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Parasympathectomy increases resting secretion of the submandibular gland in minipigs in the long term

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Abstract

Parasympathectomy leads to retrogressive alteration and dysfunction of the submandibular gland (SMG) within 1 month, but its long-term effect is unclear. Excessive secretion is observed in half of the patients 4–6 months after SMG transplantation, which completely denervates the gland. Here, we investigated the long-term effect of parasympathectomy on the secretion of SMGs in minipigs. The results showed that the resting salivary secretion of SMGs decreased by 82.9% of that in control at 2 months after denervation, but increased by 156% at 6 months. Although experiencing an atrophic period, the denervated glands regained their normal morphology by 6 months. The expression of the function-related proteins, including muscarinic acetylcholine receptor (mAChR) 3, aquaporin 5 (AQP5), tight junction protein claudin-3, and claudin-4 was decreased at 2 months after denervation. Meanwhile, the protein expression of stem cell markers, including sex-determining region Y-box 2 and octamer-binding transcription factor 4, and the number of $Ki67^+$ cells were significantly increased. However, at 6 months after denervation, the expression of mAChR3, AQP5, claudin-1, claudin-3, and claudin-4 was significantly raised, and the membrane distribution of these proteins was increased accordingly. The autonomic axonal area of the glands was reduced at 2 months after denervation but returned to the control level at 6 months, suggesting that reinnervation took place in the long term. In summary, parasympathectomy increases resting secretion of the SMGs in the long term with a possible mechanism involving improved transepithelial fluid transport. This finding may provide a new strategy for xerostomia treatment.

KEYWORDS
aquaporin 5 (AQP5), denervation, salivary gland, secretion, tight junctions

1 | INTRODUCTION

Saliva plays an important role in preserving oral health. Xerostomia caused by salivary gland hypofunction negatively affects patients' quality of life. Resting salivary secretion is maintained to create a film and replenish proteins adsorbed by the underlying oral surfaces, as well as coat and protect the oral mucosal and teeth surfaces. Submandibular gland (SMG) secretion contributes 60–65% of the volume of resting whole saliva (de Almeida Pdel, Grégio, Machado, de Lima, & Azevedo, 2008; Humphrey & Williamson, 2001). Thus, promoting the resting secretion of SMG is a promising strategy to treat xerostomia.

Parasympathetic nerves regulate salivation of salivary glands, especially the secretion of ions and fluid (Proctor & Carpenter, 2014).

Although parasympathetic denervation of the SMG is conventionally reported to lead to decreased gland weight, gland atrophy, fibrosis, and reduced saliva secretion within 1 month in rats (Azlina et al., 2010; Kang et al., 2010; Qi et al., 2017), few studies have examined the long-term effect (such as over 6 months) of denervation on SMG secretion. Autologous SMG transplantation for patients with severe dry eye provides researchers with the opportunity to study the effects of complete denervation. Approximately half of the patients develop epiphora at 4–6 months after SMG transplantation (Qin et al., 2013; Yu et al., 2004). In addition to the volume of the SMG, hypersecretion of the transplanted SMG is also an important factor resulting in postoperative epiphora. We established an autologous transplanted SMG model in rabbits and found that the secretion in transplanted glands increased at 90 and 180 days after transplantation (Yang et al., 2017). Thus, to determine the long-term effect of parasympathetic denervation, we previously severed the chorda tympani in rats and found that the resting secretion of the SMG was doubled at 6 months after denervation (Qi et al., 2017). Compared with the salivary glands of minipigs, the salivary glands of rodents display more morphological and protein expression differences from those of humans (Maruyama, Monroe, Hunt, Buchmann, & Baker, 2018; X. M. Zhang, Huang et al., 2018). Therefore, the effects of denervation on the function of salivary glands in minipigs are worthy of investigation.

Salivary fluid secretion is largely dependent on the activation of muscarinic receptors on salivary acinar cells by acetylcholine released from parasympathetic nerves. Acinar cell activation of fluid transport is achieved through transcellular and paracellular pathways (G. H. Zhang, Wu, & Yu, 2013). Water is, therefore, drawn into the duct either through aquaporin 5 (AQP5) or through tight junctions, the leakiness of which is likely to be regulated by cholinergic stimulation (Cho et al., 2015; Cong et al., 2015; G. H. Zhang et al., 2013; Ishikawa, Eguchi, Skowronski, & Ishida, 1998; L. W. Zhang et al., 2016; Mellas, Leigh, Nelson, McCall, & Baker, 2015). Because denervation alters the secretion of salivary glands, changes in the expression of the function-related proteins, such as cholinergic receptors, AQP5, and tight junction proteins, by parasympathetic denervation need to be studied.

In the current study, we investigated the long-term effect of parasympathectomy on SMGs and the underlying mechanism in minipigs, aiming to provide a novel strategy to treat xerostomia.

2 | MATERIALS AND METHODS

2.1 | Animals

The animal experimental protocols were approved by the Ethics Committee of Animal Research, Peking University (LA2016062) and were in accordance with the ARRIVE guidelines. Twelve healthy male Bama minipigs aged 8–10 months and weighing 25–30 kg were used in this study. Animals were raised in individual cages with free access to water and a regular supply of food.

2.2 | Parasympathectomy of SMGs

Animals were fasted for 24 hr and generally anesthetized by Zoletil (20 mg·kg^{-1}) and isoflurane via mask induction at 3–5%. Parasympathectomy was performed on the right SMG, and the left gland received a sham operation to serve as a within-subject control. An incision was made in the neck region to expose the SMG. The branch of the lingual nerve innervating the SMG appeared concomitantly with the Wharton's duct beneath the mylohyoid muscle. The nerve was bluntly dissected through to the hilus of the gland, and the parasympathetic ganglion located within the hilus was exposed. A segment of nerve 2–3 cm in length and the ganglion were excised. Half of the animals were randomly killed at 2 months after denervation as the short-term group, and the other half were killed at 6 months as the long-term group.

2.3 | Measurement of the flow rate of SMG

Because drugs and narcosis influence salivation, we measured the salivary flow rate of SMGs when animals were awake. A polytetrafluoroethylene cannula with a diameter of 1.2 mm was inserted into Wharton's duct and fixed to the adjacent tissue 1 day before the measurement. The animals were fed regularly after revival from anesthesia. The minipigs were placed in a specialized fixation apparatus, and the flow rates were measured between 9:00 and 11:00 a.m. To measure the resting flow rate, saliva was collected into centrifugal tubes for 1 hr. To measure the stimulated flow rate, 2 ml of 2.5% citric acid was instilled orally on the dorsum of the tongue, and the secretion was immediately collected for 5 min. The weights of the collected saliva from three tests were averaged.

2.4 | Histology, immunohistochemistry, immunofluorescence, and transmission electron microscopy

The SMGs from both sides were collected and weighed after general anesthesia. Hematoxylin and eosin staining, and alcian blue and periodic acid-Schiff (AB/PAS) staining were performed as previously reported (X. M. Zhang, Huang et al., 2018). Sections were immunostained with primary antibodies (Table 1) and processed by routine immunohistochemistry (IHC) and immunofluorescence (IF) methods. IHC images were captured using an EVOS FL Auto Cell Imaging System (Thermo Fisher Scientific, Waltham, MA). Ki67^{+} cells were counted from 10 randomly selected views of each gland. IF images were acquired by a confocal microscope (Leica TCS SP8, Wetzlar, Germany). Autonomic nerves were double stained by antibodies against protein gene product 9.5 (PGP9.5) and growth-associated protein 43 (GAP43). Ten random views of axons in gland parenchyma were captured, and the mean axon density was calculated by LAS AF software (Leica Microsystems) and presented as the ratio of axon area to total area in the image. For transmission electron microscopy, ultrathin sections were stained with 10% uranyl acetate and 1% lead citrate, and images were captured by a transmission electron microscope (Hitachi H-7000, Tokyo, Japan).

TABLE 1 Information of the primary antibodies used in this study

Antibodies	Dilution	Catalog	Source
Anti-aquaporin 5 (AQP5)	1:100 (IF)	sc9891	Santa Cruz Biotechnology, Santa Cruz, CA
Anti-AQP5	1:1,000 (WB)	178615	Merck KGaA, Darmstadt, Germany
Anti-claudin-1	1:1,000 (WB),1:100 (IF)	BS1063	Bioworld Technology, Saint Louis Park, MN
Anti-claudin-3	1:1,000 (WB),1:100 (IF)	BS1067	Bioworld Technology
Anti-claudin-4	1:1,000 (WB),1:100 (IF)	BS1068	Bioworld Technology
Anti-GAPDH	1:4,000 (WB)	ab8245	Abcam, Cambridge, UK
Anti-growth-associated protein 43 (GAP43)	1:1,000 (IF)	NB300–143	Novus Biologicals, Littleton, CO
Anti-Ki67	1:200 (IHC)	ab15580	Abcam
Anti-muscarinic acetylcholine receptor 1 (mAChR1)	1:500 (WB)	sc9470	Santa Cruz Biotechnology
Anti-muscarinic acetylcholine receptor 3 (mAChR3)	1:1,000 (WB)	ab126168	Abcam
Anti-Na-K-Cl cotransporter (NKCC1)	1:100 (IF)	13884–1-AP	Proteintech Group, Chicago, IL
Anti-octamer-binding transcription factor 4 (OCT4)	1:1,000 (WB)	ab18976	Abcam
Anti-protein gene product 9.5 (PGP9.5)	1:300 (IHC)	TA347068	OriGene Technologies, Rockville, MD
Antisex determining region Y-box 2 (SOX2)	1:1,000 (WB)	AB5603	Merck KGaA
Anti-occludin	1:1,000 (WB),1:100 (IF)	33–1500	Thermo Fisher Scientific, Waltham, MA

Note. IF: immunofluorescence; IHC: immunohistochemistry; WB: western blot.

2.5 | Western blot analysis

Total protein was extracted from the SMG using a Minute total protein extraction kit (Invent Biotechnologies, Plymouth, MN). Equal amounts of protein (30 μg) were separated by electrophoresis on a 10% or 12% sodium dodecyl sulfate-polyacrylamide gel, electro-transferred to polyvinylidene difluoride membranes, blocked with 5% nonfat milk, and probed with antibodies (Table 1) at 4°C overnight. The membranes were then incubated with horseradish-peroxidase-conjugated secondary antibodies. The bands of target proteins were detected by an enhanced chemiluminescence reagent. The membranes were stripped and reprobed with anti-GAPDH antibody to ensure equal loading of the lanes. The band density was analyzed using ImageJ software (National Institute of Health, MD) and presented as the ratio of the target protein to GAPDH.

2.6 | Statistics

Data are expressed as the mean ± standard error of the mean. Paired two-way analysis of variance and Bonferroni multiple comparisons were used to analyze the data when two conditions were involved. Two-tailed paired Student's *t* test was used to compare the difference between glands from the same animal. $P < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | The effects of parasympathectomy on SMGs

No accidental death or major complications occurred during the experimental period. To determine the effects of parasympathectomy on SMGs over time, the flow rate and morphology were evaluated at 2 and 6 months after the operation. The results showed that the resting and stimulated flow rates of SMGs decreased by 82.9% ($p < 0.01$) and 89.2% ($p < 0.05$), respectively, at 2 months after the operation. However, the resting saliva secretion was significantly increased by 156.6% ($p < 0.05$) of the control and the stimulated saliva secretion was restored to the control level at 6 months after denervation (Figure 1a,b). Under gross observation, the denervated gland was shrunken and indurated at 2 months after the operation but recovered at 6 months after the operation. The gland weight/body weight ratio of the denervated glands decreased to 33.1% of the control at 2 months after the operation ($p < 0.01$), and 67.8% of the control at 6 months after the operation ($p < 0.05$), respectively, and this ratio of the denervated glands at 6 months after the operation was higher than that at 2 months ($p < 0.01$, Figure 1c). Histologically, severe acinar atrophy, ductal enlargement, and fibrosis were observed at 2 months after denervation (Figure 1d). AB/PAS staining distinguishes serous from mucous acinar cells according to their different polysaccharide contents. The results showed that the predominantly atrophic acinar cells were serous cells, although the mucous cells also displayed atrophy to some extent at 2 months after denervation (Figure 1e). Transmission electron microscopy showed that the ultrastructure of the acini was disordered with decreased and unevenly scattered secretory granules (Figure 1f). However, the impaired glandular structure was almost completely restored at 6 months after denervation.

The Ki67 protein is a cellular proliferation marker (Yan, Cai, Sun, Gui, & Liang, 2018). At 2 months after the operation, the number of $Ki67^+$ cells in the denervated glands was significantly higher than that in the control glands ($p < 0.01$), especially in the ducts (Figure 2a,b). Western blot results showed that the protein expression of

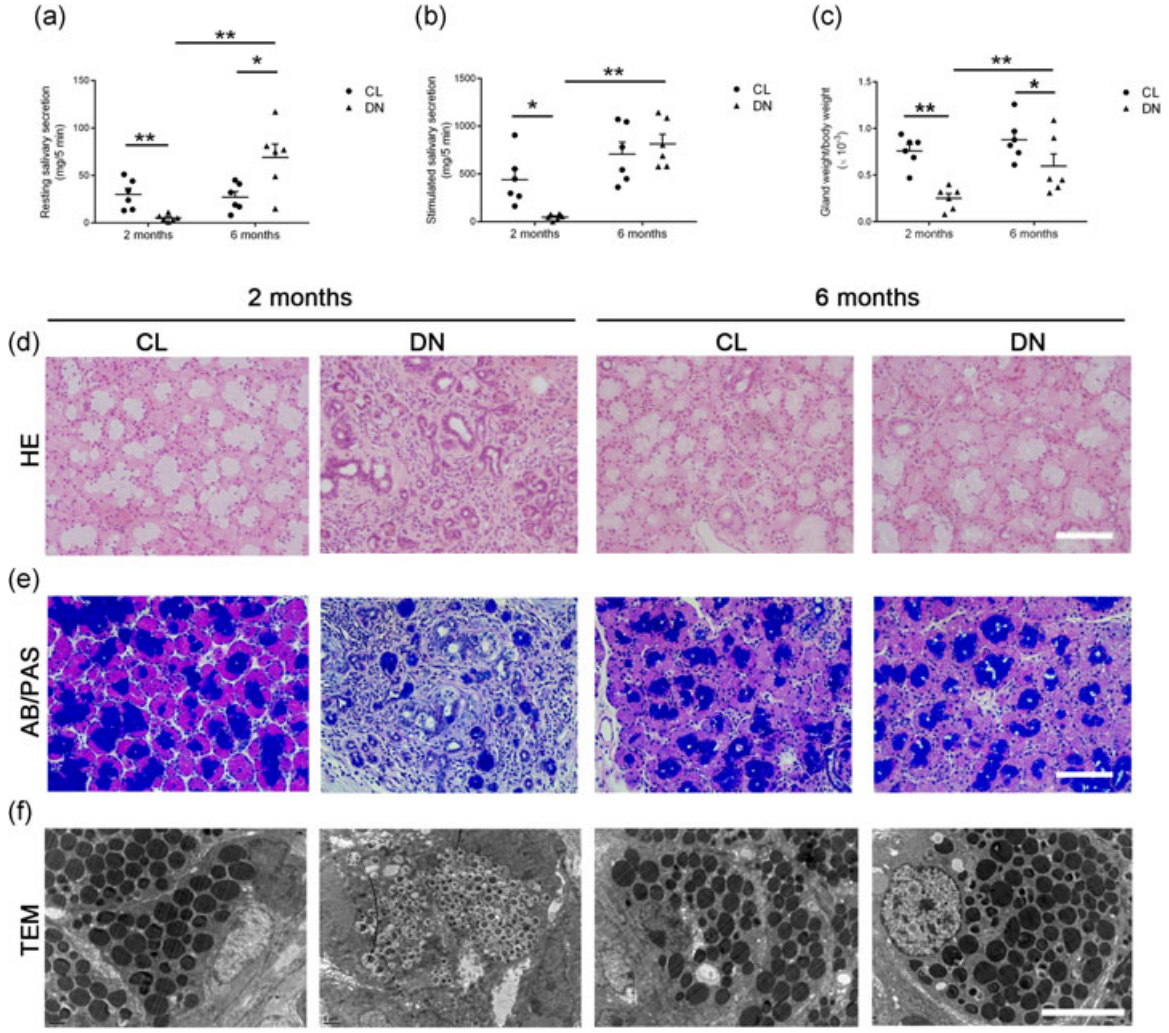

FIGURE 1 The secretion and morphology of submandibular glands (SMGs) after parasympathectomy. (a) Resting salivary secretion. (b) Acid-stimulated salivary secretion. (c) The ratio of gland weight to body weight. Data are expressed as the mean ± *SEM*. $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 6$ per group. Paired two-way ANOVA and Bonferroni multiple comparisons were used. (d) Hematoxylin and eosin (H&E) staining. Bar: 100 μm. (e) Alcian blue and periodic acid-Schiff (AB/PAS) staining. The serous acinar cells are stained purplish-red and mucus acinar cells are blue. Bar: 100 μm. (f) Transmission electron microscopy (TEM). Bar: 5 μm. ANOVA: analysis of variance; CL: contralateral; DN: denervated; *SEM*: standard error of the mean [Color figure can be viewed at wileyonlinelibrary.com]

sex-determining region Y-box 2 (SOX2) and octamer-binding transcription factor 4, the key transcription factors in stem cells (Costa et al., 2018; Rizzino, 2013), was markedly increased in the denervated glands at 2 months after the operation ($p < 0.05$ and $p < 0.01$) and recovered to the control level at 6 months after the operation (Figure 2c).

3.2 | The alteration of transepithelial fluid transport pathways after parasympathectomy of SMGs

We next explored the possible mechanism of the increase in resting salivary secretion of the SMGs after parasympathectomy in the long term. The expression of muscarinic acetylcholine receptor (mAChR) 1 in denervated SMGs was remarkably increased at 2 months ($p < 0.01$) and returned to the control level at 6 months after the operation. In contrast, the expression of mAChR3 was first reduced at 2 months ($p < 0.05$) but was higher than that in the control gland at 6 months after denervation ($p < 0.05$). Consistent with mAChR3 expression, the expression of AQP5 in the denervated glands was significantly reduced by 87.2% of that in the control at 2 months ($p < 0.05$), and increased by 166.3% of the expression in the control at 6 months after denervation ($p < 0.05$). The expression of the tight junction proteins claudin-1 and occludin was increased ($p < 0.05$), whereas the expression of claudin-3 and claudin-4 was reduced ($p < 0.05$) at 2 months after denervation. The expression of claudin-1, claudin-3, and claudin-4 was elevated ($p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively), whereas occludin content was reduced to the control level at 6 months after denervation (Figure 3).

Immunofluorescence staining showed that AQP5 was predominantly located in the apicolateral membrane of control acinar cells, with the basolateral membrane labeled by the Na-K-Cl cotransporter (Kondo et al., 2015). AQP5 staining was significantly

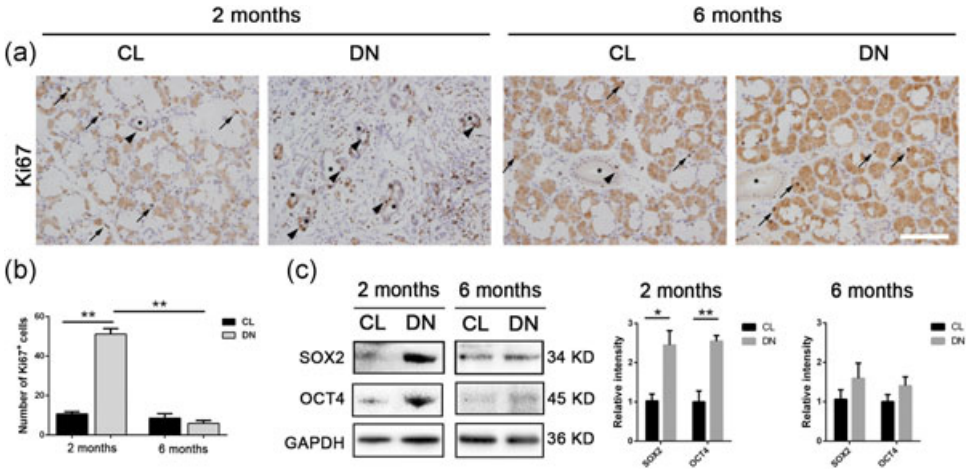

FIGURE 2 Ki67$^+$ cells and expression of stem cell transcription factors in submandibular glands after parasympathectomy. (a) Immunohistochemical staining of Ki67. Bar: 100 μm. Arrows: Ki67$^+$ cells in acini, arrowheads: Ki67$^+$ cells in ducts. (b) Statistical analysis of the counts for Ki67$^+$ cells. (c) Western blot of sex-determining region Y-box 2 (SOX2) and octamer-binding transcription factor 4 (OCT4). Data are expressed as the mean ± *SEM*. $^*p < 0.05$ and $^{**}p < 0.01$. $n = 6$ per group. Paired two-way ANOVA and Bonferroni multiple comparisons were used for (b), and paired Student's *t* test was used for (c). ANOVA: analysis of variance; CL: contralateral; DN: denervated; *SEM*: standard error of the mean [Color figure can be viewed at wileyonlinelibrary.com]

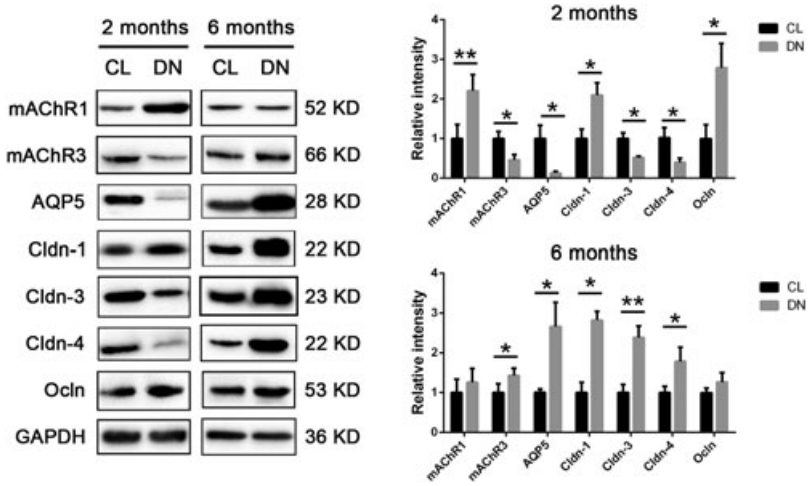

FIGURE 3 The expression of key proteins in transepithelial fluid transport after parasympathectomy. Data are expressed as the mean ± *SEM*. $^*p < 0.05$ and $^{**}p < 0.01$. $n = 6$ per group. Paired Student's *t* test was used. AQP5: aquaporin 5; CL: contralateral; Cldn: claudin; DN: denervated; Ocln: occludin; mAChR:muscarinic acetylcholine receptor; *SEM:* standard error of the mean

diminished, especially in the apicolateral region, in the 2-month denervated glands. In the 6-month denervated glands, AQP5 staining intensity was greater than that in the control glands and redistributed to the apicolateral membrane. Claudin-1, claudin-3, and claudin-4 were localized to the cell membrane of acinar cells in control glands. In comparison, claudin-1 staining was increased and diffused to the cytoplasm of the acinar cells, whereas claudin-3 and claudin-4 membrane staining was remarkably reduced in the 2-month denervated glands. Then, these claudins redistributed to the cell membrane with higher fluorescence intensity at 6 months after denervation. Occludin was located in the apicolateral membrane of acinar cells both in the control and denervated glands throughout the experimental period (Figure 4).

3.3 | The reinnervation of SMGs after parasympathectomy in the long term

PGP9.5 is a neuronal marker that is abundantly present in all neurons (Doran, Jackson, Kynoch, & Thompson, 1983). GAP43 is restricted to nonmyelinating Schwann cells and nonmyelinated axons in unlesioned adult nerves (Scherer et al., 1994). Autonomic nerve axons were double stained by PGP9.5 and GAP43 to evaluate autonomic nerve reinnervation after parasympathectomy. Immunostaining showed that ganglionic cells existed both in the stromal area of the control and denervated glands throughout the entire experimental period (Figure 5a). The axon density in the gland parenchyma was greatly reduced at 2 months (from 4.3% to 0.8%, $p < 0.01$), while it recovered to the control level at 6 months after operation (Figure 5b,c).

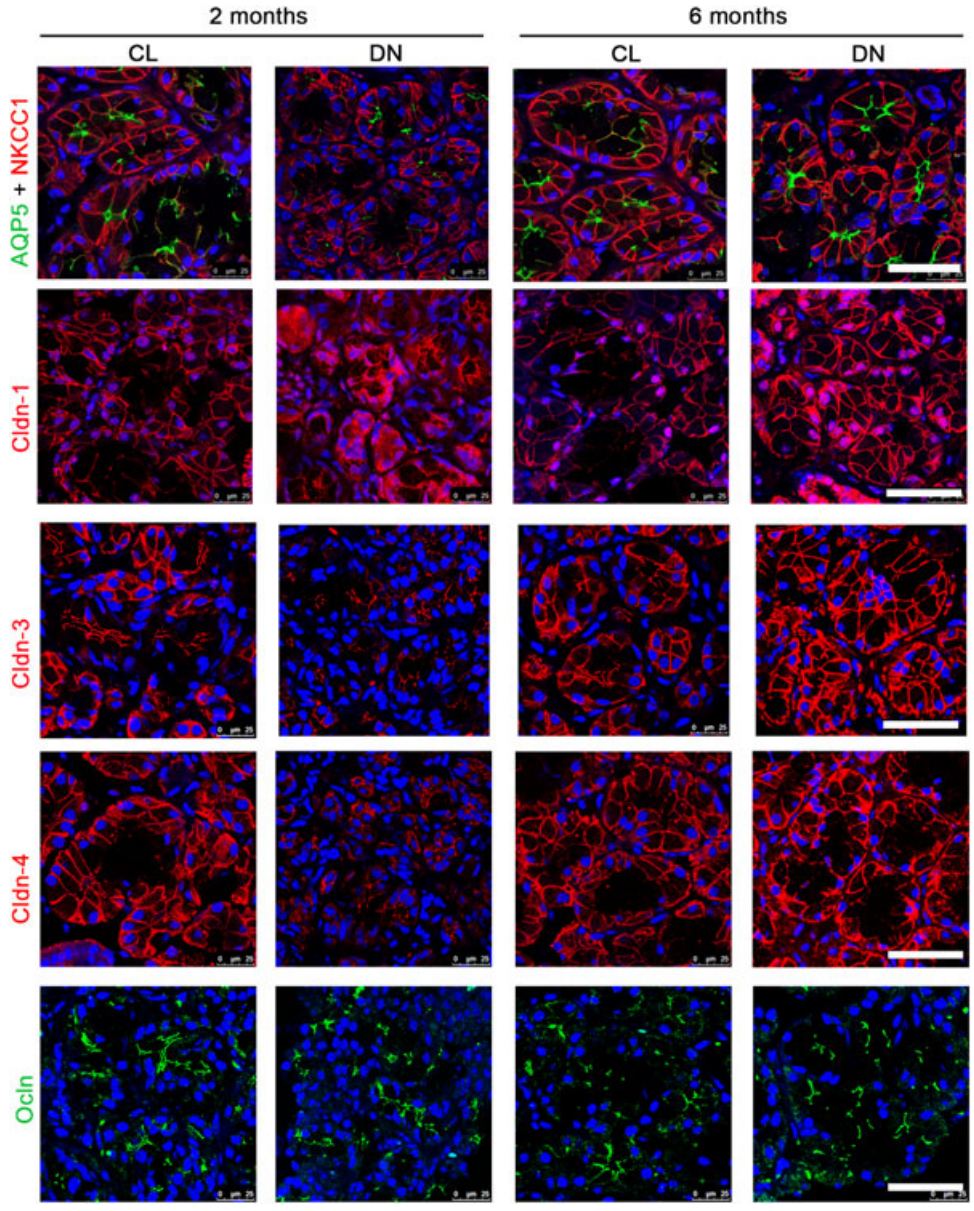

FIGURE 4 The localization of key proteins in transepithelial fluid transport after parasympathectomy. AQP5 (green) and NKCC1 (red) were double labeled. Bars: 50 μm. AQP5: aquaporin 5; CL: contralateral; Cldn: claudin; DN: denervated; Ocln: occludin; NKCC1: Na-K-Cl cotransporter [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

Maintenance of salivation is dependent on the efferent signals delivered to salivary glands in autonomic nerves. Parasympathectomy has been used to explore the role of parasympathetic nerves in regulating salivation and leads to temporary hypersecretion followed by glandular atrophy within 1 month (Garrett, 1987). However, the long-term effect (over 6 months) of parasympathetic denervation on salivary secretion remains unclear. Here, we performed parasympathectomy of SMGs in minipigs and found that the resting flow rate was doubled at 6 months after the operation, which verified that the resting secretion of SMGs could be improved after parasympathectomy in the long term, and thus may contribute a novel mechanism to regulate fluid secretion.

Fluid is secreted by salivary epithelia through transcellular and paracellular fluid transport pathways, which are mainly evoked by cholinergic stimulation. Studies have shown that mAChR3 activation induces AQP5 trafficking in parotid acinar cells (Ishikawa et al., 1998), and administration of cevimeline, a selective mAChR3 agonist, can rescue the degradation of AQP5 in rat SMGs after parasympathetic denervation (Li et al., 2008). These results suggest that mAChR3 plays an important role in modulating the function and quantity of AQP5. Using minipigs, we found that the protein levels of mAChR3 and AQP5 decreased at 2 months and increased at 6 months after parasympathectomy compared with those in control glands. The decrease and increase in the protein levels of mAChR3 and AQP5 were closely related to the changes in resting salivation after denervation, suggesting

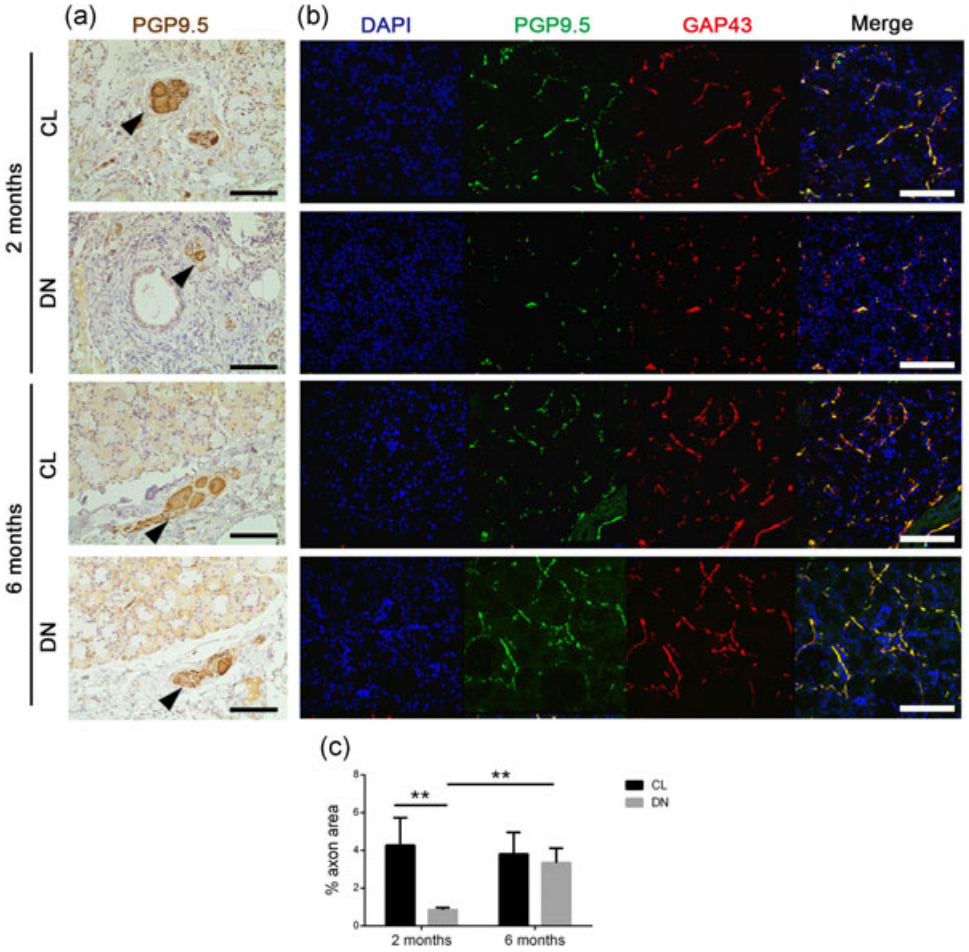

FIGURE 5 Reinnervation of the submandibular gland after parasympathectomy. (a) Ganglionic cells (arrowheads) were labeled with protein gene product 9.5 (PGP9.5) by immunochemistry. (b) Autonomic nerve axons were double stained with PGP9.5 and growth-associated protein 43 (GAP43). Bars: 100 μm. (c) The autonomic nerve axon density was presented as the ratio of the axon area to total area in the image (%). Data are expressed as the mean ± *SEM*. $^{**}p < 0.01$, $n = 6$ per group. Paired two-way ANOVA and Bonferroni multiple comparisons were used. ANOVA: analysis of variance; CL: contralateral; DN: denervated; *SEM*: standard error of the mean [Color figure can be viewed at wileyonlinelibrary.com]

that the transcellular pathway was inhibited in the short term and activated in the long term after parasympathectomy.

Tight junctions form the primary epithelial barrier and maintain the selective transepithelial ion gradients needed for salivary secretion. Overexpression of claudin-1 in MDCK T23 cells increases the permeability to 4-kDa fluorescein isothiocyanate (FITC)-dextran (McCarthy et al., 2000). Increasing expression of claudin-3 in rat alveolar cells enhances calcein and Texas Red dextran permeability (Mitchell, Overgaard, Ward, Margulies, & Koval, 2011). Cholinergic stimulation can temporarily modulate epithelial paracellular permeability by decreasing the distribution of claudin-4 in the membrane and promoting its internalization (Cong et al., 2015). Our results showed that the protein contents and membrane distribution of claudin-1, claudin-3, and claudin-4 were all increased at 6 months after parasympathectomy when resting salivary secretion was increased. Taken together, the results demonstrated that the enhanced transepithelial fluid transport capacity characterized by the upregulated expression, and redistribution of AQP5 and tight junction proteins might be involved in the promotion of resting salivary secretion after parasympathectomy in the long term.

Clinically, chronic inflammation of salivary glands is a common cause for impairment of saliva flow, and sialolithiasis is the main trigger of inflammation. When the sialolith is removed, the gland will restore its secretory function soon (Ruiz, Brygo, Nicot, & Ferri, 2018). The altered secretory mechanisms of chronic inflammation of SMG and the acute denervation of peripheral nerve due to parasympathectomy are different, while the recovery procedure has something in common. Ductal ligation is experimentally used to induce SMG inflammation (Silver et al., 2008). Studies show that ligating the major excretory duct of the salivary glands led to acinar cell apoptosis and gland dysfunction, accompanied by the proliferation of ductal cells (Cotroneo, Proctor, Paterson, & Carpenter, 2008; Takahashi et al., 2004). The initial functional ablation could be rescued after deligation through proliferation and differentiation of these ductal cells, which are presumed to be stem/progenitor cells (Pringle, Van Os, & Coppes, 2013; Vissink, van

Luijk, Langendijk, & Coppes, 2015). Two-month ligation of the main excretory duct in mice increases the expression of the stem cell marker c-kit, which decreases once the duct is deligated, whereas the expression of the acinar cell precursor gene keratin 5 increases steadily over the 2 months after deligation (Watanabe, Takahashi, Hata-Kawakami, & Tanaka, 2017). Previously, acetylcholine signaling was demonstrated to increase epithelial morphogenesis and proliferation of keratin 5^+ progenitor cells via mAChR1 signaling during SMG development (Knox et al., 2010). Here, we observed that SMGs experienced a retrogression and regeneration process after parasympathetic denervation. The number of $Ki67^+$ cells was remarkably increased at 2 months after denervation, especially in the duct system where salivary stem/progenitor cells exist and restored to the control level at 6 months after denervation. Coincidently, the protein levels of mAChR1, stem cell marker SOX2, and OCT4 were significantly increased at 2 months and recovered to normal at 6 months after denervation. These findings indicated that SMGs were potentially able to regenerate after parasympathetic denervation through stem cell proliferation and differentiation, and mAChR1 might be related to the regeneration process.

The interactions between nerves and salivary glands are considered essential in glandular development and regeneration after injury (Ferreira & Hoffman, 2013; Hai et al., 2014; Knox et al., 2013, 2010; Nedvetsky et al., 2014). Autonomic reinnervation is observed 4–6 months after SMG transplantation in humans (Jacobsen et al., 2008; X. Zhang, Yang et al., 2018) and 3 months in rabbits (X. Zhang, Yang et al., 2018). The parasympathetic axon density has been demonstrated to correlate with the acinar area and secretory function of transplanted SMGs (X. Zhang, Yang et al., 2018). In the current study, we found that although the axon density was decreased, the gland structure and secretory function disordered at 2 months after denervation and the number of $Ki67^+$ cells and the expression of transcription factors of stem cells were apparently increased, suggesting that the regeneration process had already begun at 2 months after denervation. At 6 months after denervation, autonomic axon density had recovered to the control level, the impaired gland structure was greatly relieved, and the resting salivation was doubled. Meanwhile, stimulated salivation had recovered, which has also been observed in some patients who underwent SMG transplantation (Liu, Li, Su, Xie, & Yu, 2015). These results suggest that reinnervation contributes, at least in part, to the regeneration process and the response to citric acid in long-term recovery after parasympathectomy. Thus, the origin of the reinnervated nerves, for example, the sympathetic nerves or the parasympathetic fibers from the lingual nerve, needs to be determined in the future.

In summary, we demonstrate that the resting saliva secretion of porcine SMG is increased after parasympathectomy in the long term and that the possible mechanism involves the proliferation of ductal cells and improved transepithelial fluid transport. This finding suggests that parasympathectomy might provide a potential novel way to improve the resting secretion of SMGs. We will focus our further study on the effect of parasympathectomy of SMG on the prevention or alleviation of radiation-induced xerostomia in the future.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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