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Cells in focus

Salivary epithelial cells: An unassuming target site for gene therapeutics

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ABSTRACT

Salivary glands are classical exocrine glands whose external secretions result in the production of saliva. However, in addition to the secretion of exocrine proteins, salivary epithelial cells are also capable of secreting proteins internally, into the bloodstream. This brief review examines the potential for using salivary epithelial cells as a target site for in situ gene transfer, with an ultimate goal of producing therapeutic proteins for treating both systemic and upper gastrointestinal tract disorders. The review discusses the protein secretory pathways reported to be present in salivary epithelial cells, the viral gene transfer vectors shown useful for transducing these cells, model transgenic secretory proteins examined, and some clinical conditions that might benefit from such salivary gland gene transfer.

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Cell facts

- Salivary epithelial cells are of two general types: acinar and duct.
- Salivary epithelial cells have a primary function of producing saliva, a fluid with essential digestive and protective functions.
- Salivary epithelial cells can secrete protein both into saliva and into the bloodstream.

1. Introduction

Salivary glands are classically considered to be exocrine glands whose secretions protect and facilitate the function of all oral and upper gastrointestinal tract tissues (Amerongen and Veerman, 2002). The vast majority of cells in these glands are epithelial and of two broad types: acinar and duct (Turner and Sugiya, 2002). Acinar cells are secretory and the only site of fluid secretion in the glands. Acinar cells secrete a primary fluid that is isotonic and contains ~85% of the secreted proteins found in saliva. Salivary ducts constitute an absorptive epithelium. While duct cells secrete the remaining ~15% of salivary proteins, their primary physiological role is to absorb NaCl. By the time the forming saliva exits the duct and enters the mouth, the NaCl concentration has been reduced from ~150 mequiv./L to ~25 mequiv./L.

While studies of protein production by and secretion from salivary glands most often focus on the proteins found in saliva (e.g., Helmerhorst and Oppenheim, 2007), there is a long history recognizing protein secretion into the bloodstream by salivary glands (e.g., Leonora et al., 1980; Isenman et al., 1999). Due to this duacrine (Fig. 1; both exocrine and endocrine) nature of salivary epithelial cell protein secretion, we began to study the potential applications of in situ gene transfer to salivary glands (Baum et al., 1999) for gene therapeutics. Although not typically considered a target tissue for gene therapeutics, and in particular not for systemic applications, salivary gland epithelial cells present multiple advantages as a gene transfer target site (Baum et al., 2004). They are: (i) easily accessible through the main excretory duct, which opens into the mouth; (ii) well-encapsulated limiting any spread of the gene transfer vector; (iii) well-differentiated, providing a relatively stable target site for non-integrating vectors; (iv) capable of producing significant amounts of protein for export; and (v) not-critical for life in case of the occurrence of a severe adverse event.

Our aggregate studies demonstrated two key findings. First, it is possible to deliver transgenes encoding various secretory proteins to salivary epithelial cells and find transgenic proteins in both saliva and the bloodstream (e.g., see O'Connell et al., 1996; Baum et al., 1999). Secondly, for several transgenic secretory proteins, there is no simple way to predict the direction of secretion (e.g., Adriaansen et al., 2008; Voutetakis et al., 2008; see below). Finding a way to circumvent this latter situation is essential if salivary gene therapeutics is to be clinically useful.

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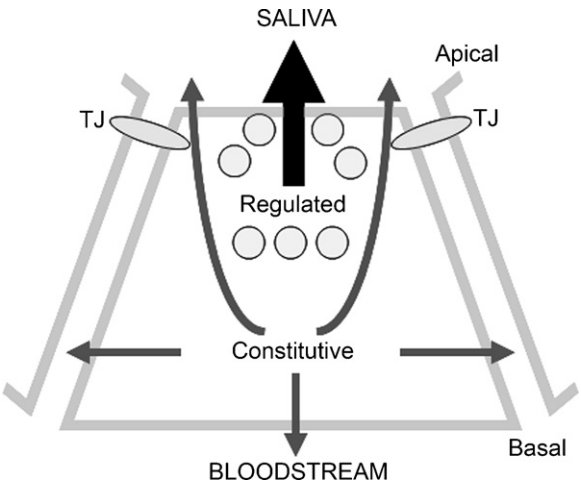

Fig. 1. General depiction of protein secretory pathways operative in salivary epithelial cells. The regulated pathway leads to exocrine protein secretion via secretory granules (circles at the apical pole of the cell). Endocrine secretion presumably occurs via a constitutive or constitutive-like pathway. TJ, tight junctions.

2. Cellular origins

Salivary glands develop from a thickening of embryonic oral epithelium, which then protrudes into the underlying mesenchyme (Tucker, 2007). This initial bud of epithelium undergoes branching morphogenesis in response to signals from the mesenchyme and extracellular matrix, so that by E14 a highly branched gland exists, with an elongated duct and formed lumen (Patel et al., 2006; mouse submandibular gland). Proacinar cells appear after E15, with acinar differentiation occurring postnatally and granular convoluted ducts becoming fully differentiated only at puberty (Patel et al., 2006; Tucker, 2007).

3. Viral vectors for use in salivary gland gene transfer

Gene transfer delivers a gene of interest into target cells or tissues using a carrier, termed a vector. Successful gene transfer requires efficient, nontoxic vectors (viral or non-viral) that provide adequate transgene expression for an appropriate duration. The simplest and least intrusive way to deliver a gene is with plasmid DNA. This method, however, is generally inefficient, especially in vivo. Viral vectors currently are important tools for in vivo gene transfer, because viruses have evolved efficient mechanisms to introduce their DNA into recipient cells.

Viral vector selection depends on the intended purpose (e.g., long- or short-term gene expression), the target cell or tissue, and the method of delivery (e.g., in vivo, ex vivo). It follows that no single virus is suitable for all gene transfer applications. There are two general classifications of viral vectors, DNA and RNA, and only the former appear useful with salivary epithelial cells. The two most often used are serotype 2 adeno-associated viral (AAV2) vectors, which are single-stranded, and serotype 5 adenoviral (Ad5) vectors, which are double-stranded (Table 1; Baum et al., 2002). Typically, recombinant viral vectors are replication incompetent with several viral genes deleted. The gene of interest and associated regulatory elements replace the deleted viral genes.

Vector delivery to salivary glands is achieved through cannulation of the main excretory ducts whose orifices are accessible in the mouth (Baum et al., 2002). Ad5 vectors possess broad cell and tissue tropism, and, following intraductal delivery, transduce both duct and acinar cells efficiently (Mastrangeli et al., 1994). However, they can provoke vigorous immune responses resulting in relatively short-term transgene expression (2–4 weeks; Kagami et al., 1998). AAV2 vectors also possess fairly broad cell and tissue tropism, but after intraductal delivery only transduce duct cells. AAV2 vectors elicit minimal immune reactivity and yield long-term transgene expression in salivary glands (Voutetakis et al., 2007).

Table 1
Key characteristics of Ad5 and AAV2 vectors.

Characteristics	Ad5	AAV2
Genome	37 kb	4.7 kb
DNA	Double-stranded	Single-stranded
Salivary cell targets	Acinar, duct	Duct
Transgene levels	High	Modest
Stability of expression	Low	High
Titers achieved	High	Modest
Immune response	Significant	Modest
Production	Easy	Laborious

A significant issue generally for gene transfer is controlling transgene expression levels. High and uncontrolled transgene expression could impair cell function and confound biological studies, as well as endanger patients in clinical studies. There are multiple levels at which control of transgene expression can be addressed. Of primary importance is the selection of key elements in the transgene cassette, e.g., the promoter, use of enhancers, introns, 5′ and 3′ untranslated regions and different poly-adenylation signals. We have tested multiple promoters in rodent salivary glands, e.g., cell-type specific (AMY, from human amylase, acinar cell specific; Kall, from human kallikrein, duct cell specific) and non-specific (e.g., CMV, cytomegalovirus; EF1α, human elongation factor 1α) that lead to a broad range of transgene expression (Zheng and Baum, 2005). Control of transgene expression, in an off/on manner, also occurs using a small molecule (drug) inducible promoter system. One such system, which we use, employs rapamycin or its non-immunosuppressive analogues (Wang et al., 2006) and is quite effective regulating transgene expression following gene transfer to rodent submandibular glands with both Ad5 and AAV2 vectors.

4. Secretory pathways used by transgenic proteins in salivary cells

Physiological exocrine protein secretion in salivary glands is primarily triggered by the sympathetic nervous system, and secondarily by the parasympathetic system (Turner and Sugiya, 2002). While protein secretion pathways can roughly be divided into exocrine and endocrine (Fig. 1), three specific pathways have been well described in salivary cells: major regulated, minor regulated and constitutive-like (Castle, 1998; Castle et al., 2002; Gorr et al., 2005). The first two are external stimulus-dependent, with proteins stored in secretory granules until stimulation and secretion is directed apically, i.e., into saliva. Both the constitutive and constitutive-like pathways are non-directional and result in continuous secretion of protein at roughly the rate of translation, i.e., not modulated by external stimuli. They account for a small proportion of protein secretion from acini (Castle et al., 2002; Gorr et al., 2005), but, presumably, all endocrine secretion from salivary cells, although the molecular details of these secretory routes are essentially non-existent.

In general, the mechanisms responsible for sorting soluble secretory proteins are not well known. The notion emerged that signals lie within the protein's secondary or tertiary structure, e.g., pro-opiomelanocortin (Cool et al., 1995), though such signals are clearly not simple to identify (Wang et al., 2005) and no universal signals have been identified. When produced as a transgenic protein following gene transfer, it is assumed the protein will maintain its secretory behavior in other tissues. However, studies with several transgenic secretory proteins in salivary epithelial cells