in salivary glands uses healthy functioning glands, turning them into endogenous bioreactors to make therapeutic proteins for benefit either systemically (endocrine application) or locally in the upper gastrointestinal tract (exocrine application). These studies have been conducted only in animals, although in many species (mouse, rat, miniature pig, macaques), but have not yet reached the clinic. There have been multiple endocrine applications suggested, including proposed treatments [gene or cDNA indicated if not obvious] for growth hormone deficiency (Hoque et al, 2001), chronic anemia (erythropoietin; Voutetakis et al, 2004), Fabry disease (α -galactosidase; Passineau et al, 2011), α 1-antitrypsin deficiency (Perez et al, 2011), and diabetes (type 1, proinsulin B10, Rowzee et al, 2013; type 2, glucagon-like peptide 1, Voutetakis et al, 2010). There have been fewer examples for exocrine use;azole-resistant candidiasis (histatin 3; O'Connell et al, 1996) and radiation-induced mucositis (keratinocyte growth factor; Zheng et al, 2009). The primary reason for the failure of this approach to reach the clinic is the lack of understanding of the process and signals by which secretory proteins in salivary cells are directed in either an endocrine or exocrine direction (Perez et al, 2010). The end result of this mechanistic conundrum is that often, but not always, the transgenic proteins are secreted inefficiently into the desired secretory pathway (e.g., see Hoque et al, 2001; Adriaansen et al, 2011). Until this conundrum is reasonably understood, it is unlikely that human clinical trials will be approved for this particular application.

In closing, it is hoped that this brief commentary conveys the real potential of gene transfer to influence future treatment strategies, and further improve patient care, for many oral and other diseases.

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