Involvement of aquaporin 5 in Sjögren's syndrome

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Key words: Aquaporin 5, Sjögren's syndrome, salivary glands, autoimmunity

ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune disease with the pathological hallmark of lymphoplasmacytic infiltration of exocrine glands - more specifically salivary and lacrimal glands - resulting in a diminished production of tears and saliva (sicca syndrome). The pathophysiology underscoring the mechanisms of the sicca symptoms in SS has still yet to be unraveled but recent advances have identified a cardinal role of aquaporin-5 (AQP5) as a key player in saliva secretion as well as salivary gland epithelial cell dysregulation. AQP5 expression and localization are significantly altered in salivary glands from patients and mice models of the disease, shedding light on a putative mechanism accounting for diminished salivary flow.

Furthermore, aberrant expression and localization of AQP5 protein partners, such as prolactin-inducible protein and ezrin, may account for altered AQP5 localization in salivary glands from patients suffering from SS and are considered as new players in SS development. This review provides an overview of the role of AQP5 in SS salivary gland epithelial cell dysregulation, focusing on its trafficking and protein-protein interactions.

Highlights :

1/ Aquaporin-5 (AQP5) plays a major role in lacrimal and salivary gland physiology.

2/ In pSS, several non-apoptotic neuroexocrine dysfunctions lead to AQP5 deregulation.

3/ AQP5 rescue by pSS treatments is associated with salivary flow restauration.

4/ Iscalimab (anti-CD40) failed to rescue AQP5 deregulation and low salivary flow in pSS.

5/ Gene and cell therapies restoring AQP5 expression are promising therapies for pSS.

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1. Introduction

Sjögren's syndrome (SS) is an autoimmune disease characterized by dysregulation of exocrine glandular tissue following lymphoplasmacytic infiltration [1–4]. SS is classified as primary (pSS) if it occurs alone and secondary (sSS) when associated with other autoimmune diseases such as rheumatoid arthritis (RA) or systemic lupus erythematous (SLE). The most common adverse manifestations are keratoconjunctivitis (dry eyes) and xerostomia (dry mouth) also termed as sicca syndrome [5–8].

Initial manifestations of keratoconjunctivitis are the consequences of both corneal and bulbar conjunctival epithelium destruction, which is generally associated with dilatation of the bulbar conjunctival vessels and glands enlargement. SS patients usually complain of a burning sensation, redness, and photophobia [5]. Likewise, xerostomia is characterized by dry oral mucosa, poor dentition, caries, unilateral or bilateral SG enlargement and decrease of saliva with concomitant difficulty to swallow, speak, and taste food [8]. Beyond the classic sicca symptoms, SS patients also display systemic involvement of other organs such as lungs, skin, kidneys and joints mainly [9–12]. A quantum leap has been made in unearthing and deciphering the inherent pathobiological mechanisms underlying SS but the main culprit in initiating disease is yet to be discovered. Nevertheless, SS is considered as an autoimmune epithelitis in which epithelial cell play a central role. In this context, salivary gland epithelial cell (SGEC) dysregulation involves alteration affecting proteins implicated in saliva formation, such as AQP5. Indeed, altered AQP5 distribution and altered expression and localization of AQP5

protein partners have been incriminated in SS [13–15]. This review summarizes the current state of knowledge concerning the involvement of AQP5 in Sjögren's syndrome.

2. Current knowledge of Sjögren's syndrome physiopathology

The etiopathogenesis of SS remains largely unknown. It is postulated that on a predisposing genetic and hormonal background, an exogenous - probably viral - or endogenous element leads to the activation of epithelial cells [3]. The latter acquire an increased propensity for apoptosis - generating apoptotic blebs rich in nucleic autoantigens - and the possibility of behaving like a non-professional antigen-presenting cell of the immune system. This leads to the attraction and activation of autoreactive T cells, which in turn activate B cells. The lymphocyte infiltrate perpetuates epithelial abnormalities and produces cytokines and autoantibodies: it is the so-called « autoimmune epithelitis » expressing histologically as a focal sialadenitis [16,17].

Extraglandular/systemic manifestations of SS are related to local complications of exocrinopathy, peri-epithelial infiltrates affecting other epithelia (i.e. bronchus, kidney), systemic autoimmunity (i.e. cryoglobulinemia, organ-specific autoantibodies) or unregulated proliferation of B lymphocytes (i.e. lymphocytic interstitial pneumonia, lymphoma).

Contrary to popular belief, glandular dysfunction resulting in SS exocrinopathy is not related to increased apoptosis of epithelial cells or destruction of glandular parenchyma by lymphocytic infiltrate. Different abnormalities of the parasympathetic neuroexocrine pathway - not mutually exclusive - have been described in SS and collectively form the "non-apoptotic theories" of SS exocrinopathy [18]. Downstream to this neuroexocrine pathway is AQP5, the aquaporin responsible for acinar apical water permeability, the dysfunction of which could be involved in the reduced glandular flows observed during pSS (Figure 1).

3. Insight into Aquaporin 5

Aquaporin-5 (AQP5) is a member of the water-channel protein family named aquaporins (AQPs) ensuring transmembrane water permeability. AQP5 was cloned from rat submandibular gland and shows a wide distribution in the human body [19]. Indeed, human AQP5 is highly expressed in various tissues including salivary, lacrimal and sweat glands [20], compartments of digestive system such as stomach, pancreas, duodenal Brunner's glands [21–23], lens fiber cells and corneal epithelium in the eye [24], lungs and sub-mucosal glands [25,26]. AQP5 (made of 256 amino acids) form a functional tetramer made of four identical monomers. Each of the AQP5 monomer composing the tetramer behaves as a water pore and exhibits six transmembrane alpha-helices (1-6) interconnected by five loops (A-E): three are extracellular and two are cytoplasmic. Both amino- and carboxyl- termini are located intracellularly within the cytoplasm. Two half-helices constituted by the folding of loops B and E enclose the two highly conserved NPA motifs (asparagine-proline-alanine, NPA) [21,27,28]. The conserved motifs are localized in the narrow central constriction of the channel, where they play a critical role together with the ar/R constriction region or selectivity filter (SF), ensuring high selectivity of the pore in water and solute permeation [28]. AQP5 trafficking to the plasma membrane is regulated by distinct independent mechanisms including phosphorylation of Ser156, protein kinase A activity and extracellular osmolarity [29].

4. Expression of AQPs in salivary and lacrimal glands

AQP5 is considered as the principal player in saliva secretion. Indeed, upon nerve stimulation, AQP5 translocates from intracellular vesicles to the plasma membrane of acinar cells, therefore allowing transcellular water passage into the acini lumen and contributing to the saliva formation [30–33]. AQP5 protein is expressed in rat, mouse and human submandibular, parotid and sublingual salivary glands [34–36]. In rat, AQP5 expression is predominantly located in the apical membrane of acinar cells [37–39]. In mice, both the apical and basolateral acinar cells membranes displayed positive AQP5 labeling [40–43]. In addition, intercalated ducts also

expressed AQP5 protein at their apical membrane, but its functional role has not been deciphered [44]. In human parotid, submandibular and labial glands, AQP5 labelling was restricted to the apical membrane of acinar cells, but absent in ductal cells [45,46].

AQP5 is expressed at the apical membrane of acinar and ductal cells of mouse extraorbital and intraorbital lacrimal glands [47,48]. In rat, AQP5 protein was exclusively located at the apical membrane of lacrimal acinar cells [39,48,49].

5. Role of AQP5 in saliva and lacrimal secretion

The key role of AQP5 in the production of saliva has been highlighted by several studies carried out on knockout mice [50,51]. Indeed, as compared to wild type mice, *Aqp5* deficient mice are characterized by a 60% reduction of pilocarpine-induced saliva flow, hypertonic (420mOsm) and more viscous saliva [50]. Furthermore, acinar cells from the parotid and sublingual glands of *Aqp5* knockout mice exhibited a reduced ability to adapt to osmotic changes (65% and 77%, respectively, compared to similar cells from wild type mice) [50,51]. Altogether, these results revealed the important role played by AQP5 in saliva secretion under physiological conditions.

Saliva is a watery secretion containing various electrolytes and proteins. Saliva secretion is triggered by a nervous cholinergic stimulation inducing Ca^{2+} release from intracellular storage sites (ER, mitochondria) and subsequent 5 to 10-fold rise in cytoplasmic Ca^{2+} concentration. Consequently, such rise in intracellular Ca^{2+} provokes the entrance of Cl^{-} into the acinar cells via the basolateral $Na^{+}/K^{+}/2Cl^{-}$ cotransporter (NKCC1) and the secretion of Cl^{-} into the acini lumen via the apical Cl^{-} channels (TMEM16/ANO1) [52] culminating in an accumulation of salt within the acini lumen [53]. The resulting osmotic gradient induces transcellular water movement into the acini lumen concomitantly to the translocation of AQP5 to the acinar apical membrane [53,54]. The resulting isotonic primary saliva generated by this mechanism is pushed

towards the ductal lumen by the contractile force of the myoepithelial cells surrounding the basal side of acini. Upon passage through the ductal lumen, the composition of the primary saliva is modified by the ductal cells which reabsorb most of the NaCl through Na^+ channels (mainly ENaC, epithelial sodium channel), Na^+/H^+ exchangers (Nhe2 and Nhe3 channels), Cl^- channels (CFTR channel and Cl^-/HCO_3^- exchangers) and secrete K^+ and HCO_3^- via Cl^-/HCO_3^- and K^+/H^+ exchangers [53–55]. Following completion of this process, the final saliva entering the mouth cavity is hypotonic.

The role of AQP5 in tear secretion remains a subject of debate considering some *Aqp5* knockout mice displayed similar [56,57] or decreased (±50%) [58] tear secretion. Therefore, further studies are necessary to clarify the role of AQP5 in tear secretion.

6. Regulation of AQP5 trafficking under physiological conditions

AQP trafficking from intracellular vesicles to the cell apical membrane is a complex process triggered by a combination of different stimuli such as post-translation modifications, binding of neurotransmitters to G protein-coupled receptor (GPCR) and interaction with several partner proteins that act as motor along microtubules or scaffold along the cell membrane [59–61]. Considering the high sequence homology between AQP5 and AQP2 (a renal AQP), the numerous studies dedicated to deciphering AQP2 trafficking responsible for renal water reabsorption upon vasopressin stimulation [62–64] have laid the foundation to unravel AQP5 translocation. To date, the current understanding of AQP5 trafficking occurring in salivary glands under physiological conditions indicates AQP5 translocates to the apical membrane in response to acetylcholine (Ach) and noradrenalin (NA) binding to M3 and adrenergic receptors, respectively [65,66]. These neurotransmitters induce a massive increase in cytoplasmic calcium and cAMP concentrations and the subsequent activation of PKC and PKA, respectively. Despite the presence of two consensus PKA sites in AQP5 cytoplasmic loop D

(Ser156) and carboxyl-terminus (Thr259), their phosphorylation has not been directly associated with the protein trafficking [29,67]. Rather, it has been suggested that AQP5 trafficking is regulated by at least three independent mechanisms involving Ser156 phosphorylation, protein kinase A activity and extracellular tonicity [29]. Still, further studies are required to further deepen of our understanding of AQP5 trafficking.

7. AQP5 interactome

Protein–protein interaction is necessary for most biological processes, including the trafficking of AQPs that is necessary to ensure several important human physiological functions such as urine concentration [68], maintenance of eye lens transparency [69], endocrine and exocrine secretions including saliva [47,70–72].

Molecular and structural analysis of AQP0 [73,74], AQP1 [75], AQP2 [76] and AQP5 [27] have revealed that C-terminal helices share an amphipathic character due to the presence of hydrophobic residues. This suggested a potential interaction between this region and considerable number of putative protein partners. The mechanism by which vesicles containing AQP2 traffic to the membrane likely involves interactions with the cytoskeleton and other chaperon partners. In this scenario, protein partners interacting with microtubules induce a substantial modifications and rearrangements of the dense cytoskeleton network facilitating the access of vesicles to the cellular membrane [77,78]. While the AQP2 interactome has been extensively studied [79–83], the AQP5 interactome has been poorly explored. Few AQP5-interacting protein partners have been identified to date and shown some modification in SS (Table 1). Some proteins of the AQP5-interactome are discussed below in more details.

Among the AQP5 interactome, prolactin-inducible protein (PIP) is a glycoprotein discovered in different human tissues. It's an immature pre-protein consisting of 146 amino acid residues of which 28 are cleaved to give rise to secreted mature protein. Due to its versatile nature, PIP

is responsible for many physiological functions such as inhibitory effect on oral bacteria growth, aspartyl proteinase and immunomodulatory role. In addition, PIP expression is regulated by hormones. Indeed, androgens and prolactin induce an over-expression of PIP, contrarily to estrogen that leads to a down-expression. PIP is highly expressed in lacrimal glands of control mice compared to SS mice in which it appears decreased [84]. Affinity chromatography using a synthetic peptide corresponding to the C-terminus of murine AQP5 revealed its binding with PIP present in lacrimal gland homogenates from control mice, while PIP was absent in lacrimal glands from SS mice. In addition, treatment of control mice with antisense PIP oligonucleotides decreased immunostaining of AQP5 at the apical membrane of lacrimal gland acinar cells [84]. The decreased AQP5 expression in lacrimal glands from SS mice [84] and SS patients [85] may result from the absence of PIP. Our laboratory has recently shown for the first time that AQP5-PIP interaction also exists in salivary glands [14]. Moreover, a significant step forward was made showing the AQP5-PIP interaction in SS human minor salivary gland (hMSG) biopsies [14]. The distribution of the AQP5-PIP complexes in hMSG acini from patients with sicca symptoms and pSS (SICCA-SS) was altered as compared to patients with sicca symptoms without SS (SICCA-NS; used as control). These data suggest that PIP may be involved in AQP5 trafficking through cytoskeleton binding and membrane vesicle mobilization and an aberrant expression of PIP could account for AQP5 mislocalization observed in SS salivary [46,86] and lacrimal glands [84,85]. Further analysis of the AQP5-PIP interaction has revealed the involvement of the AQP5 C-terminus and the N-terminal of PIP with a ratio of one molecule of PIP per AQP5 tetramer [14].

Ezrin is another interesting protein being a member of the AQP5 interactome. Ezrin belongs to the cross-linker proteins family which includes Radexin and Moesin (ERM). ERM proteins are localized near plasma membrane, in specific cell compartments such as microvilli, membrane ruffles and cell-cell contact regions suggesting an important role in cell motility, cell shape

preservation and protein trafficking. The major regulator of ERM assembly to the actin filaments is Rho, a small GTP binding protein. This interaction induces the switching of ezrin from a closed and inaccessible conformation (inactive) to an open one (active) [87]. The ezrin membrane-cytoskeleton linker role was revealed for the first-time using glutathione-S-transferase fusion proteins of truncated ezrin in affinity chromatography using actin from the cell extract and purified rabbit muscle actin. The ability of ezrin to bind actin appears to be a fundamental prerequisite for addressing the vesicles to the apical membrane [88]. The AQP5-ezrin protein-protein interaction has recently been shown for the first time by our laboratory in salivary glands from healthy and SS patients [15]. AQP5–ezrin complexes were predominantly localized at the apical side of hMSG acini from SICCA-NS patients but disrupted and mislocalized in hMSG acini from SICCA-SS patients. Considering of the role of ezrin as a linker between the cytoplasmic membrane and cytoskeleton, its loss [89] could be responsible for aberrant AQP5 trafficking and mislocalization observed in SS salivary glands [15].

SLC12A2, also named Na-K-Cl Symporter (NKCC1), has been identified as an AQP5-interacting protein partner by immunoprecipitation in human embryonic kidney 293 (HEK293) cells surexpressing both proteins [90]. While NKCC1 and AQP5 coexpression was observed at the lumen of mouse salivary gland acinar cells [90], their *in-situ* interaction remains to be corroborated by proximity ligation assay on salivary gland sections. Additional studies are necessary to further study the expression of NKCC1 in SS.

Cell-cell adhesion proteins including -catenin, tight junction protein 1 (TJP1, also named zona occludens 1 (ZO-1)), plakoglobin, and desmoglein-2 have been shown to interact with AQP5 by pull down assay using Glutathione-S-transferase (GST)-tagged AQP5 and GST-tagged AQP5 C-terminal end expressed in HEK293 cells followed by mass spectrometry analysis [91]. The expression of all these junctional proteins was reduced upon AQP5 overexpression [91]. The latter occurring in cancers may participate to epithelial-mesenchymal transition by

reducing cell-cell adhesion and to increased cell migration by facilitating higher water movement. The maintenance of cell polarity is essential for salivary gland acinar cell function (i.e. trafficking of secretory vesicles to the plasma membrane) and is regulated by a complex machinery involving tight junctions, hemidesmosomes and polarity complexes [92]. Disruption of cell polarity has been involved in SS [92]. The Wnt/ -catenin signaling pathway may be altered in SS [93] and genetic polymorphisms of some genes of the pathway have been linked to SS [94]. ZO-1 expression was reduced or absent in salivary gland areas with lymphocytic infiltration in SS mice [95,96]. Furthermore, interleukin-17 (IL17), a cytokine involved in SS, decreased the expression of ZO-1 in cultured salivary gland tissue [95]. Therefore, IL17 may account for the reduced or absent ZO-1 expression observed *in vivo*. In mouse model of SS, IL17 blockage decreased inflammation and restored saliva secretion [97] and vasoactive intestinal peptide administration decreased IL17 expression, upregulated AQP5 expression and restored saliva secretion [98]. However, it remains to assess whether these effect on saliva secretion is concomitant to a restoration of the expression of AQP5 and/or AQP5-interacting protein partners. Finally, it is still necessary to assess if AQP5 binds to cell-cell adhesion proteins *in-situ* and if such protein-protein interactions are perturbed in SS.

8. Involvement of AQPs in SS pathogenesis

Studies reported a down-regulation of AQP1 expression [99] and presence of anti-AQP1 autoantibodies [100] in SS salivary glands. However, that is unlikely to impact SG functionality as AQP1-deficient mice did not show any alterations of salivary volume or composition [101]. In this context, the role of AQP1 in SS pathology remains unclear. On the other hand, the aberrant expression and localization of AQP5 has recently been proposed as one of the mechanisms responsible for xerostomia in SS patients. For a long time, NOD mice were used as phenotypical representation of SS [102]. Although it is not a perfect model, it shares some cardinal features with SS such as spontaneous infiltration of salivary and lacrimal glands,

autoantibodies production, exocrine glands destruction and loss of salivary flow rates [103]. Eight-week-old female NOD mice without inflammation expressed AQP5 primarily at the acinar apical membrane of submandibular glands. By opposition, 24-week-old NOD mice presenting high level of inflammation showed a significant reduction in AQP5 expression in acinar cells at the apical membrane but an increase in AQP5 expression at the basal membrane [104]. Age-matched control mice (8- and 24-week-old) showed an acinar apical localization of AQP5 [104–106]. AQP5 expression in salivary glands from other SS mouse models such IQI/JIC mice [106], NOD/SCID.E2f1−/− mice [107], mice immunized with submandibular gland autoantigen [108], and specific T-cell class IA phosphoinositide 3-kinase (r1 T/r2n) knockout mice [106] confirmed the data obtained in NOD mice [104,105]. Similarly, the initial observation of altered localization of AQP5 in hMSG from SS patients [46] was corroborated [86] or not [109] by others. Patient variability and use of distinct antibodies may account for these divergent data. Still, abnormal AQP5 expression and distribution in salivary gland acini may contribute to hyposalivation.

9. Role of inflammation on AQP5 expression and localization

A link between inflammation and aberrant AQP5 distribution has been hypothesized [104,106]. Indeed, in several mice models such as 24-week-old NOD, 10- and 13-month-old IQI/JIC and r1 T/r2n with an inflammatory focus score 1, decreased apical aquaporin-5 labelling index with concomitant increased apical-basolateral, apical-cytoplasmic and/or apical-basolateral-cytoplasmic aquaporin-5 labelling indices were observed [106]. The altered distribution of AQP5 appears to be concomitant to the presence of inflammatory infiltrates and glandular epithelial tissue destruction suggesting a hypothetic role of the inflammatory environment on AQP5 aberrant trafficking [106]. Other studies have observed that TNF treatment was associated with a downregulation of AQP5 in human SG acinar cells [110] and the injection of antibodies against TNF in NOD mice reduced SG inflammatory foci and increased the

expression level of tight junction protein claudin-1 and AQP5 [111]. As well, neutralization of IFN- in anti-programmed death ligand 1 (PDL1)-treated non-obese diabetic (NOD)/ShiLtJ mice improved AQP5 expression and saliva secretion [112] corroborating the hypothesis that inflammation could be an excellent reason for AQP5 decreased expression and mislocalization in SS patients. In SS mouse models, adeno-associated viral (AAV2)-AQP1 vector [113] or cystic fibrosis transmembrane conductance regulator (CFTR) corrector C18 [114] delivery to submandibular gland resolved inflammation and restored saliva flow. Besides, IL17 blockage also decreased inflammation and improved saliva secretion [97] and vasoactive intestinal peptide administration lowered IL17 expression, increased AQP5 expression and restored saliva secretion [98] in SS mouse model.

Integrity of acini and proper expression of tight junctions between epithelial cells is essential for the establishment of apico-basal polarity and regulation of paracellular flow of ions and water [95]. In SS, proinflammatory cytokines have been shown to disrupt salivary gland acinar cell polarity by inducing an apico-basal relocation of proteins involved in the maintenance of cell polarity [95,96,115–117]. Consequently, the aberrant localization of AQP5 in acinar cells from salivary glands [46,86,104,105] or lacrimal glands [84,85] may result from decreased or lost expression of proteins involved in cell polarity concomitant to local proinflammatory cytokine production. Other mechanisms may also contribute to the AQP5 altered distribution and salivary gland hypofunction, including the aberrant lymphocytic B hyperreactivity and autoantibodies production against M3 muscarinic receptor [118–120] and anti-AQPs antibodies. Concerning anti-AQPs antibodies, SS patients presenting autoantibodies against the extracellular domain of AQP8 and AQP9 (high frequency - 39%) or against AQP1 and AQP3 (low frequency) display more severe xerophthalmia as compared to healthy controls [121]. The presence of anti-AQP5 antibodies detected in pSS patients [120,122–124] was associated with decreased AQP5 functionality [122], lower saliva flow [124] and histopathological feature of

SS salivary glands [123]. Induction of anti-AQP5 antibodies production by mice immunization with a peptide derived from the AQP5-homologous AQP of *Prevotella melaninogenica* led to saliva flow reduction [125].

Overall, inflammation and auto-immunity may on one hand affect salivary gland function by modifying AQP5 expression/distribution and on the other hand also be perpetuated by salivary gland deregulation.

10. Aquaporins as therapeutic target and therapeutic leverage

10.1. Drugs

To date, treatment for SS remains rather disappointing as they have been mainly used to relief the central “fatigue-pain-dryness” symptomatic triad and to achieve systemic immunosuppression to reduce systemic non-glandular manifestations.

Systemic administration of muscarinic agonists such as pilocarpine and cevimeline is commonly used to control xerostomia in SS patients. Fundamental research data in rodents demonstrate that chronic treatment with cevimeline can force and maintain AQP5 translocation to the apical membrane of acinar cells and increase saliva production in SS mouse model [126,127].

The effect of immunosuppressive and targeted drugs on the expression and localization of AQP5 is poorly studied. In a case report, Ring et al reported an increase in AQP5 expression in salivary glands after 3 months in a SS patient treated with Rituximab (anti-CD20) with good clinical response [128]. TNF , which represents a theoretical interesting target in SS, inhibited AQP5 expression in immortalized acinar cells line [110] and its blockade in NOD mice induced clinical remission of exocrinopathy as well as a marked increase in the expression of claudin-1 and AQP5 in submandibular glands [111]. As expected, the use of anti-TNF targeted therapy on SS-like background leads to the paradoxical increase in levels of anti-nuclear antibodies

(ANA) and anti-muscarinic receptor type 3 (M3R) autoantibodies despite the decrease in salivary gland-infiltrating T and B cells in those mice [111]. In human, randomized clinical trial failed to demonstrate a positive effect of infliximab - anti-TNF therapy - on fatigue, pain and glandular functions in pSS patients [129]. The main adverse effect of infliximab was an increase in gammaglobulin levels with the appearance of anti-dsDNA in 10.3% of patients [129]. For these reasons, anti-TNF targeted therapy is not a suitable treatment for pSS.

However, indirect targeting of TNF using anti-IL7R treatments seems promising in NOD mice as improved salivary flow rate and decreased salivary gland infiltration were observed after 3 weeks of treatment [130]. Blockade of IL-7R leads to decreased TNF production and increased claudin-1 and AQP5 expression in salivary glands, with a non-statistically significant increase in autoantibody levels [130]. Unfortunately, phase-II trial of GSK2618960 - an anti–IL-7R targeted therapy - in pSS was stopped due to portfolio prioritization [ClinicalTrials.gov Identifier: NCT03239600].

Interestingly, promising new therapies targeting the CD40-CD40L pathway inhibited sialadenitis and ectopic lymphoid structures without inducing changes in the expression and localization of AQP5 in NOD mice [131]. Phase II TWINSS trials of iscalimab (CFZ533) - an anti-CD40 antibody - are currently recruiting. A pilot study showed a positive effect of CFZ533 10 mg/kg IV on systemic activity (Eular *Sjögren* Syndrome Disease Activity index (ESSDAI) and physician global assessment (PGA)) and Schirmer's test but a marginal increase in salivary flow rate [132].

Taken together, these data seem to suggest that SS has two facets: lymphocytic infiltration (autoimmune epithelitis) and glandular dysfunction (exocrinopathy). Although the latter is may be a consequence of the former, these two facets may respond differently to a given therapy.

Considering AQP5-interacting protein partners may be involved in AQP5 trafficking to the acinar plasma membrane, future research may be warranted to develop corrector molecules capable to rescue AQP5.

10.2. Gene therapy

To overcome the limitations of conventional therapies in the treatment of SS, gene therapy provides the possibility to engineer target cells to improve or correct the disease. In SS, SG represent a key target for gene therapies due to their loss of functionality and easy access using ductal cannulation. AQPs have been considered as potential novel therapeutics to improve hyposalivation in radiation-induced salivary gland hypofunction in animals and humans [133–136]. Indeed, AQP1 gene delivery improved saliva flow in irradiated rats [133] and irradiated minipig [137]. Moreover, a clinical trial revealed that AQP1 gene delivery to PG from 11 patients who undergone radiotherapy (RT) improved the symptoms of xerostomia in five patients. In addition, gene therapy showed only minimal adverse effects, proving to be safe, well tolerated, and suitable in any future gene therapy approaches [138]. In bone morphogenetic protein 6 (BMP6) overexpressing mice, representing a model of SS, local adeno-associated viral (AAV2)-AQP1 vector delivery to their submandibular salivary glands restored saliva secretion and decreased proinflammatory cytokines expression (IFN- , IL-17, IL-23 and chemokines; typical hallmark of the pathology) [113]. In SS mouse models, submandibular gland delivery of either adeno-associated viral (AAV2)-AQP1 vector [113] or cystic fibrosis transmembrane conductance regulator (CFTR) corrector C18 [114] resolved inflammation and restored saliva flow. Current promising clinical trial has been undertaken to investigate AAV2-AQP1 gene therapy effects on salivary gland functionality in irradiated-induced xerostomia [ClinicalTrials.gov Identifier: NCT02446249]. However, further studies will be necessary to determine if AQP1 gene therapy holds its promise to treat pSS patients. It is interesting to note that other gene therapies have also been conducted to improve hyposalivation. Indeed, gene

therapy aiming at neutralizing inflammatory mediators such as B-cell activating factors and proliferation-inducing ligand showed a significantly reduction of CD138+ inflammatory cells and IgG and IgM in SG [139]. In addition, gene delivery of human keratinocyte growth factor exhibited a protective effect against irradiated-induced salivary gland dysfunction and salivary flow independently of an effect on AQP5 expression [140]. These data further emphasize the promising role of gene therapy to improve hyposalivation. Furthermore, it remains to be determined whether the use of viral vector coding for a AQP5-interacting protein partners may rescue AQP5 in SS.

10.3. Tissue regenerative medicine

Tissue regenerative medicine may provide a unique opportunity to regenerate salivary gland expressing AQP5 and displaying full functionality, i.e. saliva secretion. In recent years, the stem cell therapy has captured the attention of the scientific world as an encouraging treatment for autoimmune disorders [141]. Cell transfer therapy is expected to induce profound healing activity by modulating the immune system and pathological responses in SS pathology in which the inflammation and lymphoepithelial lesion play predominant roles [142]. The adult stem cells (also known as somatic or tissue stem cells) are rare and undifferentiated populations localized within fully developed tissues [143]. Tissue stem cells can supply new differentiated functional progeny when an adult tissue is injured (tissue plasticity), ensuring the maintenance of tissue homeostasis [142,143]. The great advantage of tissue stem cells resides in their easy harvest via minimally non-invasive procedures with limited histocompatibility and ethical concerns [144]. Tissue stem cells such as hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are capable to rescue irradiation-induced salivary gland hypofunction [142]. MSCs possess the ability to interact with immune cells [145] and to secrete multiple bioactive molecules (including growth factors, cytokines and chemokines), and thereof to have relevant effects on local cellular dynamic [146]. MSCs paracrine effects can be divided into trophic,

immunomodulatory, anti-scarring and chemoattractant [147]. Consequently, MSCs have been tested in the treatment of numerous diseases, mainly tissue injury and immune disorders [148] including pSS [149,150]. MSCs administration can improve salivary gland function [151–154]. Indeed, injection of bone marrow (BM)-MSCs in presence of anti-BMP6 antibodies has been shown to restore salivary flow in SS mice model [154]. BMP6, elevated in pSS patients, has been shown to impair the immunomodulatory action of MSCs by affecting the expression of genes related to inflammation and salivary gland function [151]. Anti-BMP6 may protect some salivary gland secreting function-related gene expression, such as AQP5 and NKCC1. In addition, MSCs injection induced a modification in electrolyte concentration, by decreasing Na+ and Cl- with a slight increase in K+ ions [151]. BM-MSCs infusion has also been shown to promote significant decrease in ESSDAI score [152].

Decisive advancement in salivary gland regenerative medicine has been made by the transplant of a bioengineered salivary gland (made from embryonic epithelium and mesenchyme) into a salivary gland-defective mouse model to restore functional salivary gland [155]. One of the most promising approaches for salivary gland bioengineering involves optimal combination of cells, matrix components and soluble cues [156,157]. Specific factors have been shown to promote growth of adult stem cells [156]. Mouse submandibular gland cells encapsulation within hydrogels (such as Matrigel, hyaluronic acid-based hydrogels) promoted the expression of NKCC1, ZO-1 and AQP5 [158]. Moreover, acini-like spheroids grown on Matrigel-coated surfaces showed the functional ability to express tight junction proteins (i.e occluding and claudin proteins, JAMA and ZO-1) [159,160]. Laminin incorporation into Matrigel has been explored to recapitulate the extracellular matrix, playing a critical role in salivary gland development and morphogenesis. In fact, the presence of laminin-111 in MSCs-conditioned media induced an increment of acinar-like structures, as well as AQP5 and keratin 14 (K14) expressions [161]. Specific soluble factors seem to provide supportive cues. Indeed, the

neurotrophic and fibroblast growth factors (FGFs) [162], as well as chemical inhibitors of Rho-associated kinase (ROCK) (PMID: 31731180), transforming growth factor receptor (TGF R) [163] and epidermal growth factor receptor (EGFR) [162] have an impact on the expression of important cell markers, including AQP5. One of the challenges of tissue engineering is to fabricate living tissues in large scale for clinical applications thanks to 3D-bioprinting [164]. Tissue engineering will likely be improved by incorporating bioprinting approaches in engineering paradigm combining with different manufacturing employing cells, growth factors, dynamic biomaterials may supply future and promising opportunities.

11. Conclusions

The passage of water and ions plays a key role in cell osmotic regulation and is of upmost importance for the cells to adapt to any sudden change in osmolarity of their surrounding environment. In the past few years, it has been possible to appreciate that salivary gland cells use their complex internal machinery composed of a myriad of proteins and intricated interactions to ensure their functionality under physiological conditions. In pathological conditions, this complex machinery can be perturbed and lead to deregulation of various processes and activate a detrimental progression that may even cell death. AQP5 is the main water channel responsible for the membrane permeability of salivary and lacrimal gland cells and it plays a major role in the production of saliva and tear. The alteration of its expression/localization due to the inflammatory microenvironment, global exocrinopathy and lymphocytic epithelitis have been well documented in SS patients and several SS animal models. In addition, it is interesting to consider that a dramatic alteration of AQP5 interactome may also explain several features observed in SS patients. Despite its central role in SS, few treatments specifically targeting AQP5 have been developed. Indeed, muscarinic receptor agonists, pilocarpine and cevimeline, can restore saliva secretion by forcing the trafficking of AQP5 to the acinar apical membrane. Other new treatments have not demonstrated any effect

on the localization of AQP5 or on the restoration of saliva flow. New innovative therapies including drug treatments, gene therapy, stem cells or glands bioengineering represent future opportunities for SS treatment despite their unique challenges. In the future, by correcting exocrinopathy and lastingly relieving the patient's dryness, these therapies will likely succeed where biotherapies and immunomodulatory therapies have failed so far.

Author Contributions: Conceptualization, C.D.; writing—original draft preparation, writing—review and editing, C.D.A., D.P., C.C., M.H., M.S., C.D.; visualization, C.D.A., D.P., C.C., M.H., M.S., C.D.; supervision, C.D.; project administration, C.D.; funding acquisition, C.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Université Libre de Bruxelles, the Fonds de la Recherche Scientifique - FNRS (Fund for Scientific Research - PINT-BILAT-P-R.P006.19 (C.D.); Foundation Jaumotte-Demoulin (C.D.); Fonds Erasme pour la recherche médicale (D.P.). D.P. is a Research Fellow of the Fonds de la Recherche Scientifique – FNRS.

Conflicts of Interest: The authors declare no conflict of interest.

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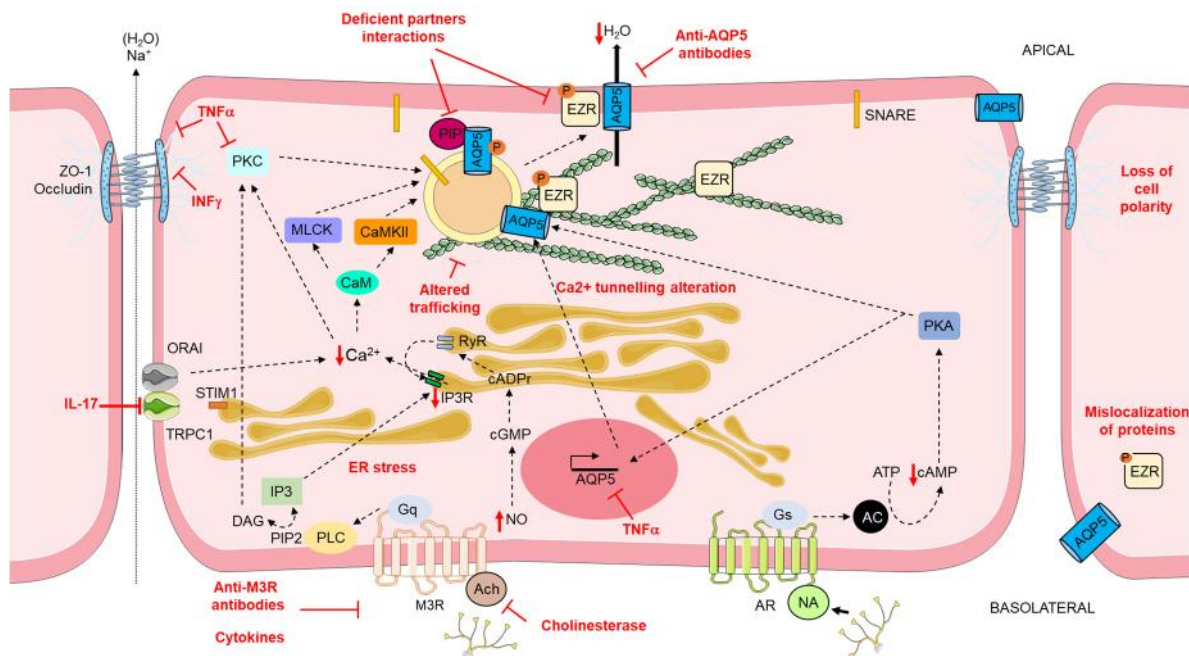
Figure 1: Aquaporin-5 physiology in healthy and Sjögren's Syndrome salivary gland acinar cells.



Under physiological conditions, release of acetylcholine from parasympathetic nerves activates the acetylcholine muscarinic M3 receptor leading to increased intracellular calcium signaling resulting in trafficking of AQP5 - whose transcription and translation is under noradrenergic control - to the apical membrane, via interaction with its partner proteins. In SS, several adverse conditions - shown in red - lead to glandular dysfunction and clinical exocrinopathy.

⇩: decrease; ⇧: increase; Ach: acetylcholine; AQP5: aquaporin-5; AR: adrenergic receptor; ATP: adenosine triphosphate; cADPr: cyclic adenosine diphosphate-ribose; CaM: calmodulin; CaMKII: Calcium/calmodulin-dependent protein kinase II; cAMP: Cyclic adenosine 3', 5' monophosphate; cGMP: Cyclic guanosine 3', 5' monophosphate; DAG: diacylglycerol; ER: endoplasmic reticulum; EZR: erzin; Gq: Gq protein; Gs: Gs protein; IL-17: interleukin-17; IP3: Inositol trisphosphate; IP3R: IP3 receptor; M3R: acetylcholine muscarinic M3 receptors; MLCK: myosin light chain kinase; NA: noradrenalin; NO: nitric oxide; ORAI: ORAI calcium release-activated calcium modulator 1; P: phosphorylation; PKA: protein kinase A; PKC: protein kinase C; PLC: phospholipase C; PIP: prolactin-inducible protein; PIP2: Phosphatidylinositol 4,5-bisphosphate; RyR: Ryanodine receptors; SNARE: Soluble N-ethylmaleimide-Sensitive Factor Attachment Proteins Receptors; STIM1: stromal interaction molecule 1; TNF : tumor necrosis factor alpha; TRPC1: Classical Transient Receptor Potential 1; INF : interferon gamma; ZO-1: Zonula occludens-1.

Table 1: AQP5-interacting protein partners.

IHC: immunohistochemistry; IP: immunoprecipitation; MS: mass spectrometry; MST: microscale thermophoresis; PLA: proximity ligation assay

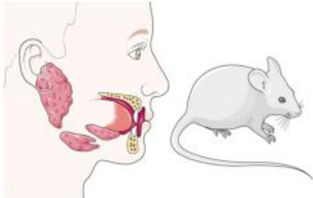
Proteins	Method	Functions	Modification in pSS	Ref
Ezrin	IP, MS, CM, PLA	Linker between proteins and cytoskeleton	Reduced expression in pSS SGs Aberrant localization in pSS SGs acini	[15,89]
PIP	IP, MS, MST, PLA	Biological role poorly understood Controversial immunomodulatory functions Involved in cell-mediate adoptive immunity	Reduced expression in LGs of SS mouse model Reduced expression in pSS SGs and saliva	[14,84,165,166]
NKCC1	IP	Sodium/Potassium/Chloride Transporter involved in saliva secretion	Decreased expression of NKCC1 in LAMP3-SS mouse model	[90,167,168]
AE2	IP	Anion exchanger involved in saliva secretion, intracellular pH regulation, bicarbonate secretion, chloride uptake	Absence of AE2 immunoreactivity in pSS SGs	[90,169]
TRPV4	IP	Ca2+ permeable, nonselective cation channel involved in the regulation of systemic osmotic pressure, saliva and tear secretions	N.D.	[170,171]
MUC5AC	IP	Extracellular matrix constituent involved in phosphatidylinositol-mediated signalling	Reduced levels in pSS tear fluid	[172,173]
CD44	IP, MS	Cell-surface glycoprotein acting as receptor for cell migration and extracellular matrix adhesion	Slight increase of CD44 serum levels in pSS	[91,174,175]
CISD2	IP, MS	Iron Sulfur Domain 2 playing a crucial role in ER-mitochondrial Ca2+ signalling. The exact mechanisms	N.D.	[91,176]

		involving intracellular Ca2+-transport systems remain unclear.	Remarks: Reduction in IP3R2 and IP3R3-protein levels and a concomitant decrease in IP3R-mediated Ca2+ release pSS SGEC	
CLCC1	IP, MS	Chloride channel CLIC-like protein-1 involved in chloride transport	N.D.	[91]
CD55	IP, MS	Complement decay-accelerating factor inhibiting complement activation.	Increased expression in pSS SGs	[91,177]
MOXD1	IP, MS	DBH-like monooxygenase protein-1. Function unknown	N.D.	[91]
DSG2	IP, MS	Desmoglein-2 Main desmoglein in desmosomes Promotes cell-cell adhesion	Upregulated during the early-onset phases in SS mouse model	[91,178, 179]
RPN1	IP, MS	Part of 26S proteasome in RER	N.D. Remark: LPS-induced AQP5 mRNA decrease is blocked by 26S proteasome inhibitor	[91,180]
HSPA5 (GRP78)	IP, MS	ER chaperone protein from HSP70 family	Conflicting data on protein variation in pSS SGs (⇩?) HSPA5 co-localize with Mucin1 in pSS SGs HSPA5 binds Ro-52 and may trigger autoimmunity HSPA5-ATF6-CHOP apoptosis pathway in pSS SGs	[91,181–183]
ERLIN2	IP,MS	Lipid raft-associated protein at ER location Critical role in ER-associated degradation of activated IP3 receptors	N.D. Remarks: IP3R deficit described in pSS SGs; IP3R KO mice display pSS phenotype	[91,184, 185]
FLOT1 FLOT2	IP, MS, IHC	Caveolae-associated, integral membrane protein Plays a role in vesicle trafficking and cell morphology Co-localized with AQP5 and increased by Cevimeline in rat parotids; Role in M3R internalization in SGEC	N.D.	[91,186, 187]
JUP	IP, MS	Part of desmosomes and intermediate junctions	N.D.	[91]
LRRC59	IP, MS	Enables RNA and cadherin binding activity Modulates type I interferon signalling	N.D.	[91,188]
PELP1	IP, MS	Estrogen receptor coactivator	N.D. Remark: Expressed in salivary duct carcinoma	[91,189]

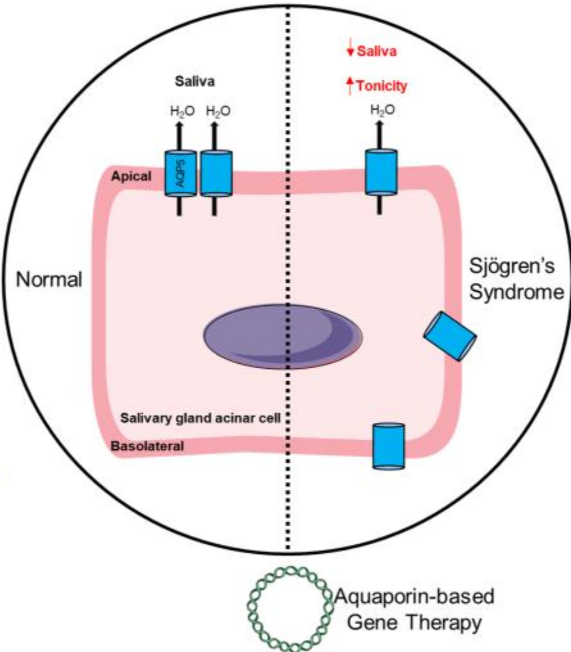
CNIH4	IP, MS	Enables CCR5 chemokine receptor binding activity Involved in ER to Golgi vesicle-mediated transport	N.D.	[91]
FYN	IP, MS	Protein-tyrosine kinase oncogene	N.D.	[91]
CACNA2D1	IP, MS	alpha-2/delta subunit protein involved in the voltage-dependent calcium channel complex	N.D. Remark: Role in central hyperalgesia of dry eye related pain in Sicca	[91,190]

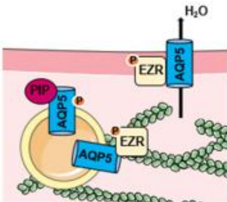
Graphical Abstract

AQUAPORIN-5 IN SJÖGREN SYNDROME

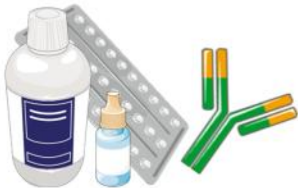


Altered expression and localization of AQP5 in Sjögren's Syndrome patients and mouse models





Role of AQP5-interacting partner proteins



Symptomatic and immunomodulatory drugs

Aquaporin-based Gene Therapy

Regenerative Medicine MSCs and 3D biopriting