RESEARCH REPORTS

Biological

J.C. Hsu¹, G. Di Pasquale², J.S. Harunaga¹,
T. Onodera¹, M.P. Hoffman¹, J.A.
Chiorini², and K.M. Yamada^{1*}

¹Laboratory of Cell and Developmental Biology and
²Molecular Physiology and Therapeutics Branch, National
Institute of Dental and Craniofacial Research, National
Institutes of Health, Building 30, Room 426, 30 Convent
Drive, MSC 4370, Bethesda, MD 20892-4370, USA; *corre-
sponding author, kenneth.yamada@nih.gov

J Dent Res 91(2):197-202, 2012

ABSTRACT

Branching morphogenesis is essential for the formation of salivary glands, kidneys, lungs, and many other organs during development, but the mechanisms underlying this process are not adequately understood. Microarray and other gene expression methods have been powerful approaches for identifying candidate genes that potentially regulate branching morphogenesis. However, functional validation of the proposed roles for these genes has been severely hampered by the absence of efficient techniques to genetically manipulate cells within embryonic organs. Using *ex vivo* cultured embryonic mouse submandibular glands (SMGs) as models to study branching morphogenesis, we have identified new vectors for viral gene transfer with high efficiency and cell-type specificity to developing SMGs. We screened adenovirus, lentivirus, and 11 types of adeno-associated viruses (AAV) for their ability to transduce embryonic day 12 or 13 SMGs. We identified two AAV types, AAV2 and bovine AAV (BAAV), that are selective in targeting expression differentially to SMG epithelial and mesenchymal cell populations, respectively. Transduction of SMG epithelia with self-complementary (sc) AAV2 expressing fibroblast growth factor 7 (Fgf7) supported gland survival and enhanced SMG branching morphogenesis. Our findings represent, to our knowledge, the first successful selective gene targeting to epithelial vs. mesenchymal cells in an organ undergoing branching morphogenesis.

KEY WORDS: AAV, embryonic, gene transfer, organogenesis, salivary glands, transduction.

DOI: 10.1177/0022034511429346

Received July 28, 2011; Last revision September 30, 2011;
Accepted October 3, 2011

A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

© International & American Associations for Dental Research

Viral Gene Transfer to Developing Mouse Salivary Glands

INTRODUCTION

The oral cavity requires a continuous supply of saliva from the salivary glands to maintain oral health. When this function is missing and xerostomia occurs as a result of Sjögren's disease, surgery, or irradiation to treat oral cancer, the patients' quality of life decreases dramatically. An understanding of the biological mechanisms underlying embryonic development of salivary glands not only may advance our understanding of developmental biology but also could provide novel approaches to restore function. This restoration could potentially be achieved through tissue engineering to regenerate salivary glands or to develop an artificial salivary gland (Baum and Tran, 2006).

Mammalian salivary glands are generated during embryonic development by the process of branching morphogenesis. The gland starts as a simple spherical epithelial structure attached to a single epithelial stalk and is surrounded by a condensed mesenchyme at embryonic day 12 (E12). The salivary epithelium then continuously remodels itself through repetitive rounds of cleft and bud formation, as well as bud/duct elongation, to generate a highly branched structure (Patel *et al.*, 2006; Tucker, 2007; Hsu and Yamada, 2010; Harunaga *et al.*, 2011). During branching morphogenesis, dynamic interactions occur within, as well as between, the salivary epithelium and the mesenchyme (two major cell populations found in the early stage gland). Branching morphogenesis also creates other branched organs, such as lungs, kidneys, and mammary glands (Affolter *et al.*, 2009; Andrew and Ewald, 2010; Costantini and Kopan, 2010; Morrissey and Hogan, 2010). Multiple complex regulatory mechanisms regulate the molecular and morphological processes responsible for branching morphogenesis.

The mouse salivary gland, and particularly the submandibular gland (SMG), has been used extensively as a research model to study branching morphogenesis, since it effectively recapitulates this process in 3D *ex vivo* organ culture. Recent research in this area has identified a variety of candidate regulatory and effector molecules, as well as potential signaling pathways that may play a key role in regulating SMG embryonic development (Melnick *et al.*, 2009; Larsen *et al.*, 2010; Harunaga *et al.*, 2011). Many more candidate genes will likely be identified through the Salivary Gland Molecular Anatomy Project database (SGMAP at <http://sgmap.nidcr.nih.gov/>; Musselmann *et al.*, 2011) and through other approaches. A major obstacle in direct testing of the functions of these candidates is currently the absence of effective and efficient experimental methods for genetic manipulation of cells within developing salivary glands. Attempts at adenoviral transduction and non-viral transfection of embryonic whole SMG cultures have been conducted multiple times by several investigators with little success, except for labeling a few individual cells for cell migration studies (Larsen *et al.*, 2006; J.C. Hsu and M.P. Hoffman, unpublished observations).

Figure 1. Summary of mouse embryonic salivary gland viral transduction results. E12 and E13 salivary glands were transduced with the indicated vector and cultured for 48 hrs on top of porous membrane filters floating on growth medium. Representative confocal single-plane images showed the expression of enhanced green fluorescent protein (eGFP) in either the epithelium, the mesenchyme, or both. The levels of eGFP expression were scored on a scale of - to ++. Epi, Epithelium, Mes Mesenchyme. Scale bar, 200 μ m.

In this study, we set out to find new vector(s) for viral gene transfer with high efficiency to specific regions of developing SMGs. We searched for a viral vector that could specifically target gene expression to a distinct region of embryonic SMGs with high efficiency. We tested a range of viral vectors, from adenovirus and lentivirus to various types of adeno-associated viruses, or AAVs (see Appendix for details). We demonstrated the biological feasibility of the leading candidate vector for viral gene transfer to embryonic SMGs through proof-of-principle experiments.

MATERIALS & METHODS

Cell Culture, Recombinant Vector Preparation, and Quantification

HEK293T cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. The media contained 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin. Cells were maintained at 37°C under a 5% CO₂ humidified atmosphere.

All recombinant vectors used in this study (see Appendix for details) encoded enhanced green fluorescent protein (eGFP), which was used as a marker to determine the level of viral gene expression in SMGs. The eGFP detection limit with optimized confocal microscope settings was $\geq 2 \mu$ g/mL. Recombinant AAV vectors were produced by a three- or four-plasmid procedure, as previously described (Di Pasquale *et al.*, 2005; see Appendix for details).

We prepared recombinant adenovirus by cloning into the pAd CMV shuttle plasmids that contain adenovirus map units 0-1 and 9.2-16, flanking an expression cassette of a CMV promoter driving eGFP. The shuttle and the backbone plasmids were digested and co-transfected into HEK293. The initial viral lysate was amplified in HEK293 cells, and the final viral lysate was purified by two rounds of CsCl gradient ultracentrifugation. Virus particles were dialyzed, diluted to 1×10^{12} particles/mL, and then frozen at -80°C. The presence of replication-competent adenovirus was checked by plaque formation on the wild-type permissive cell line A549 for at least 14 days. Lentiviral vectors were purchased from Capital Biosciences (Rockville, MD, USA; Lv105-eGFP, titer 1×10^{10} TU/mL).

Ex vivo Submandibular Gland Organ Culture

E12 or E13 SMGs and mesenchyme-free epithelial rudiments were isolated and cultured as previously described (Onodera *et al.*, 2010; see Appendix for details).

Immunofluorescence Microscopy

SMGs were fixed and stained as previously described (Onodera *et al.*, 2010; see Appendix for details).

Statistical Analyses

Results are reported as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed by two-tailed Student's t test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Identification of Tissue-specific Viral Gene Transfer Vectors for Cultured Embryonic Salivary Glands

We directly compared 13 different viral gene transfer vectors for efficacy and tissue specificity of expression; we assayed eGFP expression after 48 hrs of incubation with E12 and E13 mouse SMGs.

As a standard for comparison, we first determined the transduction efficiency of adenovirus serotype 5 (the most commonly used adenovirus) and lentivirus, as determined by eGFP expression detected by fluorescence microscopy. Following 48 hrs of incubation of SMGs with viruses, adenovirus transduced only peripheral mesenchymal cells, without effective transduction of the epithelial cells of SMGs (Fig. 1). In contrast, SMG transduction by lentivirus was not detectable in either epithelial or mesenchymal cells.

We then compared the efficacy and specificity of 11 different isolates of AAVs in E12 and E13 SMGs. Two AAVs showed effective expression and tissue specificity. AAV2 transduced epithelial cells selectively (Fig. 1). In contrast, BAAV showed the opposite specificity, almost exclusively targeting mesenchymal cells. In fact, isolated salivary epithelial rudiments devoid of mesenchyme could not be transduced by BAAV (Appendix Fig. 1). Moreover, only mesenchymal cells were transduced by BAAV, with no evidence for transduction of neuronal or endothelial cells. Neither AAV2 nor BAAV showed evidence of cytotoxicity (Appendix Fig. 2).

Of the remaining 9 AAVs tested, 4 (AAV1, AAV7, AAV8, and AAVx1) showed no detectable expression of eGFP. In 5 others (AAV4, AAV5, AAV12, AAVx5, and AAVx25), detectable eGFP expression was found only in a limited number of SMG cells (Fig. 1). We focused on the epithelial-specific AAV2 because of the biological importance of salivary gland epithelial cells in branching morphogenesis and the formation of secretory acini. To determine how our results compare with those from a non-viral approach, we conducted preliminary experiments testing the effectiveness of the Amara system to target eGFP expression to E12 SMGs. Our results showed only random labeling of a few salivary gland cells (Appendix Fig. 3), which is consistent with published findings in adult rat SMGs (Sramkova *et al.*, 2009).

Enhancement of AAV2-targeted SMG Epithelial Transduction with scAAV2

Since the expression of eGFP after AAV2 transduction was relatively low, we compared the ability of the self-complementary form of AAV2 (scAAV2) to promote gene transfer to SMGs. scAAV2 displayed substantially enhanced eGFP expression in the epithelium of SMG (Fig. 2). This confirms the epithelium-selective targeting property of the AAV2 virus with enhanced expression using scAAV2, which is modified to express transduced genes more rapidly (McCarty *et al.*, 2001). The SMG epithelial-selective pattern of transduction by scAAV2 could be enhanced further by co-incubation with tyrphostin 23 (Fig. 2) or epoxomicin (Appendix Fig. 4). Both of these drugs have been demonstrated to enhance AAV2-mediated expression by inhibiting viral degradation (Zhong *et al.*, 2007).

Time-course and Dose-response Profiles of scAAV2-mediated Transduction

Next, we characterized the time-courses of eGFP expression mediated by scAAV2 and BAAV. Following 12 hrs of incubation of SMG with scAAV2, scattered SMG cells throughout the gland

Figure 2. Augmentation of salivary epithelial viral transduction with self-complementary AAV2 (scAAV2) and tyrphostin 23 (Tyr-23). E12 SMGs were transduced with eGFP-encoding vectors. (A) AAV2, (B) scAAV2, and (C) scAAV2 in the presence of 10 μ M Tyr-23. (A) AAV2 selectively targeted eGFP expression to salivary epithelium. (B) The targeted eGFP expression was enhanced with scAAV2. (C) The eGFP expression level was augmented further by the tyrosine kinase inhibitor Tyr-23. Representative confocal single-plane images are shown. Scale bar, 100 μ m.

began to express eGFP (Fig. 3). At 24 hrs and reaching a maximum at 48 hrs, scAAV2 transduction selectively increased in the SMG epithelial buds and the duct/stalk, as determined by prominent eGFP expression. In contrast, BAAV exhibited more delayed transduction kinetics, with maximal transduction in the mesenchyme at 72 hrs (Appendix Fig. 5). When we determined the dose-response relationship of scAAV2-mediated transduction of SMG epithelial cells, we found that transduction efficiency was approximately linear within a one-log range of virus titers (Fig. 3E).

Expression of Fgf7 by scAAV2 Permits Survival and Increases Branching of Isolated Epithelial Rudiments

Next, we conducted proof-of-principle experiments to determine whether the scAAV2 vector we had identified as epithelium-selective could produce biologically effective gene transfer.

Figure 3. Time-course and dose-response profiles of scAAV2-mediated embryonic salivary gland transduction. SMGs at various stages between early E12 and E13 were transduced for (A) 2 hrs, (B) 12 hrs, (C) 24 hrs, or (D) 48 hrs with scAAV2-eGFP vector. Maximal scAAV2-mediated salivary epithelial eGFP expression occurred at 24 to 48 hrs, and (E) at the highest dose tested. Representative confocal single-plane images are shown. Salivary epithelium was stained with anti-E-cadherin antibody (red), and the basement membrane is marked by a white dashed line. Scale bars: (A-D) 100 μ m and (E) 10 μ m.

We tested whether epithelial overexpression of fibroblast growth factor 7 (Fgf7) could rescue salivary gland development in the absence of the normal mesenchymal source of Fgfs. The coding sequence of Fgf7 was cloned into the scAAV2 vector. We first tested it for expression by transient transfection into 3T3 cells. Quantitative (q)RT-PCR and antibody staining demonstrated a substantial induction of Fgf7 mRNA transcript expression in transfected cells; the cloned gene was expressed correctly by the plasmid DNA vector, as demonstrated by strong cytoplasm-specific staining with anti-c-myc antibodies, which target the myc tag co-expressed with Fgf7 (Appendix Fig. 6).

Next, the scAAV2-Fgf7 vectors were packaged into a scAAV2 virus. Following virus packaging and purification, scAAV2-Fgf7

or the control scAAV2-eGFP vectors were incubated with intact SMG, or with isolated E12 SMG epithelial rudiments. As expected, the presence of endogenous growth factors secreted by mesenchymal cells in intact SMGs masked any effects of the Fgf produced by scAAV2-Fgf7 on gland growth or morphology (Appendix Fig. 7). Using isolated SMG epithelia stripped of mesenchyme, we first tested whether epithelial expression of Fgf7 by viral gene transfer could support survival *ex vivo* in the complete absence of mesenchyme. Epithelial rudiments cultured with control scAAV2-eGFP vectors and without exogenous growth factors underwent darkening due to necrosis and died within 12 hrs (Fig. 4A). In contrast, rudiments cultured with Fgf7-producing scAAV2-Fgf7 vectors survived and were morphologically indistinguishable from rudiments cultured with a high dose of exogenously added Fgf7 protein.

In isolated epithelial culture, recombinant Fgf7 stimulates epithelial budding, whereas Fgf10 stimulates ductal morphogenesis, and the combination of both results in increased budding (Steinberg *et al.*, 2005). When control scAAV2-eGFP-transduced epithelial rudiments were cultured with Fgf10, the virus did not affect Fgf10-mediated morphogenesis (Fig. 4B). In contrast, epithelial rudiments transduced with scAAV2-Fgf7 had an increased number of epithelial buds (Figs. 4B, 4C), and appeared similar to epithelia cultured with both recombinant Fgf7 and Fgf10 (Steinberg *et al.*, 2005). Thus, our experiments demonstrate that scAAV2-mediated overexpression of Fgf7 in the epithelium allows the epithelium to survive in the absence of exogenous growth factors, and it stimulates morphogenesis in a manner similar to that of the exogenous recombinant protein.

DISCUSSION

The goal of this study was to develop novel tools to efficiently target gene expression to specific regions of early-stage embryonic SMGs. Because of difficulties transfecting embryonic SMGs, we turned to viral approaches for gene transfer. Contrary to previous findings regarding adult tissues, which have demonstrated the success of lentivirus or adenovirus in transducing adult mouse SMGs following direct cannulation of the glands (Shai *et al.*, 2002), we found that when these viruses were cultured *ex vivo* with E12 or E13 SMGs, neither virus was able to transduce detectable eGFP gene expression in the SMG epithelium. The

limited transduction of the adenovirus is due to the (outer) mesenchymal cell layer serving as a physical barrier to virus penetration, since direct epithelial contact with the adenovirus results in epithelial adenoviral transduction (Larsen *et al.*, 2006; Onodera *et al.*, 2010).

We identified two AAVs, BAAV and AAV2, that efficiently target gene expression to embryonic SMG mesenchyme and epithelium, respectively. These differences were not due to an inability of BAAV to penetrate through mesenchyme to the epithelium, because isolated epithelia without mesenchyme could not be transduced by BAAV. The difference in the tropism/cell type selectivity between these two vectors is likely due to the different cellular factors or receptors each vector requires for transduction. BAAV uses gangliosides as a receptor and gp96 for transcytosis activity (Schmidt and Chiorini, 2006; Di Pasquale *et al.*, 2010). In contrast, AAV2 transduction requires heparan sulfate proteoglycan (Summerford and Samulski, 1998). Additional proteins, including fibroblast growth factor receptor 1, $\alpha\beta 5$ integrin, and hepatocyte growth factor receptor, are involved in AAV2 transduction as well (Mizukami *et al.*, 1996; Qing *et al.*, 1999; Summerford *et al.*, 1999; Kashiwakura *et al.*, 2005).

We focused on characterizing the epithelial targeting by AAV2, since genetic manipulation of SMG epithelium will be a useful research tool to study branching morphogenesis, as well as other processes involved in the repetitive formation of clefts, ducts, and end buds (Patel *et al.*, 2006; Tucker, 2007; Hsu and Yamada, 2010; Harunaga *et al.*, 2011). Since the salivary epithelial expression of eGFP following AAV2 transduction was relatively low, we generated the self-complementary form of AAV2 (scAAV2), which circumvents the rate-limiting requirement for complementary-strand synthesis (McCarty *et al.*, 2001). The resulting higher levels of scAAV2-mediated eGFP expression in SMG epithelium mirror those described in *in vitro* cell culture systems (Zhong *et al.*, 2007). One drawback of scAAV2 is that it can transduce only genes smaller than 2 Kb, so gene transfer of larger-sized genes may require different approaches.

Figure 4. scAAV2-Fgf7-mediated expression of Fgf7 supports survival and promotes branching of SMG epithelial rudiments. SMG epithelial rudiments were cultured in the presence or absence of growth factors and the scAAV2 vector. **(A)** Rudiments cultured in the presence of scAAV2-eGFP (control vector) without growth factors died within 12 hrs, similar to controls with no growth factors. In contrast, rudiments cultured with an Fgf7-producing vector (scAAV2-Fgf7) survived equally as well as rudiments treated with a high dose of exogenous Fgf7 (200 ng/mL). **(B)** Rudiments cultured in the presence of both exogenously added Fgf10 (500 ng/mL) to maintain gland survival and scAAV2-eGFP (control vector) displayed a growth pattern similar to that seen with Fgf10 alone. In contrast, rudiments cultured in the presence of both exogenously added Fgf10 and the Fgf7-producing scAAV2-Fgf7 displayed an increased number of epithelial buds and a lack of bud elongation, which are characteristics of glands cultured with exogenous Fgf7. The numbers of new epithelial buds that formed between 4 hrs and 24 hrs in scAAV2-eGFP- and scAAV2-Fgf7-transduced glands were statistically analyzed and compared. **(C)** The numbers of buds at each time-point were also enumerated and compared. scAAV2-eGFP (empty circles), scAAV2-Fgf7 (filled circles). ** $P < 0.01$, $n = 3$. Scale bar, 100 μ m. Error bars represent standard error of the mean (SEM).

Pharmacological agents such as Tyr-23 and epoxomicin also increased scAAV2 transduction efficiency in SMGs, although the effects of pharmacological agents on the natural development of SMGs need to be considered (Kashimata and Gresik, 1997). In proof-of-principle experiments with scAAV2, we used isolated epithelial rudiments (with the mesenchyme removed) to transduce the Fgf7 gene. We did this to distinguish the effects of scAAV2-produced Fgf7 from those mediated by the high levels of endogenous Fgf7 produced by the SMG mesenchymal cells at stages E12 to E13 (Hoffman et al., 2002). We also tested the transduction of scAAV2 in another branching organ, the embryonic lung, but interestingly, scAAV2 targeted the mesenchyme of the developing lung (Appendix Fig. 8). Consequently, it is important to characterize the virus tropism for each tissue in any organ other than the salivary gland. We emphasize that tissue selectivity is relative rather than absolute. Consequently, attempts to attribute a function solely to one tissue (e.g., only epithelium) will need to use complementary approaches, such as transduction of isolated epithelium followed by recombination with non-transduced mesenchyme by classic reconstitution approaches (Grobstein, 1953).

In summary, we have developed a useful research tool for genetic manipulation of embryonic SMGs. This will enable researchers to test directly the functions of the genes being identified, either through literature searches or by using databases such as the Salivary Gland Molecular Anatomy Project database (SGMAP at <http://sgmap.nidcr.nih.gov/>; Musselmann et al., 2011).

ACKNOWLEDGMENTS

The authors thank Shelagh Johnson for excellent editing. This work was supported by the Division of Intramural Research, National Institute of Dental and Craniofacial Research, National Institutes of Health, Grant no. ZIA DE000525. The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES

- Affolter M, Zeller R, Caussinus E (2009). Tissue remodelling through branching morphogenesis. *Nat Rev Mol Cell Biol* 10:831-842.
- Andrew DJ, Ewald AJ (2010). Morphogenesis of epithelial tubes: insights into tube formation, elongation, and elaboration. *Dev Biol* 341:34-55.
- Baum BJ, Tran SD (2006). Synergy between genetic and tissue engineering: creating an artificial salivary gland. *Periodontol* 2000 41:218-223.
- Costantini F, Kopan R (2010). Patterning a complex organ: branching morphogenesis and nephron segmentation in kidney development. *Dev Cell* 18:698-712.
- Di Pasquale G, Rzdadzinska A, Schneider ME, Bossis I, Chiorini JA, Kachar B (2005). A novel bovine virus efficiently transduces inner ear neuroepithelial cells. *Mol Ther* 11:849-855.
- Di Pasquale G, Kaludov N, Agbandje-McKenna M, Chiorini JA (2010). BAAV transcytosis requires an interaction with beta-1-4 linked-glucosamine and gp96. *PLoS One* 5:e9336.
- Grobstein C (1953). Inductive epitheliomesenchymal interaction in cultured organ rudiments of the mouse. *Science* 118:52-55.
- Harunaga J, Hsu JC, Yamada KM (2011). Dynamics of salivary gland morphogenesis. *J Dent Res* 90:1070-1077.
- Hoffman MP, Kidder BL, Steinberg ZL, Lakhani S, Ho S, Kleinman HK, et al. (2002). Gene expression profiles of mouse submandibular gland development: FGFR1 regulates branching morphogenesis in vitro through BMP- and FGF-dependent mechanisms. *Development* 129:5767-5778.
- Hsu JC, Yamada KM (2010). Salivary gland branching morphogenesis—recent progress and future opportunities. *Int J Oral Sci* 2:117-126.
- Kashimata M, Gresik EW (1997). Epidermal growth factor system is a physiological regulator of development of the mouse fetal submandibular gland and regulates expression of the alpha6-integrin subunit. *Dev Dyn* 208:149-161.
- Kashiwakura Y, Tamayose K, Iwabuchi K, Hirai Y, Shimada T, Matsumoto K, et al. (2005). Hepatocyte growth factor receptor is a coreceptor for adeno-associated virus type 2 infection. *J Virol* 79:609-614.
- Larsen M, Wei C, Yamada KM (2006). Cell and fibronectin dynamics during branching morphogenesis. *J Cell Sci* 119(Pt 16):3376-3384.
- Larsen M, Yamada KM, Musselmann K (2010). Systems analysis of salivary gland development and disease. *Wiley Interdiscip Rev Syst Biol Med* 2:670-682.
- McCarty DM, Monahan PE, Samulski RJ (2001). Self-complementary recombinant adenoassociated virus (scAAV) vectors promote efficient transduction independently of DNA synthesis. *Gene Ther* 8:1248-1254.
- Melnick M, Phair RD, Lapidot SA, Jaskoll T (2009). Salivary gland branching morphogenesis: a quantitative systems analysis of the Eda/Edar/NFkappaB paradigm. *BMC Dev Biol* 9:32.
- Mizukami H, Young NS, Brown KE (1996). Adeno-associated virus type 2 binds to a 150-kilodalton cell membrane glycoprotein. *Virology* 217:124-130.
- Morrissey EE, Hogan BL (2010). Preparing for the first breath: genetic and cellular mechanisms in lung development. *Dev Cell* 18:8-23.
- Musselmann K, Green JA, Sone K, Hsu JC, Bothwell IR, Johnson SA, et al. (2011). Salivary gland gene expression atlas identifies a new regulator of branching morphogenesis. *J Dent Res* 90:1078-1084.
- Onodera T, Sakai T, Hsu JC, Matsumoto K, Chiorini JA, Yamada KM (2010). Btd7 regulates epithelial cell dynamics and branching morphogenesis. *Science* 329:562-565.
- Patel VN, Rebutini IT, Hoffman MP (2006). Salivary gland branching morphogenesis. *Differentiation* 74:349-364.
- Qing K, Mah C, Hansen J, Zhou S, Dwarki V, Srivastava A (1999). Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. *Nat Med* 5:71-77.
- Schmidt M, Chiorini JA (2006). Gangliosides are essential for bovine adeno-associated virus entry. *J Virol* 80:5516-5522.
- Shai E, Falk H, Honigman A, Panet A, Palmon A (2002). Gene transfer mediated by different viral vectors following direct cannulation of mouse submandibular salivary glands. *Eur J Oral Sci* 110:254-260.
- Sramkova M, Masedunskas A, Parente L, Molinolo A, Weigert R (2009). Expression of plasmid DNA in the salivary gland epithelium: novel approaches to study dynamic cellular processes in live animals. *Am J Physiol Cell Physiol* 297:C1347-C1357.
- Steinberg Z, Myers C, Heim VM, Lathrop CA, Rebutini IT, Stewart JS, et al. (2005). FGFR2b signaling regulates ex vivo submandibular gland epithelial cell proliferation and branching morphogenesis. *Development* 132:1223-1234.
- Summerford C, Samulski RJ (1998). Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *J Virol* 72:1438-1445.
- Summerford C, Bartlett JS, Samulski RJ (1999). AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. *Nat Med* 5:78-82.
- Tucker AS (2007). Salivary gland development. *Semin Cell Dev Biol* 18:237-244.
- Zhong L, Zhao W, Wu J, Li B, Zolotukhin S, Govindasamy L, et al. (2007). A dual role of EGFR protein tyrosine kinase signaling in ubiquitination of AAV2 capsids and viral second-strand DNA synthesis. *Mol Ther* 15:1323-1330.