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ABSTRACT

Autophagy is a catabolic pathway utilized to maintain a balance among the synthesis, degradation, and recycling of cellular components, thereby playing a role in cell growth, development, and homeostasis. Previous studies revealed that a conditional knockout of essential member(s) of autophagy in a variety of tissues causes changes in structure and function of these tissues. Acinar cell-specific expression of knocked-in Cre recombinase through control of *aquaporin 5* (*Aqp5*) promoter/enhancer (*Aqp5-Cre*) allows us to specifically inactivate Atg5, a protein necessary for autophagy, in salivary acinar cells of *Atg5^{fl};Aqp5-Cre* mice. There was no difference in apoptotic or proliferation levels in salivary glands of *Atg5/Cre* mice from each genotype. However, H&E staining and electron microscopy studies revealed modestly enlarged acinar cells and accumulated secretory granules in salivary glands of *Atg5^{fl};Aqp5-Cre* mice. Salivary flow rates and amylase contents of *Atg5/Cre* mice indicated that acinar-specific inactivation of ATG5 did not alter carbachol-evoked saliva and amylase secretion. Conversely, autophagy intersected with salivary morphological and secretory manifestations induced by isoproterenol administration. These results identified a role for autophagy as a homeostasis control in salivary glands. Collectively, *Atg5^{fl};Aqp5-Cre* mice would be a useful tool to enhance our understanding of autophagy in adaptive responses following targeted head and neck radiation or Sjögren syndrome.

KEY WORDS: salivary gland, apoptosis, animal models, isoproterenol, proliferation.

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Deletion of ATG5 Shows a Role of Autophagy in Salivary Homeostatic Control

INTRODUCTION

Macroautophagy (hereafter referred to as autophagy) is a catabolic pathway that is required for cellular homeostasis. It is induced during metabolic stress and is necessary to provide energy to cells under these conditions (Glick *et al.*, 2010). Autophagy also works to remove misfolded proteins or damaged organelles from intracellular compartments (Glick *et al.*, 2010). Analysis of available data suggests that autophagy is necessary to maintain cellular homeostasis and increase survival capacity of stressed cells (Mathew *et al.*, 2007).

ATG5 plays a key role in the autophagic process by binding to ATG12 and inducing curvature of the phagophore. ATG7 acts like an E1 ubiquitin-activating enzyme. A non-canonical pathway of autophagy has been identified *in vitro* by demonstrating the formation of autophagosomes and autolysosomes in *Atg5^{-/-}* and *Atg7^{-/-}* MEFs (Nishida *et al.*, 2009). However, *Atg5^{-/-}* mice do not survive the neonatal starvation period, showing that this ATG5-independent pathway does not provide sufficient nutrients for survival *in vivo*. This ATG5/ATG7-independent pathway has not been definitively described *in vivo*.

Autophagy has a 'double-edged sword' effect and has been shown to play a role in the prevention and pathogenesis of a variety of diseases, including Alzheimer's, Parkinson's, diabetes mellitus, and cancer. It has been shown that activation of autophagy occurs with ligation-induced atrophy (Silver *et al.*, 2010), showing its potential role in salivary gland disease states. The efficiency of autophagy and its ability to degrade damaged proteins and organelles slows as an organism ages. Longevity pathways such as FoxO3 have been shown to be positive regulators of autophagy. Inhibitors of autophagy such as mTOR and NF-κB have been shown to expedite the aging process (Bonawitz *et al.*, 2007).

Previous studies utilizing conditional knockouts of autophagy in a variety of tissues have found developmental and/or functional manifestations in targeted tissues when compared with those in wild-type mice. Mice with a conditional knockout of *Atg5* in cardiomyocytes suffer from functional and structural changes in cardiac tissue, including cardiac hypertrophy, left ventricular dilation, and

systolic dysfunction, when compared with wild-type mice (Nakai *et al.*, 2007). Mice with a conditional knockout of *Atg5* in skeletal muscle have severe muscle-wasting, profound kyphosis, and growth retardation (Raben *et al.*, 2008). A conditional knockout of *Atg7* in β -cells of the pancreas had decreased β -cell mass and pancreatic insulin content due to increased apoptosis and decreased proliferation (Jung *et al.*, 2008). Mice with a conditional knockout of *Atg7* in adipose tissue have decreased white adipose tissue mass, altered fatty acid metabolism, and increased insulin sensitivity (Zhang *et al.*, 2009).

The role of autophagy in salivary gland development and homeostatic control has not been addressed, partly because of the lack of proper animal models. We therefore developed a *Atg5^{fl};Aqp5-Cre* mouse model by crossing *Aqp5-Cre* mice (Flodby *et al.*, 2010) with *Atg5^{fl}* mice (Hara T 2006). AQP5 is the primary aquaporin water channel protein and is localized to the apical membranes of acinar cells but not ductal cells. In these mice, the Cre recombinase is knocked-in to endogenous *aquaporin 5* (*Aqp5*) context, thereby preferentially expressed in salivary acinar cells to inactivate *Atg5* in these *Atg5^{fl};Aqp5-Cre* mice. In the present study, we examined the effects of conditional *Atg5* knockout in regulating post-natal salivary gland development and function.

MATERIALS & METHODS

Production of *Atg5^{fl};Aqp5-Cre* Mice

The *Aqp5-Cre* mouse line contains recombinase Cre knocked-in to exon 1 of one copy of endogenous *Aqp5* gene (Flodby *et al.*, 2010). The *Aqp5-Cre* mouse line was crossed with the mouse line containing floxed *Atg5* gene (*Atg5^{fl}*) (Hara *et al.*, 2006) to create *Atg5^{fl};Aqp5-Cre* and *Atg5^{+/+};Aqp5-Cre*. The *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{fl}*, *Atg5^{fl};Aqp5-Cre*, *Atg5^{fl}*, and *Atg5^{fl};Aqp5-Cre* genotypes were screened by PCR using tail DNA. Primer pairs purchased from IDT (San Jose, CA, USA) were used, one for *Atg5* forward 5'-AAGCACCTAGTCACACCA CAT-3', *Atg5* reverse 5'-CACGTGTGAGTGATGGTTGGC-3' and one for *Cre* recombinase forward 5'-TGCCCAAGAAGAA GAGGAAGGTGT-3', *Cre* recombinase reverse 5'-GCCGCATA ACCAGTGAACAGCAT-3'.

Histology

Salivary gland tissues were fixed in formalin, followed by 70% ethanol, then embedded in paraffin and sectioned (4 μ m). Serial tissue sectioning and hematoxylin and eosin (H&E) staining were performed by the Histology Service Laboratory in the UA Department of Cellular and Molecular Medicine or the COH Pathology Laboratory.

Quantification of Caspase-3 Activation and PCNA Levels

Serial sectioned slides were stained for activated caspase-3 or Proliferating Cellular Nuclear Antigen (PCNA) as previously described (Martin *et al.*, 2012). A Leica DM5500 and 4 megapixel Pursuit camera were used to capture images.

Saliva Collection and Composition

Mice were injected i.p. with carbachol (0.25 mg/kg body weight) and collected as previously described (Limesand *et al.*, 2010). Total protein composition of saliva was determined by use of the Bio-Rad Experion System (Bio-Rad, Hercules, CA, USA) as previously described (Grundmann *et al.*, 2010).

Immunofluorescence Microscopy

Frozen sublingual gland (SLG) tissue sections from *Atg5^{fl};Aqp5-Cre* and *Atg5^{+/+};Aqp5-Cre* mice were fixed for 10 min in cold acetone and blocked in 5% BSA-PBS for 20 min at r.t. The slides were reacted with primary antibody against mucin 5B (MUC5B) (clone Y-20, Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by Alexa Fluor 488-conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA). Actin filaments were stained with TRITC-conjugated phalloidin (Invitrogen), and nuclei were demarcated with DAPI (Invitrogen) staining. After being washed, the tissues were mounted in anti-fading solution and viewed with a Zeiss LSM700 laser-scanning confocal microscope image system.

Western Blot Analyses

Whole-cell lysates were prepared with sodium dodecylsulfate (SDS) lysis buffer, subjected to SDS-PAGE, and immunoblotted with antibodies against ATG5 (Novus Biologicals, Littleton, CO, USA), LC3 (NanoTools GmbH, Munich, Germany), p62/SQSTM1 (American Research Products, Waltham, MA, USA), and actin (Millipore, Billerica, MA, USA). Blots were visualized with a chemiluminescence detection kit (ECL-Plus, GE Healthcare) and a VersaDoc 5000 Imaging System (Bio-Rad). Western analyses shown are representative of 2-4 independent experiments.

Statistical Analysis

All statistical analyses were conducted by one-way analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test with InStat GraphPad 3 (San Diego, CA, USA).

RESULTS

No Significant Changes in Activated Caspase-3 and PCNA Levels in Salivary Glands of *Atg5^{fl};Aqp5-Cre* Mice

Previous studies have found that levels of apoptosis increase in certain tissues with a conditional knockout of autophagy (Hou *et al.*, 2010). We sought to determine if our conditional knockout caused similar changes in cell turnover events. Activated caspase-3 was quantified in parotid (PAGs) and submandibular glands (SMGs) of all mouse genotypes. There was no statistically significant difference ($p > .05$) in levels of apoptosis between genotypes (Figs. 1A, 1B), as confirmed with TUNEL staining (Appendix Figs. 2A, 2B). We found that salivary acinar and ductal proliferation levels, reflected by PCNA levels, were

Figure 1. Conditional inactivation of ATG5 fails to affect salivary apoptosis and proliferation. (A-F) Salivary gland tissues were collected from four- to six-week-old naïve *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{+/+}*, and *Atg5^{+/+};Aqp5-Cre* mice, formalin-fixed and paraffin-embedded. Percentages of positive cells were determined from the means of 3 to 5 images *per mouse*. (A) Quantification of positive cleaved caspase-3 staining of naïve PAGs. (B) Positive cleaved caspase-3 staining of naïve SMGs. (C) Positive PCNA staining of naïve PAG acinar cells. (D) Positive PCNA staining of naïve PAG ductal cells. (E) Positive PCNA staining of naïve SMG acinar cells. (F) Positive PCNA staining of untreated SMG ductal cells. (A-F) $p > .05$; $n \geq 4$ *per genotype*. (G) Representative images of cleaved caspase-3 staining. (H) Representative images of positive PCNA staining. Scale bar: 50 μ m.

not statistically significantly different ($p > .05$) between genotypes of *Cre/Atg5* mice (Figs. 1C-1F), as confirmed with BrdU staining and immunoblotting for anti-pAKT and anti-cyclin B1 (Appendix Figs. 2C-2E). These results suggest that a conditional knockout of autophagy in the salivary glands does not affect apoptotic or proliferation rates in salivary acinar or ductal cells.

Conditional Inactivation of ATG5 Results in Salivary Acinar Morphological and Structural Manifestations

Previous conditional knockouts of autophagy have shown changes in tissue structure (Komatsu *et al.*, 2005). H&E-stained slides and EM studies were used to determine if structural changes occurred in salivary glands of conditional knockout mice. H&E-stained sections from each genotype at 4 wks of age showed no gross differences in tissue structure in PAGs, SLGs, or SMGs between genotypes (Fig. 2A). However, detailed examination (TEM) revealed that serous acinar cells in PAGs and mucous acinar cells in SLGs of male *Atg5^{+/+};Aqp5-Cre* mice are modestly hypertrophic compared with those of *Atg5^{+/+}* mice. In SLGs of male *Atg5^{+/+}* mice, the ducts appeared to include some granular cells (Fig. 2A). TEM images of PAG acinar cells show an apparent increase in the number of serous secretory granules in *Atg5^{+/+};Aqp5-Cre* mice (Fig. 2B). *Atg5^{+/+}* granules

look typical for mouse PAGs, with a dark peripheral ring and one or more dark areas in the granules (Fig. 2B, upper panels). In contrast, PAG acinar granules from *Atg5^{+/+};Aqp5-Cre* mice had a more homogeneous content. TEM images of *Atg5^{+/+};Aqp5-Cre* mice SLGs show mucous acinar cells packed with increased granules of various sizes (Fig. 2B, lower panels). SLG mucous and serous granules tended to be relatively homogeneous in *Atg5^{+/+}* mice.

To validate that SLG mucous acinar cells were packed with more mucous granules in *Atg5^{+/+};Aqp5-Cre* mice than in *Atg5^{+/+};Aqp5-Cre* cells, we performed immunofluorescence microscopy with anti-MUC5B. As shown in Fig. 2C, more MUC5B signals (green, right panel) were observed in mucous acinar cells of *Atg5^{+/+};Aqp5-Cre* mice. It also appears that more nuclei (blue, left panel; Fig. 2C) *per field* could be visualized in *Atg5^{+/+};Aqp5-Cre* mice, implying that SLG cells were relatively smaller.

Atg5 Inactivation-induced Acinar Hypertrophy Persists through the Aging Process

We compared the morphology of salivary glands during age progression from 6- to 18-month-old mice to determine if autophagy deficiency affected salivary gland structure.

Figure 2. Conditional knockout of *Atg5* causes enlarged salivary acinar cells. **(A)** Salivary gland tissues were collected from four- to six-week-old naïve *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{Δ/+}*, *Atg5^{Δ/+};Aqp5-Cre*, *Atg5^{Δ/Δ}* and *Atg5^{Δ/Δ};Aqp5-Cre* mice, formalin-fixed and paraffin-embedded for H&E studies. **(B)** Dissected PAGs and SLGs from *Atg5^{Δ/Δ}* and *Atg5^{Δ/Δ};Aqp5-Cre* mice were fixed in glutaraldehyde and analyzed by transmission electron microscopy (TEM). Two representative TEM images with different magnifications (1100x and 2700x) from each gland are shown. **(C)** Frozen sections of dissected SLGs from *Atg5^{Δ/+};Aqp5-Cre* and *Atg5^{Δ/+};Aqp5-Cre* mice were stained with an anti-MUC5B antibody and visualized with an Alexa Fluor 488-conjugated secondary antibody. F-actin and nuclei were respectively stained with TRITC-conjugated phalloidin and DAPI. Scale bar: 20 μm. **(D)** Salivary glands of naïve male mice aged to 6 mos (upper two panels) and 18 mos (lower two panels) were analyzed for changes in structure using H&E slides. Magnification: 40x. Arrows indicate hypertrophic cells. Scale bar: 50 μm.

Consistent with their young (Fig. 2A) counterparts, serous acini in PAGs and mucous acini in SLGs were modestly enlarged in six-month-old autophagy-deficient mice (indicated by white

arrowheads) compared with their age-matched control mice (Fig. 2D). Moreover, H&E staining revealed less basophilic, more pinkish PAG serous acini and whitish SLG mucous acini in *Atg5^{ff};Aqp5-Cre* mice (Fig. 2D). The histological difference between these genotypes in mixed serous/mucous acini of SMGs was less pronounced. As aging progressed, decreased basophilic staining and hypertrophy in acinar cells were more notable in PAG and SLG salivary glands of 18-month-old *Atg5^{ff};Aqp5-Cre* mice (Fig. 2D, indicated by white arrowheads). While this hypertrophy and histological change observed in acinar cells may, at least partly, reflect the defective autophagy-mediated clearance of aggregates, the exact nature of this change remains to be further investigated.

Conditional Knockout of *Atg5* Does Not Alter Carbachol-induced Amylase Secretion

Mice with conditional knockouts of autophagy suffer from a change in tissue function (Jung *et al.*, 2008). Carbachol-stimulated saliva was collected, and these normalized salivary flow rates showed that there was no significant difference ($p > .05$) between genotypes in both genders (Fig. 3A). Saliva was analyzed for total protein composition for every genotype (Fig. 3B), and no statistically significant differences in the percentage of amylase were detected (Fig. 3C; $p > .05$).

Compromised Autophagy Intersects with Isoproterenol (Ipr)-increased Acinar Cell Hypertrophy and Ipr-activated Secretion

We investigated whether ATG5 affects isoproterenol-induced adaptive response and secretion. While chronic daily administration of Ipr, a β -adrenergic receptor agonist, is known to induce salivary gland hypertrophy (Ann *et al.*, 1997), acute administration of Ipr activates salivary protein secretion (Nishiyama *et al.*, 2007). H&E images showed that there was no apparent change in gross salivary gland structure

between vehicle- and Ipr-injected *Atg5^{f/f}* or *Atg5^{f/f};Aqp5-Cre* mice (Fig. 4A). However, a greater extent of acinar hypertrophy of both PAGs and SMGs of Ipr-treated *Atg5^{f/f}; Aqp5-Cre* mice

was observed (Fig. 4A). Chronic Ipr treatment of *Atg5^{fl/+};Aqp5-Cre* (WT) mice caused a decrease in LC3-II, while p62/SQSTM1 and ATG5 protein levels remained unchanged in the PAGs and SMGs (Fig. 4B). In contrast, the p62/SQSTM1 level was increased, and the conversion of LC3-I to LC3-II was impaired (indicators of lack of autophagy activation; Klionsky *et al.*, 2012), in the PAGs and SMGs of *Atg5^{fl/-};Aqp5-Cre* mice. Therefore, whether autophagic flux is regulated by chronic Ipr treatment is inconclusive.

We used TEM to examine the ultrastructural changes of PAG and SLG acinar cells harvested 30 min post-Ipr administration from *Atg5^{fl/fl}* and *Atg5^{fl/+};Aqp5-Cre* mice (Appendix Fig. 1). Compromised autophagy reduced the Ipr-induced secretory granule numbers in both PAGs (63% decrease for *Atg5^{fl/fl}* mice vs. 23% decrease for *Atg5^{fl/+};Aqp5-Cre* mice) and SLGs (90% decrease for *Atg5^{fl/fl}* mice vs. 67% decrease for *Atg5^{fl/+};Aqp5-Cre* mice) (Fig. 4C). Analysis of these data suggests that ATG5-dependent autophagy could play a critical role in regulating β -receptor-mediated protein secretion.

DISCUSSION

Our results demonstrate that autophagy plays important roles in the maintenance of the salivary acinar cell mass by regulating, at least in part, the sizes and numbers of their secretory granules. Based on previous conditional knockouts of models of autophagy in a variety of tissues (Nakai *et al.*, 2007; Raben *et al.*, 2008), it is clear that autophagy is involved in diverse physiological and pathological processes with immense relevance in human development, diseases, and aging. Autophagy is characterized as a fundamental eukaryotic process with multiple cytoplasmic homeostatic roles through its catabolic, lysosomal-degradative process (Mizushima *et al.*, 2011); our findings expanded these roles to governing salivary cell mass homeostasis through regulating acinar cell secretion.

Our results further suggest that autophagy intersects with Ipr-stimulated, but not carbachol-activated, secretion. Carbachol was utilized to stimulate saliva production in this study, since Ipr has been shown to regulate autophagy in certain tissues (Aránguiz-Urroz *et al.*, 2011). The results of accumulated

Figure 3. Inactivation of *Atg5* in salivary acinar cells does not affect carbachol-stimulated salivary gland function. Stimulated salivary flow rates were normalized to *Atg5^{+/+}* mice for their respective gender. **(A)** Carbachol-stimulated saliva was collected from four- to six-week-old female (upper panel) and male (lower panel) *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{fl/+}*, *Atg5^{fl/+};Aqp5-Cre*, *Atg5^{fl/-}* and *Atg5^{fl/-};Aqp5-Cre* mice. $p > .01$; $n \geq 10$ per genotype. **(B)** Representative image of ExperiChip analyses of saliva collected from four- to six-week-old carbachol-treated female and male *Atg5/Cre* mice with 6 different genotypes and analyzed for percentage amylase determined by the 54- to 56-kD band. $n \geq 10$ per genotype. **(C)** The amylase levels from stimulated saliva from female (left panel) and male (right panel) *Atg5^{+/+}*, *Atg5^{+/+};Aqp5-Cre*, *Atg5^{fl/+}*, *Atg5^{fl/+};Aqp5-Cre*, *Atg5^{fl/-}* and *Atg5^{fl/-};Aqp5-Cre* mice (one representative profile is shown in **B**) were summarized. $p > .01$; $n \geq 10$ per genotype.

MUC5B in SLGs (Fig. 2C) and more secretory granules detected in both PAGs and SLGs of naïve and Ipr-injected *Atg5^{fl/+};Aqp5-Cre* mice (Fig. 4C) suggested the possibility that autophagy is involved in the regulation of mucous and/or serous granule biogenesis and secretion. These results are consistent with those from the study by Masedunskas *et al.* (2011), who demonstrated that exocytosis was induced by Ipr, but not carbachol, through collapsing of secretory granules with apical plasma membrane. However, additional experiments are needed to elucidate the

Figure 4. Autophagy intersects with Ipr-triggered morphological and functional manifestations. **(A)** Four- to six-month-old male mice were given daily injections (i.p.) of Ipr or vehicle for 10 consecutive days. Salivary tissues were collected and sections stained for hematoxylin and eosin (H&E) on the day following the last injection: representative H&E of PAGs, SMGs, and SLGs from untreated *Atg5^{fl/fl}* mice (top panels); representative H&E of PAGs, SMGs, and SLG from Ipr-injected *Atg5^{fl/fl}* mice (2nd-row panels); representative H&E of PAGs, SMGs, and SLGs from untreated *Atg5^{fl/fl};Aqp5-Cre* mice (3rd-row panels); and representative H&E of PAGs, SMGs, and SLGs from Ipr-injected *Atg5^{fl/fl};Aqp5-Cre* mice (bottom panels). Scale bar: 50 μ m. **(B)** The effect of chronic Ipr administration on salivary glands of autophagy-deficient mice. *Atg5^{-/-};Aqp5-Cre* (KO) and *Atg5^{fl/+};Aqp5-Cre* (WT) mice were subjected to Ipr injections for 10 days as in (A). Western blot analyses of PAG and SMG tissue lysates were performed with the indicated primary antibodies. NT; no treatment control. The underlying cause for the abnormal migration of ATG5-ATG12 and p62/SQSTM1 in PAGs of Ipr-injected mice, albeit reproducible, was unclear. **(C)** PAGs and SLGs were harvested from vehicle- and Ipr-injected *Atg5^{fl/fl};Aqp5-Cre* (*Atg5^{-/-}*) and *Atg5^{fl/fl}* mice at 30 min post-administration. Dissected tissues were examined by TEM. Quantification of secretory granules in PAGs and SMGs per field of TEM images (magnification 1,100 $\times$; n = 25; one representative image is shown in Appendix Fig. 1) of vehicle- and Ipr-injected *Atg5^{fl/fl};Aqp5-Cre* (*Atg5^{-/-}*) and *Atg5^{fl/fl}* mice is shown as mean $\pm$ SD.

molecular mechanisms underlying the role of autophagy in acute Ipr-induced secretion and chronic Ipr-induced adaptive responses.

Emerging evidence has uncovered numerous intersections of autophagy with conventional, unconventional, and regulated secretion of biologically active cargo (Deretic *et al.*, 2012), supporting our notion. For example, the biogenesis of several specialized tissues is notably influenced by alterations of members of the autophagic machinery, including bone remodeling by osteoclasts (DeSelm *et al.*, 2011), melanosome maturation in skin melanocytes and retinal pigment epithelial cells (Ganesan *et al.*, 2008), and secretion of inflammatory mediators. Autophagy also directly or indirectly diminished insulin secretion in pancreatic β -cells by either reduced numbers of insulin granules or lack of compensatory increase in β -cell mass (Jung *et al.*, 2008). This is contrary to our observations that ATG5 inactivation led to increased acinar cell mass and accumulated baseline secretory granules as well as reduced Ipr-stimulated secretion. It has been suggested that autophagy could entail a highly specialized step in the maturation of secretory organelles and maintenance of their integrity, since the Golgi apparatus is involved in both autophagy and secretory granule biogenesis (Deretic *et al.*, 2012). However, analysis of our data suggests that compromised autophagy could be involved in the retention of salivary secretory granules. Moreover, the molecular mechanisms underlying the differential involvement of autophagy in Ipr-stimulated and carbachol-activated salivary secretion remain to be addressed.

Yousefi *et al.* (2006) have demonstrated that elevated ATG5 levels were associated with increased caspase-3 activity. We have shown here that the Cre/*Atg5* mice have no differences in apoptosis, proliferation, salivary flow rates, and saliva composition at baseline. It is still unclear whether autophagy plays a protective or harmful role in regulating salivary adaptive response to stress. *Atg5^{fl/fl};Aqp5-Cre* mice are a useful model to determine whether ATG5-dependent autophagy can be precisely modulated to prevent or alleviate salivary dysfunction associated with targeted head and neck radiation or Sjögren syndrome.

In summary, we speculate that the accumulation of ubiquitinated proteins, secretory granules, and dysfunctional organelles contributes to observed salivary acinar hypertrophic manifestations.

Autophagy could be a key homeostatic regulator for cellular organelles and proteins in salivary acinar cells.

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