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Review

Novel approaches for rescuing function of the salivary gland epithelium in primary Sjögren's syndrome

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ABSTRACT

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease characterised by dysfunction and inflammatory lymphocytic infiltration of exocrine glands, namely the salivary and lacrimal glands. pSS patients often suffer from sicca (dry mouth) complaints, including dental caries, and difficulties in eating, sleeping and speaking. A large body of literature points to a central role for salivary gland (SG) epithelial cells in the development of this pathology. Here we summarise recent studies concerning the role of SG epithelial cells in pSS, which strongly indicate their intrinsic activation and early involvement during the disease process. Based on that, we propose possible future interventions targeting SG epithelial cells, to treat SG dysfunction pSS.

Introduction

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease. Salivary and tear gland dysfunction with lymphocytic infiltration and autoantibody production, are among its common characteristics (1). A serious complication is lymphoma development, particularly mucosa-associated lymphoid tissue (MALT) lymphoma (2).

Salivary glands (SGs) and lacrimal glands are primary pSS targets. Ninetyfive percent of pSS patients present with symptoms of dry mouth and/or eyes (3, 4). The early phases of SG damage leading to hyposalivation in pSS remains poorly studied, most likely due to a mean 7-year delay between symptom presentation and pSS diagnosis (5). pSS treatment options are limited and traditionally focus on symptoms alleviation (*e.g.* using artificial saliva to alleviate dryness). In the last two decades, studies tried to interfere with the inflammatory process in pSS. However, all these measures show limited influence on SG function. An unmet clinical need therefore exists, in terms of understanding pSS SG pathology, and exploration of feasible strategies to rescue pSS patient SGs at various-stages of the disease (6).

The role of epithelium in pSS salivary gland dysfunction

The functional part of SG is epithelial parenchyma, responsible for producing and channeling saliva to the mouth (Fig. 1A, 1D). In pSS, glandular lymphocytic infiltration is a well-established feature, and the infiltrate is characteristically associated with striated ducts, forming periductal foci (Fig. 1B, 1E). In earlier stages of pSS, most of infiltrating cells are T cells (especially $CD4^+$ T cells) (7). As infiltration severity increases, more $Foxp3^+$ Tregs are detected, together with increasing B cells (7). When foci are present in the SG, lymphoepithelial lesions (LELs) can develop, whereby B cells invade and proliferate within the striated ducts, accompanied by the proliferation of basal ductal cells (Fig. 1C, 1F) (8). pSS lymphocytic infiltration is strongly associated with autoantibody positivity, but not with hyposalivation (9, 10), raising the question about which mechanisms exactly are responsible for loss of saliva production in pSS, and how we can best tackle this therapeutically.

In the context of pSS, *in vitro* cultured striated duct epithelial cells are often referred to as SG epithelial cells (SGECs), ignoring that fact that acinar cells, intercalated ducts, myoepithelial cells and excretory ducts are also epithelial in nature. For clarity of this manuscript, we will maintain this terminology when we refer to *in vitro* cultured epithelial cells.

SG epithelium acts as immunological

target in pSS. Enhanced apoptosis of salivary gland epithelium in pSS has been explored by many studies, and reviewed by several papers (11-13). Briefly, pro-apoptotic molecules (*e.g.* Fas and Bax) increase, and anti-apoptotic Bcl-2 decreases in human pSS SG epithelial cells, compared to healthy individuals and sicca controls (14-16). TUNEL assays shows elevated levels of apoptotic acinar and ductal epithelial cells in pSS patient SGs (17). Endoplasmic reticulum stress, resulting in autophagy and apoptosis, may lead to redistribution of Ro/SSA and La/SSB autoantigens, initially to the cell surface and finally in apoptotic blebs, as shown in *in vitro* cultures of pSS-SGECs (18). These autoantigens are upregulated in pSS SGECs and are regulated by TLR/IFN type I signalling (19). Notably, epithelial apoptosis in pSS can happen at an early stage (11, 20), supporting the early role of SG epithelium in pSS. Besides through apoptotic blebs, exosomes (small membrane vesicles), containing autoantigens, are also secreted by pSS SGECs (21, 22). Epithelial cells are not only an important source for the signature pSS autoantigens Ro/SSA and La/SSB, they also express MHC class I and II and T-cell co-stimulatory molecules (CD80/CD86), rendering them equipped to act as auto-antigen presenting cells (23-25). pSS SG epithelium produces a wide variety of pro-inflammatory cytokines and chemokines (IL-1, IL-6, TNF-α, type I and type III IFNs, CXCL3, CXCL10, etc.), which has been recently thoroughly reviewed (26-28). Noticeably, IL-6 and the co-stimulatory molecule ICOSL expressed by SGECs from pSS patients contribute to the induction of follicular helper T cells, which are critical for B cell activation and differentiation (29). IFNλs are more highly expressed in minor SG epithelium from pSS patients than non-SS controls (28). IFNλ stimulation of *in vitro* cultured SG epithelial cells can also dose-dependently induce the expression of B cell activating factor (BAFF) and CXCL10, suggesting a role of type III IFNs in development of SG pathology in pSS (28). Together, available evidence shows that the activated SG epithelial cells not only are an important immunological target but also exert critical immune functions involved in pSS pathogenesis by initiation and perpetuation of the inflammatory (auto-)immune response in SGs.

Salivary gland progenitor cells in pSS

As in other organs, progenitor cells in SG maintain glandular homeostasis through their self-renewal and differentiation. Several niches (basal striated duct (BSD), intercalated duct (ID), and acinar compartment) for salivary gland progenitor cells (SGPCs) have been suggested, mostly through lineage tracing studies in the mouse (Table I). SG ducts have been long suspected as a niche for SGPCs, with initial hierarchical theories, presuming that those in the BSDs differentiate into ID cells, and these cells further into acinar cells. Cytokeratin 14 (KRT14) in human SG labels the BSD and ID cells (Table I). More recent studies however suggested that distinct populations of SGPCs may exist, each with a responsibility for maintenance of a particular mature cell type. For example, $KRT14^+c\text{-}kit^-$ BSD cells generate only ductal cells, whereas $KRT14^+c\text{-}kit^+$ cells present in the IDs may generate not only ID cells but also acinar cells (30-34). Studies using irradiated SG, whereby functionality is also lost, show that all ductal cells and also acinar cell types are responsible for endogenous regeneration after this severe cell loss (30, 35, 36). SGPCs may be also present within the acinar cell population, where $Sox2^+$ acinar cells proliferate and restore the acinar compartment after radiation damage, or maintain its healthy conditions (36, 37).

Salivary gland progenitor cell senescence

Through the employment of organoid culture techniques, we discovered that pSS patient SGPCs displayed deficient self-renewal and differential abilities compared with controls (38). Moreover, pSS SGPCs exhibited shorter telomeres than normal, suggesting a replicative-stress induced senescence phenotype (38). Additionally, pSS-associated pro-inflammatory cytokines IFNα, TNF-α, and IL-6, promoted SGPC proliferation (38), indicative of their potential culpability in development of senescence. We also found increased $p16^+$ expression, a marker of senescence, in both the BSD progenitor cell niche and the total parotid gland epithelium (39). The proportion of $p16^+$ BSD cells correlated with pSS patient saliva production, lymphocytic infiltration, and SG ultrasound total score, implying that SGPCs senescence may be of critical importance in the pathogenesis of pSS. Senescent cells express and secrete pro-inflammatory cytokines (the so-called senescence associated secretory phenotype, SASP) playing a role in spreading senescence and promoting tissue inflammation (40). When the NF-κB innate immune signalling pathway was constitutively activated in $KRT14^+$ cells (thus comprising SGPCs) in the mouse, lymphocytic infiltration of the SGs was observed, implying again that activated epithelial cells in the SG progenitor cell niche play a central role in pSS SG pathology development (41).

Salivary gland progenitor cells and lymphoepithelial lesion formation

In pSS SGs, LELs formation can be observed, associated with the periductal foci. A LEL is the summation of B cell invaded striated ducts, accompanied by proliferation of both the B cells and basal striated duct cells (8). Considering that the basal striated duct (BSD) layer is a proposed SGPC niche, SGPCs may also be central to LEL development. The infiltrating B cells express FcRL4, a phenotype shared with MALT lymphomas (42). These $FcRL4^+$ B cells are chronically activated B cells that may secrete pro-inflammatory cytokines involved in epithelial proliferation (43). We speculate that neoplastic B cells of the MALT lymphoma likely arise from the epithelium associated B cells. Recent developments in this field have been reviewed by Verstappen and Pringle *et al*. (unpublished observations), to which the reader is referred for more detail.

Thus besides being target of the disease and its immunological functions, the regenerative capability of the SG is severely affected in pSS. For this rea-

son, realistic treatment options for SG hypofunction in pSS should place the SG epithelium as a central target.

Effect of existing drugs on the salivary gland epithelium

Currently, standard-of-care pSS treatment decisions are based on the evaluation of glandular dryness and systemic symptoms. Here we review the observed effects of existing non-biologic and biologic drugs on the SG epithelium, as far this has been evaluated in studies.

Non-biologic drugs used in treating pSS and their potential effects on the salivary gland epithelium

Non-biologic drugs are clinically used for treating pSS, either to stimulate saliva production or to suppress the (systemic) inflammatory responses, largely to inhibit systemic manifestations.

• *Muscarinic signalling agonists (pilocarpine, cevimeline)*

Muscarinic receptors type 1 and 3 (M1R/M3R) are major muscarinic acetylcholine receptors in SG acinar cells. The activation of M1R/M3R induces intracellular Ca^{2+} influx, and further stimulates K^+ and Cl^- channels, the Na^+-K^+-$2Cl^-$ cotransporter and Na^+/H^+ and $Cl^-/HCO3^-$ exchangers, finally initiates primary saliva secretion by acinar cells (44, 45). Pilocarpine is a muscarinic agonist, binds muscarinic receptors and increases intracellular Ca^{2+} levels (46). In pSS, a randomised controlled trial (RCT) study revealed that administration of pilocarpine improved the saliva flow significantly compared with placebo (47). Pilocarpine also partially restored reduced salivary proteins production of pSS patients (48). Whilst short-term stimulation with pilocarpine may increase SG acinar cell size, long-term administration may exhaust the secretion ability of SG, limiting its long-term applicability to pSS patients (49). Cevimeline, another muscarinic agonist, specifically activates epithelial Na^+/H^+ exchange, used clinically to treat hyposalivation (50). pSS patients taking cevimeline showed significantly improvement of dry mouth symptoms, and objectively increased saliva flow (51).

• *Anti-inflammatory drugs: glucocorticoids*

Glucocorticoids bind to glucocorticoid receptors which are nuclear receptors, widely expressed by many cell types, including lymphocytes (52). Their binding results in increased transcription of anti-inflammatory genes (*e.g.* IL-10, lipocortin-1). The anti-inflammatory proteins inhibit the expression of inflammatory genes (encoding for cytokines, enzymes and adhesion molecules). Glucocorticoids are widely used for chronic inflammatory autoimmune diseases (53). Six-weeks administration of glucocorticoids resulted in improved salivary flow in some pSS patients, however generally failed to improve the histological (*e.g.* focus score, the percentage of gland epithelium) or functional parameters of SGs (54). A nearly 4-year-prospective study followed 60 pSS patients found that the decreased of stimulated saliva was not improved by corticosteroids (55). Therefore, not surprisingly, the anti-inflammatory drug of glucocorticoids cannot significantly influence pSS patient SG function.

• *Immunosuppresive drugs: cyclosporin A, 6-mercaptopurine, azathioprine, leflunomide, hydroxycloroquine*

Cyclosporin A inhibits IL-2 activity of T cells, by interfering with calcineurin, required for IL-2 gene transcription (56, 57). Cyclosporin A treatment showed only limited and unclear benefits for sicca features and systemic manifestations of pSS (58). One study suggests that cyclosporin A down-regulated HLA-DR antigen expression on the labial minor SG epithelium, potentially limiting the immune response (59). The immunosuppressive drugs 6-mercaptopurine, and its derivative azathioprine, act as antagonists of purines, which are essential components of DNA, RNA and certain co-enzymes. They inhibits of the synthesis of proteins, DNA and RNA (60), and have both been employed for treating inflammatory bowel diseases (61, 62). When employed in pSS, no significant improvements in sicca symptoms and other manifestations were found (63). Notably, daily administration of 6-mercaptopurine for 2 weeks caused severe degenerative changes in rabbit SGs with disarranged acini, atrophied and shrunken striated ducts (64). Leflunomide inhibits pyrimidine biosynthesis, decreasing naïve and memory $CD4^+$ T cells, B cells proliferation and NF-kB activation (65, 66). Hydroxycholorquine interferes with antigen processing and blocks T cell activation (67). The combination treatment with leflunomide and hydroxychloroquine in pSS significantly increased the unstimulated and stimulate whole saliva production at certain time points compared with the placebo (68). The effect of single administration of hydroxychloroquine on pSS patient saliva flow vary from reports of both increased flow and no improvement (69, 70). Single leflunomide treatment did not increase the parotid saliva flow in a placebo-controlled study (71).

Biologic drugs used in treating pSS

Various biologicals interfering with critical steps in different pathogenic immunoregulatory pathways have been used or are currently being tested in clinical trials to treat pSS patients (72). We summarise some biologic drugs used in trials with examining their effects on SG function/histopathology in Table II, and describe some of them briefly below.

• *Rituximab*

Rituximab is a monoclonal antibody targeting CD20 on B cells. CD20 is involved in the growth regulation of B lymphocytes following activation. Rituximab treatment in pSS patients depleted peripheral blood B cells and reduced SG B lymphocytes numbers. In patients with residual SG function, saliva production could be rescued, but this important clinical outcome was not consistent between various studies (73). Besides reducing B cell numbers in SGs, and as a consequence also smaller infiltrates, rituximab treatment also decreased LEL number and severity in pSS parotid gland (74, 75). Also acinar cell proliferation was reduced after treatment (75, 76). In a study of co-culturing syngeneic SGECs and B cells from pSS patients found rituximab could interfere with the Raf-1/ERK1/2 pathway and decreased cytokine pro-

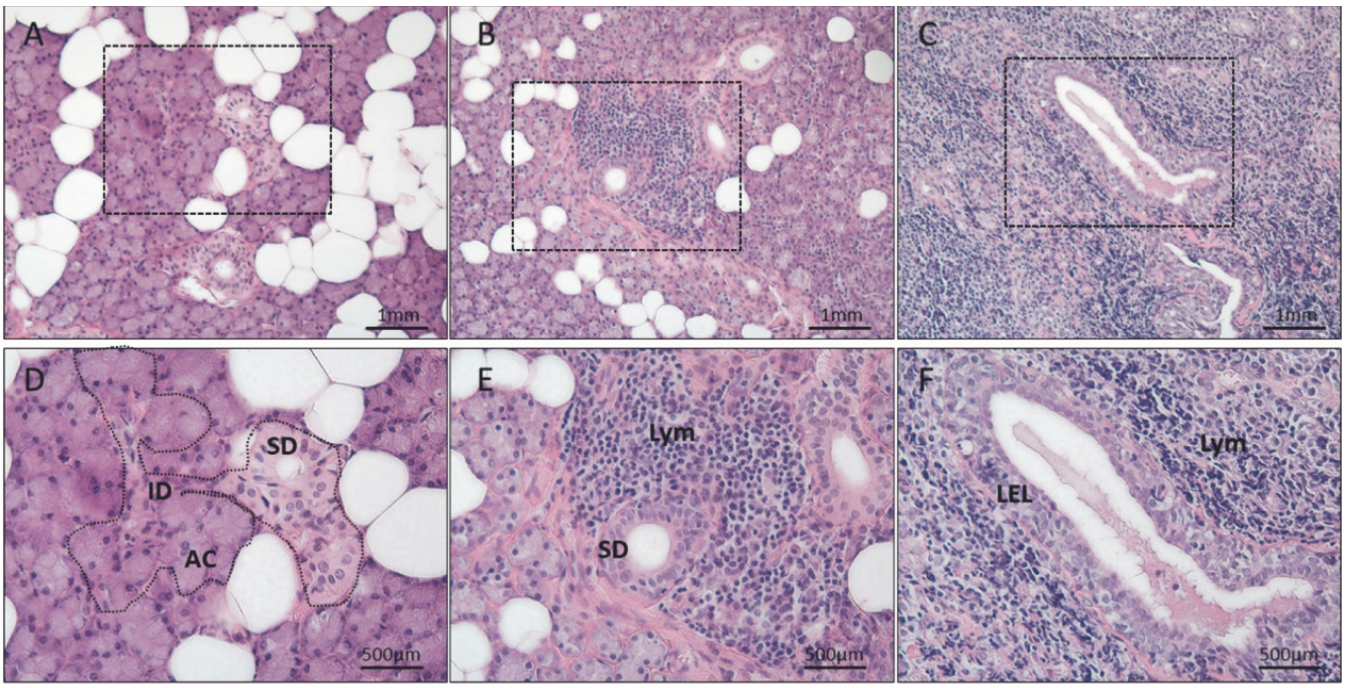

Fig. 1. Healthy control and pSS salivary glands.
A: Healthy control salivary gland. **B**: pSS salivary gland with a lymphocytic focus. **C**: pSS salivary gland with massive lymphocytic infiltrations.
D-F: High resolution images of black square in **A-C**.
Tissues were obtained with relevant ethical board approval. HE staining was performed according to routine steps.
SD: striated duct; ID: intercalated duct; AC: acinus; Lym: lymphocyte; LEL: lymphoepithelial lesion.

duction by SGECs (77). Rituximab treatment of pSS patients may thus contribute to local inflammation reduction and LEL formation, herewith stabilising, or even improving, saliva production.

• *Belimumab*
Belimumab inhibits soluble BAFF. BAFF is an important B cell survival factor and controls its homeostasis (78). In pSS SGs, BAFF is produced and secreted by activated SG epithelial cells (79, 80). An open-label phase II study of belimumab treatment in pSS showed significantly decreased ESSDAI and ESSPRI scores, however, the saliva flow remained unchanged, suggesting minimal benefits on SG function (81). Theoretically, BAFF produced by SG epithelium is inhibited by belimumab as well, although no studies specifically examining this have been reported.

• *Abatacept*
Abatacept (CTLA4-Ig) binds to co-stimulatory molecules CD80/CD86, blocking the binding of these molecules with CD28 on T cells (82). A pilot open-label study found significantly decreased ESSDAI, ESSPRI and IgG levels during abatacept treatment, however, salivary and lacrimal gland function remained stable (83). Meanwhile, abatacept treatment in this study resulted in a decrease of germinal centres and an increase in the number of IgM plasma cells/mm^2, but had no effect on focus score, amount of lymphocytic infiltrate and LELs (84). The primary outcome (as measured by ESSDAI) of a phase III study of abatacept in early active pSS patients did not significantly differ between patients receiving abatacept or placebo, including SG functionality (85).

• *Iscalimab*
Iscalimab targets CD40, which is constitutively expressed on B lymphocytes, monocytes, macrophages, dendritic cells and parenchymal cells, including epithelia (86). CD40 is a co-stimulatory molecule, involved numerous downstream effects depending upon the cell type, after binding with CD154 on activated CD4$^+$ T cells (87). A RCT assessing intravenous iscalimab treatment in pSS showed more reduction in ESSDAI score than placebo controls (88). Unstimulated and stimulated salivary flow were increased, but not statistically significant (88). The effect of iscalimab on glandular epithelial cells remain to be investigated.

• *Summary*
There is a poor correlation between lymphocytic infiltration and saliva production (10, 89, 90). The limited effects on saliva production seen in trials with the variety of the above mentioned biological drugs that interfere with B- and T-cell function are in line with this notion. Reduction in infiltrate size or function of B- and T-cells does not automatically lead to improved functionality of the glandular epithelium, with one exception: rituximab. So far, to our knowledge, rituximab is the only biological with demonstrated effect on the histopathology of the salivary glands, by reducing LELs and restoring the affected ductal morphology. For a complete restoration of SG function in pSS, including saliva production and LEL formation, we must learn to think outside of the box, and away from the shadow of immune infiltration. This will necessitate placing the SG epithelium,

Table I. Salivary gland stem/progenitor cell markers.

Salivary gland stem/progenitor cell markers	Expression	Subjects	Salivary glands	Reference
C-kit/CD117	IDs, larger ED and SDs	Human	SMG, SLG, PG	(121, 122)
	IDs, GDs, and EDs	Mouse	SMG	(113, 114) (122)
Sca-1	EDs and SDs	Mouse	SMG	(114)
Musashi-1	EDs and SDs	Mouse	SMG	(114)
CD24	EDs	Mouse	SMG	(113)
CD29/CD49f	EDs	Mouse	SMG	(113)
CD133	EDs	Mouse	SMG	(113)
KRT5	the basal layer of ductal epithelial cells	Mouse	Developing SMG	(123)
KRT14	myoepithelial cells, the acinar-ID and ID- GD junctions	Mouse	SMG	(122)
	basal cells of the SD and EDs, IDs and SMA^+ myoepithelial cells	Human	SMG, SLG, PG	(122)
SOX2	Acini	Mouse	SMG, SLG, PG	(32, 124)

ED: excretory duct; SD: striated duct; ID: intercalated duct; GD: granulated duct (mouse); SMG: submandibular gland; SLG: sublingual gland; PG: parotid gland.

Table II. The target of biologic drugs and their effects on salivary gland function and histopathology.

Biologic drugs	Target molecule	Effects on salivary gland function	Effects on salivary gland histopathology
Abatacept	CD80/CD86	No change[83]	Resulted in a decrease of germinal centres and an increase in the number of IgM plasma cells/mm^2, but had no effect on focus score , lymphoepithelial lesions and lymphocytic infiltrates[84]
Rituximab	CD20	Increased saliva flow[75,76,125,126].	Reduced the proliferation of parotid gland acinar cells and decreased the amount of lymphoepithelial lesions[75].
Belimumab	B-cell activating factor (BAFF)	No change[81]	No data
Iscalimab (CFZ533)	CD40	Not significantly increased[88].	No data

including its progenitor cells, as equal, if not first priority, in arrangement of treatment strategies, and seriously considering new treatment possibilities with potential to rescue the epithelium.

New avenues for treating pSS by targeting epithelium

Given the limited effects of agonists of the muscarinic receptors and drugs that target the chronic lymphocytic inflammatory responses on saliva production, in combination with the central role of the epithelium in pSS, we propose several future promising directions for treating pSS SG dysfunction. We place the SG epithelial centrally in this treatment strategy, and consider the potential benefit on saliva production, and salivary gland progenitor cells (Fig. 4).

Small molecule inhibitors

Small molecule inhibitors (SMIs) are small in size and weight, and easily transported intracellularly. They promise more accurate, rapid and steerable targeting, with designs that can be inexpensively and easily modified (91). Only very few SMIs are tested in clinical trials for pSS treatment (92). Ongoing trials include SMIs targeting NF-κB modulators and JAK1 (92). Through intertwining existing knowledge on epithelial cell biology with that of the SMI mechanisms of action, we have recently proposed that SMIs interfering with the NF-κB pathway may represent the tactic (92). Of the SMIs, we believe the NF-κB inhibitors may induce the least collateral damage to the SG epithelium, and therefore the greatest chance of improvement of SG functional properties (92). Other targeted pathways (*e.g.* toll-like receptor signalling, JAK-STAT signalling, and epithelial cell survival mechanism) also interfere with a series of signalling pathways that critical to epithelial homeostasis, thereof are less recommended. Therefore, to develop more accurate targeting by exploring more precise targeted pathways or proteins to reduce the possible side effects on SG epithelium can be a future direction.

Modulation of the hypoxic state in the salivary gland

Hypoxia is a state of reduced oxygen availability. It can be caused by reduced blood flow, anaemia, metabolic changes, and inflammation (42, 93-96). Hypoxia has been documented to contribute to epithelial cell apoptosis (97), to down-regulate electrogenic Cl^- secretion causing decreased epithelial fluid transportation activity, and to lead to disruption of inter-epithelial cell tight junctions (TJ) (98). In an experimental hypoxia situation, carbachol-stimulated Ca^{2+} signals, which are fundamentally important for saliva secretion, were reduced in *in vitro* mouse parotid acinar cells (99). Expression of HIF1α is often employed as a marker of hypoxia, although recent data suggest that its expression in epithelial cells is protective against hypoxia-induced loss of TJ integrity, and epithelial cell secretory function. Presence of p582S variant in the genome specifically has been linked to decreased pSS risk, suggesting that HIF1α activity may be complicit in pSS disease development (100).

We have examined HIF1α and LDHA (downstream target of HIF1α) expression for the first time in the parotid SG of pSS patients. Nuclear expression of HIF1α and LDHA, indicating their activity, is present in pSS SGs containing lymphocytic infiltration (Fig. 3). This increased expression was most notable in the acinar cells, and was not observed in SGs without infiltration. If hypoxia induces secretory dysregulation in acinar cells of the SGs, and HIF1α expression is also protective, its expression is apparently not enough (or expression of a less active variant) to maintain saliva secretion, conside-

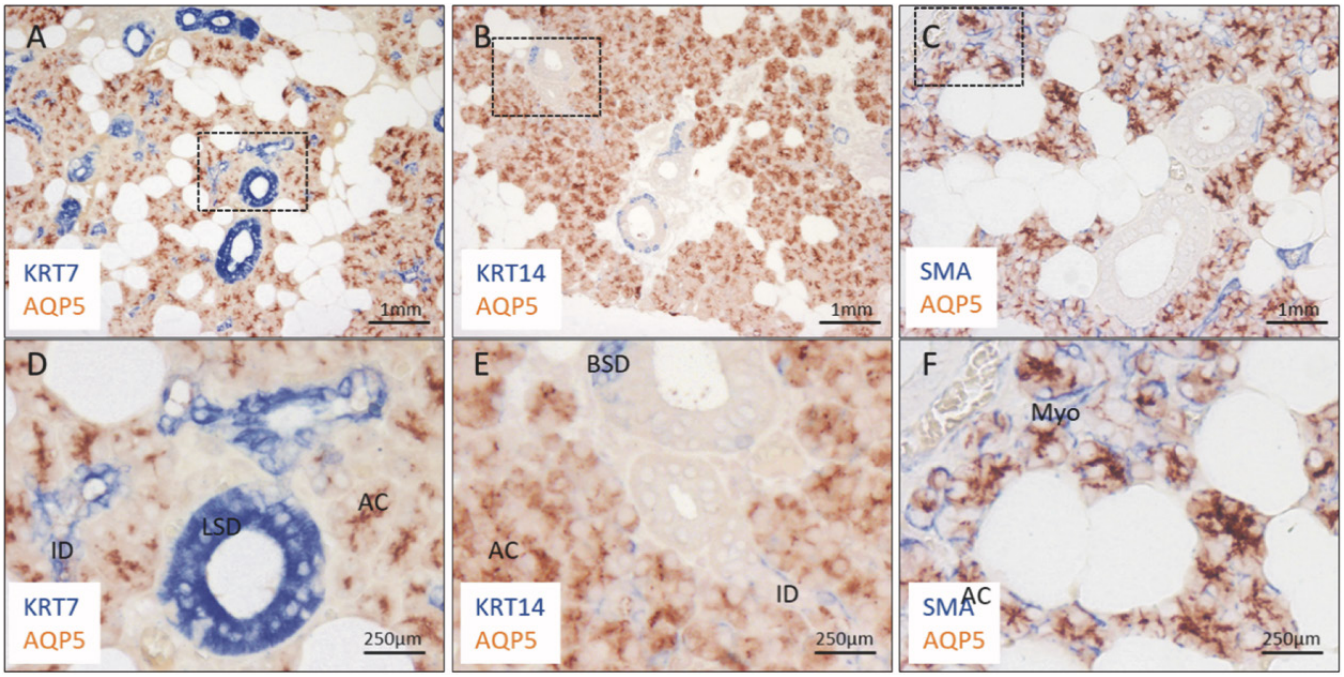

Fig. 2. Parenchymal cell type of human salivary gland.
A: KRT7 and AQP5 in healthy control salivary gland. **B**: KRT14 and AQP5 in healthy control salivary gland. **C**: SMA and AQP5 in healthy control salivary gland. **D-F**: High resolution images of black square in **A-C**. Tissues were obtained with relevant ethical board approval. Antigen retrieval was performed for 20 minutes with a solution of 1 mM EDTA (pH 8.0) on paraffin sections. KRT7 (Millipore, clone RCK105, 1:100), KRT14 (Sigma, clone LL002, 1:200), or SMA (DAKO, clone 1A4, 1:100) were counterstained with used with AQP-5 (Abcam, clone EPR3747, 1:200) using the Lab Vision MultiVision Polymer Detection System anti Mouse-AP anti-rabbit-HRP staining kit (Thermofisher) to detect salivary gland parenchymal cells.
BSD: basal striated duct; LSD: luminal striated duct; ID: intercalated duct; AC: acinus; Myo: myoepithelial cell.

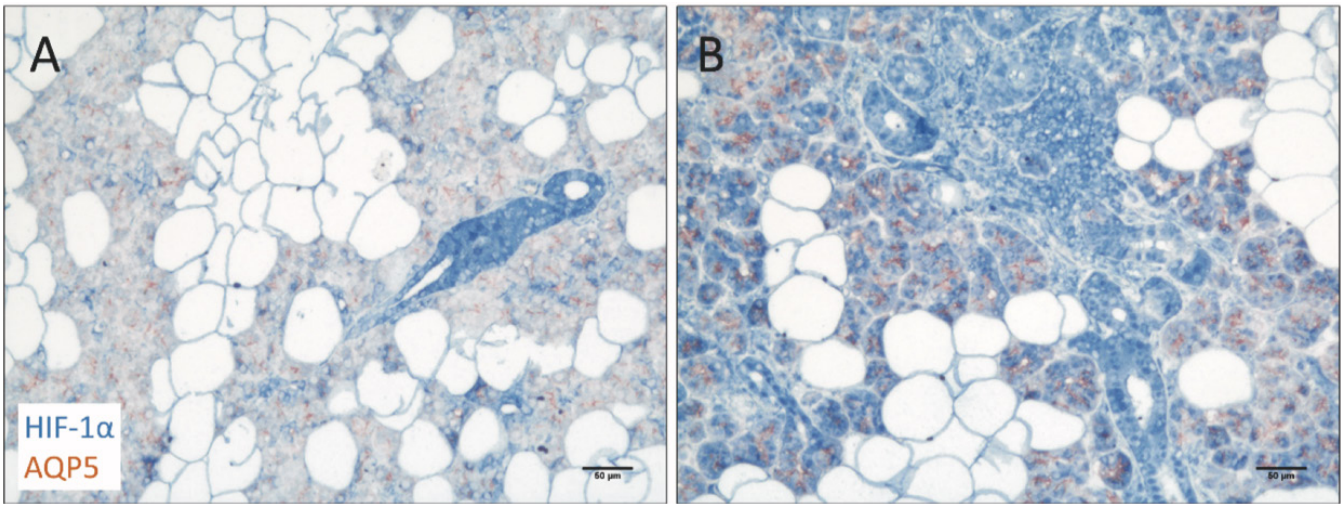

Fig. 3. Parenchymal cell type of human salivary gland.
A: HIF-1α and AQP5 in sicca control salivary gland. **B**: HIF-1α and AQP5 in pSS salivary gland. Bars = 50µm. Tissues were obtained with relevant ethical board approval. Antigen retrieval was performed for 10 minutes with a solution of 10 mM sodium citrate (pH 6.0) on paraffin sections. HIF-1α (Abcam, clone ESEE122, 1:100) was counterstained with AQP-5 (Abcam, clone EPR3747, 1:200, acinar cell marker) using the Lab Vision MultiVision Polymer Detection System anti Mouse-AP anti-rabbit-HRP staining kit (Thermofisher).

ring these patients suffer from hyposalivation. HIF1α genomic variation in pSS patients can obviously not be changed, thus a potential therapeutic option may be blockade of hypoxia development. Application of DMOG and FG-4497 hypoxia stabilisers have shown promising effects in inflammatory bowel disease (IBD), with decreased inflammation (again suggesting the pivotal role of hypoxia in inflammation development), decreased apoptosis of intestinal epithelial cells, and subsequently enhanced the intestinal barrier function (101-103). A more thorough understanding of the extent and timing of hypoxia development in pSS SGs will be critical, before incorporation of anti-hypoxia treatment regimens can be seriously considered.

Senolytics

Senescence is a permanent state of cell cycle arrest, with the upregulation of

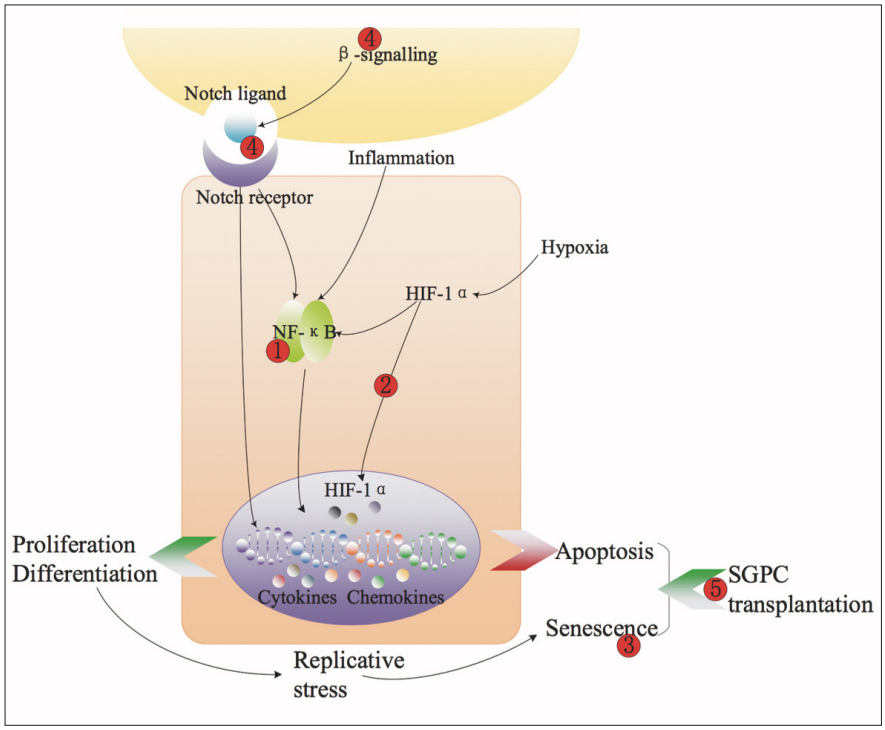

Fig. 4. New avenues for treating pSS by targeting epithelium.

anti-apoptotic pathways (104). In terms of progenitor cells, a senescent progenitor cell can no longer proliferate and differentiate. As described in section 3.1, pSS SGPCs tend to be senescent, with the senescent SG microenvironment maintained by for example, SASPs (38, 40).

Therefore, depleting senescent cells and prohibiting the spreading of senescence might be beneficial to pSS SGs. Senolytics are a group of drugs that selectively eliminate senescent cells (105). Currently reported senolytics such as navitoclax, dasatinib and quercetin, work by inhibiting pro-survival pathways (*e.g.* Bcl-2 and Bcl-X_L), which are up-regulated in senescent cells, resulting in senescent cell death (106). Senolytic treatment rescued senescence-induced dysfunction in mice (105). Another possible target of senolytics is HSP90, involved in the upregulation of the survival pathway. Treatment of Ercc1$^{-/\Delta}$ mice (a mouse model of human accelerated aging) with a HSP90 inhibitor, delayed the onset of several age-related symptoms and reduced the expression of senescence marker p16 (106). Timing of senolytics use is likely to be of critical importance if applied to pSS. In the advanced stage of pSS (*e.g.* high degree of B-cell rich lymphocytic infiltration), patient SGPCs have very limited self-renewal and differentiation abilities (38), and may not possess enough regenerative potential to fully rejuvenate patient SGs. Therefore, timely rejuvenation of SGs using senolytics to target senescent cells is expected to achieve more effective therapy for pSS.

Manipulation of Notch pathway

Notch signalling is important for stem cell maintenance, cell proliferation and differentiation, and cell fate in a variety of epithelia (107-110). Notch pathway activation requires cell-to-cell contact, where the ligand on one cell binds to the Notch receptors on neighboring cells (111). In normal healthy SGs, Notch pathway ligands and receptors are actively expressed in ductal cells and occasionally weakly expressed by acinar cells, and are critical for the proliferation and differentiation of a human intercalated duct-like cell line (112). In a rat experimental SG trauma model, Notch receptors and ligands are expressed in the regenerating epithelial acinar cells *in vivo* and cultured SGECs (112). We have recently demonstrated that the activity of progenitor cells within the intercalated ducts of human parotid SG are likely to be dependent on Notch signalling for proliferation and differentiation into acinar cells (manuscript under review). The proliferation and differentiation machinery of these progenitor cells can be inhibited by use of β-adrenergic blockers such as metoprolol and shows hyposalivation. Manipulating the Notch pathway may thus potentially represent a very promising target for treating pSS SG. Possible interventions include promoting the proliferation and differentiation abilities of SGPCs residing in the intercalated ducts by either directly targeting the ligands (*e.g.* DLLs, JAGs) and receptors (*e.g.* Notch 1-4) of Notch, or upregulating its upstream signalling pathways, such as β-adrenergic signalling by agonists, such as isoproterenol.

Salivary gland progenitor cell transplantation

The secretory abilities of SGs from pSS patients are often severely deteriorated when clinically diagnosed. A strategy for rescuing SG function when severely damaged may be SGPCs transplantation. Directly transplanted autologous SGPCs are capable of rescuing hyposalivation following irradiation in an *in vivo* mouse model (34, 35, 113, 114). For pSS treatment, the autoimmune microenvironment, unlike irradiated SG, still exists. This might not be a friendly environment for the transplanted SGPCs. Furthermore, the SG biopsies are usually small, to obtain enough SGPCs to achieve major/minor glandular recovery, and upscaling transplantable cells numbers using tissue culture techniques such organoid culture in the bioscaffold Matrigel may be necessary (38, 115). Preliminary success has been made in several mouse studies showing those 3D mini SGs are able to connect with the recipient SG duct and function as native tissue (116, 117). In pSS, we have previously shown that SGPCs isolated from patient SGs had significantly reduced proliferation and differentiation abilities (38, 39). The use of autologous SGPCs as therapy and their expansion *in vitro* may therefore be limited, unless patients are treated very early in pSS progression. An alternative would be to

manufacture fresh SGPCs from pluripotent stem cells (PSCs), an embryologically primitive cell type capable of unlimited proliferation and of differentiation (118, 119). Through the initial generation of PSCs from somatic cell sources (skin cells, cheek swabs, blood cells), unlimited sources of patient-matched SGPCs can in theory be generated. Proof of principle of this strategy has been demonstrated using mouse PSCs. When transplanted *in vivo*, the PSC-derived SG rudiment showed both morphology and function similar to normal murine SGs (120). Optimisation of these protocols for use with human PSCs remains to be demonstrated, and may provide promising therapeutic options for irreversibly damaged SGs, together with dampening of the proinflammatory environment, to provide a better SG microenvironment.

Conclusions

The majority of articles discussing dysfunction of the SG in pSS will outline the nature and extent of its lymphocytic infiltration, and how this is a defining feature of the autoimmune disease. The correlation between lymphocytic infiltration and SG function is moderate at best, and has been demonstrated multiple times. Whilst this is certainly true, and critical for discussion of MALT development, the function of the SG epithelium is an entity to itself. The SG epithelium as a whole, appears to be severely compromised by apoptosis, senescence, and potentially hypoxia, in addition to its immune activation and active participation in the immune process. Any progress towards durable resolution of sicca symptoms in pSS is likely to result from addressing several if not all of these issues, in a multi-pronged approach to regeneration of SGs in pSS.

References

1. BRITO-ZERÓN P, THEANDER E, BALDINI C *et al*.: Early diagnosis of primary Sjögren's syndrome: EULAR-SS task force clinical recommendations. *Expert Rev Clin Immunol* 2016; 12: 137-56.
2. NOCTURNE G, MARIETTE X: Advances in understanding the pathogenesis of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2013; 9: 544-56.
3. PRICE EJ, RAUZ S, TAPPUNI AR *et al*.: The British Society for Rheumatology guideline for the management of adults with primary Sjögren's syndrome. *Rheumatology* 2017; 56: e24-48.
4. TASHBAYEV B, RUSTHEN S, YOUNG A *et al*.: Interdisciplinary, comprehensive oral and ocular evaluation of patients with primary Sjögren's syndrome. *Sci Rep* 2017; 7: 1-9.
5. FOX RI: Sjögren's syndrome. *Lancet* 2005; 366: 321-31.
6. CAFARO G, CROIA C, ARGYROPOULOU OD *et al*.: One year in review 2019: Sjögren's syndrome. *Clin Exp Rheumatol* 2019; 37 (Suppl. 112): S3-15.
7. CHRISTODOULOU MI, KAPSOGEORGOU EK, MOUTSOPOULOS HM: Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010; 34: 400-7.
8. VAN GINKEL MS, HAACKE EA, BOOTSMA H *et al*.: Presence of intraepithelial B-lymphocytes is associated with the formation of lymphoepithelial lesions in salivary glands of primary Sjögren's syndrome patients. *Clin Exp Rheumatol* 2019; 37 (Suppl. 112): S42-8.
9. DANIELS TE, COX D, SHIBOSKI CH *et al*.: Associations between salivary gland histopathologic diagnoses and phenotypic features of Sjögren's syndrome among 1,726 registry participants. *Arthritis Rheum* 2011; 63: 2021-30.
10. JONSSON R, KRONELD U, BÄCKMAN K *et al*.: Progression of sialadenitis in Sjögren's syndrome. *Br J Rheumatol* 1993; 32: 578-81.
11. PATEL YI, MCHUGH NJ: Apoptosis—new clues to the pathogenesis of Sjögren's syndrome? *Rheumatology* 2000; 39: 119-21.
12. HUMPHREYS-BEHER MG, PECK AB, DANG H *et al*.: The role of apoptosis in the initiation of the autoimmune response in Sjögren's syndrome. *Clin Exp Immunol* 1999; 116: 383-7.
13. HAYASHI Y, ARAKAKI R, ISHIMARU N: Apoptosis and estrogen deficiency in primary Sjögren syndrome. *Curr Opin Rheumatol* 2004; 16: 522-6.
14. KONG L, OGAWA N, NAKABAYASHI T *et al*.: Fas and Fas ligand expression in the salivary glands of patients with primary Sjögren's syndrome. *Arthritis Rheum* 1997; 40: 87-97.
15. MANGANELLI P, QUAINI F, ANDREOLI AM *et al*.: Quantitative analysis of apoptosis and bcl-2 in Sjögren's syndrome. *J Rheumatol* 1997; 24: 1552-7.
16. KONG L, OGAWA N, MCGUFF HS *et al*.: Bcl-2 family expression in salivary glands from patients with primary Sjögren's syndrome: Involvement of Bax in salivary gland destruction. *Clin Immunol Immunopathol* 1998; 88: 133-41.
17. POLIHRONIS M, TAPINOS NI, THEOCHARIS SE *et al*.: Modes of epithelial cell death and repair in Sjögren's syndrome (SS). *Clin Exp Immunol* 1998; 114: 485-90.
18. KATSIOUGIANNIS S, TENTA R, SKOPOULI FN: Endoplasmic reticulum stress causes autophagy and apoptosis leading to cellular redistribution of the autoantigens Ro/Sjögren's syndrome-related antigen A (SSA) and La/SSB in salivary gland epithelial cells. *Clin Exp Immunol* 2015; 181: 244-52.
19. KYRIAKIDIS NC, KAPSOGEORGOU EK, GOURZI VC *et al*.: Toll-like receptor 3 stimulation promotes Ro52/TRIM21 synthesis and nuclear redistribution in salivary gland epithelial cells, partially via type I interferon pathway. *Clin Exp Immunol* 2014; 178: 548-60.
20. KILLEDAR SY, ECKENRODE SE, MCINDOE RA *et al*.: Early pathogenic events associated with Sjögren's syndrome (SjS)-like disease of the nod mouse using microarray analysis. *Lab Investig* 2006; 86: 1243-60.
21. KAPSOGEORGOU EK, ABU-HELU RF, MOUTSOPOULOS HM *et al*.: Salivary gland epithelial cell exosomes: a source of autoantigenic ribonucleoproteins. *Arthritis Rheum* 2005; 52: 1517-21.
22. GREENING DW, GOPAL SK, XU R *et al*.: Exosomes and their roles in immune regulation and cancer. *Semin Cell Dev Biol* 2015; 40: 72-81.
23. ARELLANO-GARCIA ME, MISUNO K, TRAN SD *et al*.: Interferon-γ induces immunoproteasomes and the presentation of MHC I-associated peptides on human salivary gland cells. *PLoS One* 2014; 9: e102878.
24. FRANCO A, VALESINI G, BARNABA V *et al*.: Class II MHC antigen expression on epithelial cells of salivary glands from patients with Sjögren's syndrome. *Clin Exp Rheumatol* 1987; 5: 199-203.
25. KAPSOGEORGOU EK, MANOUSSAKIS MN: Salivary gland epithelial cells (SGEC): carriers of exquisite B7-2 (CD86) costimulatory molecules. *J Autoimmun* 2010; 35: 188-91.
26. MANOUSSAKIS MN, KAPSOGEORGOU EK: The role of intrinsic epithelial activation in the pathogenesis of Sjögren's syndrome. *J Autoimmun* 2010; 35: 219-24.
27. MITSIAS DI, KAPSOGEORGOU EK, MOUTSOPOULOS HM: The role of epithelial cells in the initiation and perpetuation of autoimmune lesions: lessons from Sjögren's syndrome (autoimmune epithelitis). *Lupus* 2006; 15: 255-61.
28. HA Y-J, CHOI YS, KANG EH *et al*.: Increased expression of interferon-λ in minor salivary glands of patients with primary Sjögren's syndrome and its synergic effect with interferon-α on salivary gland epithelial cells. *Clin Exp Rheumatol* 2018; 36 (Suppl. 112): S31-40.
29. GONG Y-Z, NITITHAM J, TAYLOR K *et al*.: Differentiation of follicular helper T cells by salivary gland epithelial cells in primary Sjögren's syndrome. *J Autoimmun* 2014; 51: 57-66.
30. WENG P-L, AURE MH, MARUYAMA T *et al*.: Limited regeneration of adult salivary glands after severe injury involves cellular plasticity. *Cell Rep* 2018; 24: 1464-70.e3.
31. KWAK M, ALSTON N, GHAZIZADEH S: Identification of stem cells in the secretory complex of salivary glands. *J Dent Res* 2016; 95: 776-83.
32. EMMERSON E, MAY AJ, BERTHOIN L *et al*.: Salivary glands regenerate after radiation injury through SOX2-mediated secretory cell replacement. *EMBO Mol Med* 2018; 10: e8051.
33. NANDURI LSY, BAANSTRA M, FABER H *et al*.: Purification and ex vivo expansion of

fully functional salivary gland stem cells. *Stem Cell Reports* 2014; 3: 957-64.
34. PRINGLE S, MAIMETS M, VAN DER ZWAAG M *et al*.: Human salivary gland stem cells functionally restore radiation damaged salivary glands. *Stem Cells* 2016; 34: 640-52.
35. NANDURI LSY, LOMBAERT IMA, VAN DER ZWAAG M *et al*.: Salisphere derived c-Kit+ cell transplantation restores tissue homeostasis in irradiated salivary gland. *Radiother Oncol* 2013; 108: 458-63.
36. AURE MHH, KONIECZNY SFF, OVITT CEE: Salivary gland homeostasis is maintained through acinar cell self-duplication. *Dev Cell* 2015; 33: 231-7.
37. RUSAKIEWICZ S, NOCTURNE G, LAZURE T *et al*.: NCR3/NKp30 contributes to pathogenesis in primary Sjögren's syndrome. *Sci Transl Med* 2013; 5: 195ra96.
38. PRINGLE S, WANG X, VERSTAPPEN GMPJPJ *et al*.: Salivary gland stem cells age prematurely in primary Sjögren's syndrome. *Arthritis Rheumatol* 2018; 71: 133-42.
39. WANG X, BOOTSMA H, TERPSTRA J *et al*.: Progenitor cell niche senescence reflects pathology of the parotid salivary gland in primary Sjögren's syndrome. *Rheumatology* 2020; keaa012.
40. ACOSTA JC, BANITO A, WUESTEFELD T *et al*.: A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat Cell Biol* 2013; 15: 978-90.
41. WANG X, SHAALAN A, LIEFERS S *et al*.: Dysregulation of NF-kB in glandular epithelial cells results in Sjögren's-like features. *PLoS One* 2018; 13: e0200212.
42. HAACKE EA, BOOTSMA H, SPIJKERVET FKL *et al*.: FcRL4 + B-cells in salivary glands of primary Sjögren's syndrome patients. *J Autoimmun* 2017; 81: 90-8.
43. VERSTAPPEN GM, ICE JA, BOOTSMA H *et al*.: Gene expression profiling of epithelium-associated FcRL4+ B cells in primary Sjögren's syndrome reveals a pathogenic signature. *J Autoimmun* 2020; 109: 102439.
44. BAUM BJ: Principles of saliva secretion. *Ann N Y Acad Sci* 1993; 694: 17-23.
45. AMBUDKAR IS: Calcium signalling in salivary gland physiology and dysfunction. *J Physiol* 2016; 594: 2813-24.
46. NARITA T, QI B, MURAKAMI M *et al*.: Pilocarpine induces the residual secretion of salivary fluid in perfused submandibular glands of rats. *PLoS One* 2019; 14: e0221832.
47. VIVINO FB, AL-HASHIMI I, KHAN Z *et al*.: Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjögren syndrome: A randomized, placebo-controlled, fixed- dose, multicenter trial. *Arch Intern Med* 1999; 159: 174-81.
48. PELUSO G, DE SANTIS M, INZITARI R *et al*.: Proteomic study of salivary peptides and proteins in patients with Sjögren's syndrome before and after pilocarpine treatment. *Arthritis Rheum* 2007; 56: 2216-22.
49. STURGESS J, REID L: The effect of isoprenaline and pilocarpine on (a) bronchial mucus-secreting tissue and (b) pancreas, salivary glands, heart, thymus, liver and spleen. *Br J Exp Pathol* 1973; 54: 388-403.
50. KONDO Y, NAKAMOTO T, MUKAIBO T *et al*.: Cevimeline-induced monophasic salivation from the mouse submandibular gland: decreased Na+ content in saliva results from specific and early activation of Na+/H+ exchange. *J Pharmacol Exp Ther* 2011; 337: 267-74.
51. PETRONE D, CONDEMI JJ, FIFE R *et al*.: A double-blind, randomized, placebo-controlled study of cevimeline in Sjögren's syndrome patients with xerostomia and keratoconjunctivitis sicca. *Arthritis Rheum* 2002; 46: 748-54.
52. PUJOLS L, MULLOL J, ROCA-FERRER J *et al*.: Expression of glucocorticoid receptor α- and β-isoforms in human cells and tissues. *Am J Physiol Physiol* 2002; 283: C1324-31.
53. BARNES PJ: Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin Sci* 1998; 94: 557-72.
54. FOX PC, DATILES M, ATKINSON JC *et al*.: Prednisone and piroxicam for treatment of primary Sjögren's syndrome. *Clin Exp Rheumatol* 1993; 11: 149-56.
55. PIJPE J, KALK WWI, BOOTSMA H *et al*.: Progression of salivary gland dysfunction in patients with Sjögren's syndrome. *Ann Rheum Dis* 2006; 66: 107-12.
56. KRONKE M, LEONARD WJ, DEPPER JM: Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. *Proc Natl Acad Sci USA* 1984; 81: 5214-8.
57. POWER WJ, MULLANEY P, FARRELL M *et al*.: Effect of topical cyclosporin A on conjunctival T cells in patients with secondary Sjögren's syndrome. *Cornea* 1993; 12: 507-11.
58. RAMOS-CASALS M, BRITO-ZERÓN P, SISÓ-ALMIRALL A *et al*.: Topical and systemic medications for the treatment of primary Sjögren's syndrome. *Nat Rev Rheumatol* 2012; 8: 399-411.
59. DALAVANGA YA, DETRICK B, HOOKS JJ *et al*.: Effect of cyclosporin A (CyA) on the immunopathological lesion of the labial minor salivary glands from patients with Sjögren's syndrome. *Ann Rheum Dis* 1987; 46: 89-92.
60. LENNARD L: The clinical pharmacology of 6-mercaptopurine. *Eur J Clin Pharmacol* 1992; 43: 329-39.
61. STOCCO G, PELIN M, FRANCA R *et al*.: Pharmacogenetics of azathioprine in inflammatory bowel disease: a role for glutathione-S-transferase? *World J Gastroenterol* 2014; 20: 3534-41.
62. PEARSON DC, MAY GR, FICK GH *et al*.: Azathioprine and 6-mercaptopurine in Crohn disease. A meta-analysis. *Ann Intern Med* 1995; 123: 132-42.
63. PRICE EJ, RIGBY SP, CLANCY U *et al*.: A double blind placebo controlled trial of azathioprine in the treatment of primary Sjögren's syndrome. *J Rheumatol* 1998; 25: 896-9.
64. AL-MOULA AD, AL-MASHHADANE FA, MAMMDOH JK: Effects of 6-Mercaptopurine on salivary glands in rabbit. *Al-Rafidain Dent J* 2012; 12: 266-73.
65. BRUNEAU JM, YEA CM, SPINELLA-JAEGLE S *et al*.: Purification of human dihydroorotate dehydrogenase and its inhibition by A77 1726, the active metabolite of leflunomide. *Biochem J* 1998; 336: 299-303.
66. MANNA SK, AGGARWAL BB: Immunosuppressive leflunomide metabolite (A77 1726) blocks TNF-dependent nuclear factor-kappa B activation and gene expression. *J Immunol* 1999; 162: 2095-102.
67. GOLDMAN FD, GILMAN AL, HOLLENBACK C *et al*.: Hydroxychloroquine inhibits calcium signals in T cells: a new mechanism to explain its immunomodulatory properties. *Blood* 2000; 95: 3460-6.
68. VAN DER HEIJDEN EHM, BLOKLAND SLM, HILLEN MR *et al*.: Leflunomide–hydroxychloroquine combination therapy in patients with primary Sjögren's syndrome (RepurpSS-I): a placebo-controlled, double-blinded, randomised clinical trial. *Lancet Rheumatol* 2020; 2: e260-9.
69. FOX R, DIXON R, GUARRASI V *et al*.: Treatment of primary Sjögren's syndrome with hydroxychloroquine: a retrospective, open-label study. *Lupus* 1996; 5: 31-6.
70. GOTTENBERG J-E, RAVAUD P, PUÉCHAL X *et al*.: Effects of hydroxychloroquine on symptomatic improvement in primary Sjögren syndrome: the JOQUER randomized clinical trial. *JAMA* 2014; 312: 249-58.
71. VAN WOERKOM JM, KRUIZE AA, GEENEN R *et al*.: Safety and efficacy of leflunomide in primary Sjögren's syndrome: a phase II pilot study. *Ann Rheum Dis* 2007; 66: 1026-32.
72. MAVRAGANI CP, MOUTSOPOULOS HM: Sjögren's syndrome: Old and new therapeutic targets. *J Autoimmun* 2020; 110: 102364.
73. VERSTAPPEN GM, VAN NIMWEGEN JF, VISSINK A *et al*.: The value of rituximab treatment in primary Sjögren's syndrome. *Clin Immunol* 2017; 182: 62-71.
74. DELLI K, HAACKE EA, KROESE FGM *et al*.: Towards personalised treatment in primary Sjögren's syndrome: baseline parotid histopathology predicts responsiveness to rituximab treatment. *Ann Rheum Dis* 2016; 75: 1933-8.
75. PIJPE J, MEIJER JM, BOOTSMA H *et al*.: Clinical and histologic evidence of salivary gland restoration supports the efficacy of rituximab treatment in Sjögren's syndrome. *Arthritis Rheum* 2009; 60: 3251-6.
76. PIJPE J, VAN IMHOFF GW, SPIJKERVET FKL *et al*.: Rituximab treatment in patients with primary Sjögren's syndrome: An open-label phase II study. *Arthritis Rheum* 2005; 52: 2740-50.
77. LISI S, SISTO M, D'AMORE M *et al*.: Co-culture system of human salivary gland epithelial cells and immune cells from primary Sjögren's syndrome patients: an *in vitro* approach to study the effects of Rituximab on the activation of the Raf-1/ERK1/2 pathway. *Int Immunol* 2015; 27: 183-94.
78. MACKAY F, BROWNING JL: BAFF: a fundamental survival factor for B cells. *Nat Rev Immunol* 2002; 2: 465-75.
79. ITTAH M, MICELI-RICHARD C, ERIC GOTTENBERG J- *et al*.: B cell-activating factor of the tumor necrosis factor family (BAFF) is expressed under stimulation by interferon in salivary gland epithelial cells in primary Sjögren's syndrome. *Arthritis Res Ther* 2006; 8: R51.
80. ITTAH M, MICELI-RICHARD C, GOTTEN-

BERG J-EE *et al*.: Viruses induce high expression of BAFF by salivary gland epithelial cells through TLR- and type-I IFN-dependent and -independent pathways. *Eur J Immunol* 2008; 38: 1058-64.
81. MARIETTE X, SEROR R, QUARTUCCIO L *et al*.: Efficacy and safety of belimumab in primary Sjögren's syndrome: Results of the BELISS open-label phase II study. *Ann Rheum Dis* 2015; 74: 526-31.
82. TSUBOI H, MATSUMOTO I, HAGIWARA S *et al*.: Efficacy and safety of abatacept for patients with Sjögren's syndrome associated with rheumatoid arthritis: Rheumatoid Arthritis with Orencia Trial toward Sjögren's syndrome Endocrinopathy (ROSE) trial—an open-label, one-year, prospective study—Interim analysis of 32 patients for 24 weeks. *Mod Rheumatol* 2015; 25: 187-93.
83. MEINERS PM, VISSINK A, KROESE FGM *et al*.: Abatacept treatment reduces disease activity in early primary Sjögren's syndrome (open-label proof of concept ASAP study). *Ann Rheum Dis* 2014; 73: 1393-6.
84. HAACKE EA, VAN DER VEGT B, MEINERS PM *et al*.: Abatacept treatment of patients with primary Sjögren's syndrome results in a decrease of germinal centres in salivary gland tissue. *Clin Exp Rheumatol* 2017; 35: 317-20.
85. VAN NIMWEGEN JF, MOSSEL E, VAN ZUIDEN GS *et al*.: Abatacept treatment for patients with early active primary Sjögren's syndrome: a single-centre, randomised, double-blind, placebo-controlled, phase 3 trial (ASAP-III study). *Lancet Rheumatol* 2020; 2: e153-63.
86. CORDOBA F, WIECZOREK G, AUDET M *et al*.: A novel, blocking, Fc-silent anti-CD40 monoclonal antibody prolongs nonhuman primate renal allograft survival in the absence of B cell depletion. *Am J Transplant* 2015; 15: 2825-36.
87. VAN KOOTEN C, BANCHEREAU J: CD40-CD40 ligand. *J Leukoc Biol* 2000; 67: 2-17.
88. FISHER BA, SZANTO A, NG W-F *et al*.: Assessment of the anti-CD40 antibody iscalimab in patients with primary Sjögren's syndrome: a multicentre, randomised, double-blind, placebo-controlled, proof-of-concept study. *Lancet Rheumatol* 2020; 2: e142-52.
89. MIGNOGNA MD, FEDELE S, RUSSO L LO *et al*.: Sjögren's syndrome: the diagnostic potential of early oral manifestations preceding hyposalivation/xerostomia. *J Oral Pathol Med* 2005; 34: 1-6.
90. SOTO-ROJAS AE, KRAUS A: The oral side of Sjögren syndrome. Diagnosis and treatment. A review. *Arch Med Res* 2002; 33: 95-106.
91. KHERA N, RAJPUT S: Therapeutic potential of small molecule inhibitors. *J Cell Biochem* 2017; 118: 959-61.
92. PRINGLE S, WANG X, BOOTSMA H *et al*.: Small-molecule inhibitors and the salivary gland epithelium in Sjögren's syndrome. *Expert Opin Investig Drugs* 2019; 28: 605-16.
93. KRÜGER B, KRICK S, DHILLON N *et al*.: Donor toll-like receptor 4 contributes to ischemia and reperfusion injury following human kidney transplantation. *Proc Natl Acad Sci USA* 2009; 106: 3390-5.
94. ELTZSCHIG HK, ECKLE T: Ischemia and reperfusion-from mechanism to translation. *Nat Med* 2011; 17: 1391-401.
95. TAYLOR CT, COLGAN SP: Regulation of immunity and inflammation by hypoxia in immunological niches. *Nat Rev Immunol* 2017; 17: 774-85.
96. JIANG BH, SEMENZA GL, BAUER C *et al*.: Hypoxia-inducible factor 1 levels vary exponentially over a physiologically relevant range of O2 tension. *Am J Physiol Physiol* 1996; 271: C1172-80.
97. YAMAMOTO K, TOMITA N, YOSHIMURA S *et al*.: Hypoxia-induced renal epithelial cell death through caspase-dependent pathway: role of Bcl-2, Bcl-xL and Bax in tubular injury. *Int J Mol Med* 2004; 14: 633-40.
98. TAYLOR CT, LISCO SJ, AWTREY CS *et al*.: Hypoxia inhibits cyclic nucleotide-stimulated epithelial ion transport: role for nucleotide cyclases as oxygen sensors. *J Pharmacol Exp Ther* 1998; 284: 568-75.
99. PARK HS, BETZENHAUSER MJ, ZHANG Y *et al*.: Regulation of Ca 2+ release through inositol 1,4,5-trisphosphate receptors by adenine nucleotides in parotid acinar cells. *Am J Physiol Liver Physiol* 2012; 302: G97-104.
100. HERNÁNDEZ-MOLINA G, RODRÍGUEZ-PÉREZ JM, FERNÁNDEZ-TORRES J *et al*.: HIF1A (rs11549465) and AKNA (rs10817595) Gene Polymorphisms Are Associated with Primary Sjögren's Syndrome. *Biomed Res Int* 2017; 2017: 1-8.
101. TAMBUWALA MM, CUMMINS EP, LENIHAN CR *et al*.: Loss of prolyl hydroxylase-1 protects against colitis through reduced epithelial cell apoptosis and increased barrier function. *Gastroenterology* 2010; 139: 2093-101.
102. CUMMINS EP, SEEBALLUCK F, KEELY SJ *et al*.: The hydroxylase inhibitor dimethyloxalylglycine is protective in a murine model of colitis. *Gastroenterology* 2008; 134: 156-165.e1.
103. ROBINSON A, KEELY S, KARHAUSEN J *et al*.: Mucosal protection by hypoxia-inducible factor prolyl hydroxylase inhibition. *Gastroenterology* 2008; 134: 145-55.
104. CHILDS BG, DURIK M, BAKER DJ *et al*.: Cellular senescence in aging and age-related disease: From mechanisms to therapy. *Nat Med* 2015; 21: 1424-35.
105. XU M, PIRTSKHALAVA T, FARR JN *et al*.: Senolytics improve physical function and increase lifespan in old age. *Nat Med* 2018; 24: 1246-56.
106. FUHRMANN-STROISSNIGG H, LING YY, ZHAO J *et al*.: Identification of HSP90 inhibitors as a novel class of senolytics. *Nat Commun* 2017; 8: 422.
107. NAKAMURA T, TSUCHIYA K, WATANABE M: Crosstalk between Wnt and Notch signaling in intestinal epithelial cell fate decision. *J Gastroenterol* 2007; 42: 705-10.
108. KAWANO S, MOROTOMI T, TOYONO T *et al*.: Establishment of dental epithelial cell line (HAT-7) and the cell differentiation dependent on Notch signaling pathway. *Connect Tissue Res* 2002; 43: 409-12.
109. BOURAS T, PAL B, VAILLANT F *et al*.: Notch signaling regulates mammary stem cell function and luminal cell-fate commitment. *Cell Stem Cell* 2008; 3: 429-41.
110. WANG X, LEOW C, ZHA J *et al*.: Notch signaling is required for normal prostatic epithelial cell proliferation and differentiation. *Dev Biol* 2006; 290: 66-80.
111. CHIBA S: Concise review: Notch signaling in stem cell systems. *Stem Cells* 2006; 24: 2437-47.
112. DANG H, LIN AL, ZHANG B *et al*.: Role for notch signaling in salivary acinar cell growth and differentiation. *Dev Dyn* 2009; 238: 724-31.
113. NANDURI LSY, MAIMETS M, PRINGLE SA *et al*.: Regeneration of irradiated salivary glands with stem cell marker expressing cells. *Radiother Oncol* 2011; 99: 367-72.
114. LOMBAERT IMA, BRUNSTING JF, WIERENGA PK *et al*.: Rescue of salivary gland function after stem cell transplantation in irradiated glands. *PLoS One* 2008; 3: e2063.
115. HUCH M, KOO B-K: Modeling mouse and human development using organoid cultures. *Development* 2015; 142: 3113-25.
116. OGAWA M, OSHIMA M, IMAMURA A *et al*.: Functional salivary gland regeneration by transplantation of a bioengineered organ germ. *Nat Commun* 2013; 4: 1-10.
117. PRADHAN-BHATT S, HARRINGTON DA, DUNCAN RL *et al*.: Implantable three-dimensional salivary spheroid assemblies demonstrate fluid and protein secretory responses to neurotransmitters. *Tissue Eng Part A* 2013; 19: 1610-20.
118. KAWAKAMI M, ISHIKAWA H, TACHIBANA T *et al*.: Functional transplantation of salivary gland cells differentiated from mouse early ES cells *in vitro*. *Hum Cell* 2013; 26: 80-90.
119. ONO H, OBANA A, USAMI Y *et al*.: Regenerating salivary glands in the microenvironment of induced pluripotent stem Cells. *Biomed Res Int* 2015; 2015: 1-11.
120. TANAKA J, OGAWA M, HOJO H *et al*.: Generation of orthotopically functional salivary gland from embryonic stem cells. *Nat Commun* 2018; 9: 4216.
121. VAN LUIJK P, PRINGLE S, DEASY JO *et al*.: Sparing the region of the salivary gland containing stem cells preserves saliva production after radiotherapy for head and neck cancer. *Sci Transl Med* 2015; 7: 305ra147.
122. MAY AJ, CRUZ-PACHECO N, EMMERSON E *et al*.: Diverse progenitor cells preserve salivary gland ductal architecture after radiation-induced damage. *Development* 2018; 145: dev166363.
123. KNOX SM, LOMBAERT IMA, REED X *et al*.: Parasympathetic innervation maintains epithelial progenitor cells during salivary organogenesis. *Science* 2010; 329: 1645-7.
124. ARNOLD K, SARKAR A, YRAM MA *et al*.: Sox2 + adult stem and progenitor cells are important for tissue regeneration and survival of mice. *Cell Stem Cell* 2011; 9: 317-29.
125. MEIJER JM, MEINERS PM, VISSINK A *et al*.: Effectiveness of rituximab treatment in primary Sjögren's syndrome: A randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* 2010; 62: 960-8.
126. DEVAUCHELLE-PENSEC V, MARIETTE X, JOUSSE-JOULIN S *et al*.: Treatment of primary Sjögren syndrome with rituximab: a randomized trial. *Ann Intern Med* 2014; 160: 233-42.