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Mouse salivary glands and human β-defensin-2 as a study model for antimicrobial gene therapy: technical considerations☆

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Abstract

Transduction of salivary glands with antimicrobial peptide genes has great potential for oral infection control. Our ultimate goal is to introduce antimicrobial peptide genes into salivary glands that secrete these peptides into saliva to control bacterial/fungal infection in the oral cavity. However, an animal study model to test this potential has not been established. Therefore, we determined to test (i) whether the potent antimicrobial peptide human β-defensin-2 (hBD-2) can be overexpressed in saliva after transduction of salivary glands and (ii) whether oral fungal infection can be developed in a NOD/SCID murine model. Lentiviral vector SIN18cPPTRhMLV bearing hBD-2 cDNA was introduced into SCID mouse submandibular glands via cannulation. Reverse transcription polymerase chain reaction (RT-PCR), immunohistochemistry or enzyme-linked immunosorbent assay (ELISA) were performed to detect hBD-2 expression in glands or in saliva. *Candida albicans* 613p was inoculated orally into SCID mice to establish oral candidiasis. Whilst expression of hBD-2 was detected in mouse salivary glands by RT-PCR and immunohistochemistry 1 day or 1 week following delivery of lentivirus, hBD-2 was not detected in saliva. There was recoverable *C. albicans* from the oral cavity and gastrointestinal tract 4 days to 4 weeks after infection, but there was no establishment of observable oral candidiasis in SCID mice under a stereomicroscope. Our data indicate that lentiviral vectors transduce mouse salivary glands, but not at a sufficient level to allow hBD-2 detection in saliva. Other vectors for gene transduction and additional treatment of SCID mice to establish oral candidiasis are needed in order to utilise mouse salivary glands to test antimicrobial gene therapy.

Keywords: hBD-2; Lentiviral vectors; Mouse salivary glands; *Candida albicans*; SCID mice

1. Introduction

Since the discovery of natural antimicrobial peptides/proteins, researchers have tried not just to understand how these peptides/proteins work and applied them in topical use for infection control, but have also explored the possibility of linking their use to gene-based therapeutics, i.e. transducing cells with these peptide genes to augment the innate resistance of tissues/organs against infection [1–5]. Tracheal antimicrobial peptide (TAP), a member of the β-defensin family of antibiotic peptides found in the tracheal mucosa of cows, was used to generate transgenic animals to produce TAP in milk that is antimicrobial [3]. Among a long list of naturally occurring antimicrobial peptides, some well characterised ones are encoded by single genes and their products do not need post-translational processing, making them good candidates for antimicrobial gene therapy. Human β-defensins (hBDs or DEFB) have drawn considerable attention for this purpose as they have potent antimicrobial abilities and some also exert inhibitory effects on viral infections [6–13]. Although only four hBDs (hBD-

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1–4) have been well characterised, up to 28 hBDs may exist based on genomic surveys using a computational search strategy [14,15]. Fourteen hBD or DEFB gene transcripts (DEFB-1, -4 and -103–114) were investigated using reverse transcription polymerase chain reaction (RT-PCR) to detect their expression in gingival keratinocytes [16]. Transduction of epithelial cells in skin or oral mucosa with hBDs has been tested for its potential to enhance infection control [8,12,17]. Primary fibroblasts have also been proposed as target cells to express hBDs, but the amount of antimicrobial peptide secreted may be insufficient to exert an effect [17,18].

Salivary glands have been considered an excellent target organ for gene-based therapeutics compared with other tissues or organs (e.g. liver and skeletal muscle) both for systemic and upper gastrointestinal tract gene therapeutic applications. Salivary glands are well encapsulated, which limits undesired extraglandular vector dissemination. In addition to being an exocrine secretion organ, physiological existence of endocrine secretory pathways in these tissues has been reported [19–21]. In 1996, O'Connell et al. [2] first utilised salivary glands as a target organ for antimicrobial gene-based therapy to control oral infection. The gene encoding the anticandidal protein histatin 3 was transferred to rat salivary glands using an adenovirus-directed approach, and up to 1 mg/mL of histatin exerting a strong candicidal effect was detected in saliva. However, the study did not test whether the secreted histatin 3 in saliva controls oral fungal infection in vivo.

hBDs are expressed in salivary glands and are detected in saliva [22–25]. They are presumably among a plethora of defence mechanisms in the oral cavity to combat infections. hBD-2 is a potent antimicrobial peptide effective against a broad spectrum of bacteria, including Gram-positive bacteria, Gram-negative bacteria and fungi. Among these microbes, *Streptococcus mutans* and *Lactobacillus acidophilus*, which are associated with dental caries, and certain strains of periodontal bacteria such as *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* have been shown to be sensitive to hBD-2 [18,26]. hBD2 is chemotactic for T-cells, immature dendritic cells, mast cells and tumour necrosis factor-alpha (TNF-α)-primed neutrophils [10,27,28]. Its expression in saliva may enhance not only the innate but also adaptive immunity of the oral mucosa. Together with its ability to inhibit human immunodeficiency virus (HIV) infectibility [11,13], these characteristics make hBD-2 an ideal candidate for a study model of antimicrobial gene therapy to control oral infection.

Transduction of salivary glands with antimicrobial peptide genes has great potential for oral infection control. Our ultimate goal is to introduce antimicrobial peptide genes into salivary glands, which secrete these peptides into saliva to control bacterial/fungal/viral infection in the oral cavity. However, an animal study model to test this potential has not yet been established. In the present study, our objective was to address two questions before establishing this model: (i) whether the potent hBD-2 can be expressed in saliva after transduction of salivary glands using lentiviral vectors; and (ii) whether oral fungal infection can be developed in a NOD/SCID murine model. Our findings provide an insightful understanding on the technical issues and concerns for future investigation regarding salivary gland antimicrobial gene therapy for oral infection control.

2. Materials and methods

2.1. In vitro antimicrobial assays

The antimicrobial activity of recombinant (r)hBD-2 was assayed as previously described [6,18]. The following microorganisms were used for the assays: *Candida albicans* 613p (from Dr C. Haidaris, University of Rochester, Rochester, NY) [29]; *P. gingivalis* ATCC 33277 (from Dr S.K. Haake, UCLA, Los Angeles, CA); and *Escherichia coli* ML-35p (from Dr T. Ganz, UCLA) [6]. *Candida albicans* 613p was grown in yeast extract–peptone–dextrose (YEPD) broth, *P. gingivalis* was grown in mycoplasma broth base (Becton Dickinson, Franklin Lakes, NJ) supplemented with 0.5 μg/mL hemin SS (Sigma, St Louis, MO) and 0.5 μg/mL menadione (Sigma) and *E. coli* ML-35p was grown in Lauria–Bertani broth. Microorganisms were cultured at 37 °C in aerobic conditions (except *P. gingivalis*, which used anaerobic conditions), grown to exponential phase, washed with phosphate-buffered saline (PBS) twice and resuspended in low or high salt buffer (10 mM Na phosphate, pH 7.4 or 100 mM Na phosphate, pH 7.4, respectively, each containing 0.03% trypticase soy broth) or in filter-sterilised (0.2 μm) human saliva from healthy donors. The input concentration was adjusted to 2×10^6 cells/mL and the suspension was incubated with high-performance liquid chromatography (HPLC)-purified rhBD-2 (from Dr T. Ganz) with final hBD-2 concentrations of 0, 0.1, 1 and 10 μM. Mixtures of microbes and hBD-2 were then incubated under aerobic or anaerobic (*P. gingivalis*) conditions for 1 h at 37 °C. Subsequently, the mixtures were serially diluted and plated onto appropriate agar plates to quantitate surviving microbes for colony-forming unit (CFU) analysis.

2.2. Degradation of hBD-2 in saliva

Whole saliva from healthy mouse or human donors was collected. Mouse saliva was collected by administering 0.5 mg/kg pilocarpine via subcutaneous injection to induce saliva secretion. Saliva was filtered (0.2 μm) and stored at −80 °C until use. Forty-five microlitres of saliva was mixed with 5 μL of hBD-2 (16 ng/mL) and incubated at 37 °C for 0, 10, 30 and 60 min. At each time point, samples were snap frozen in liquid N_2 until enzyme-linked immunosorbent assay (ELISA).