A comprehensive review of the influence of Epigallocatechin gallate on Sjögren's syndrome associated molecular regulators of exocytosis (Review)

ABDELMOUNAIM ERRACHID[1,2], MICHAL NOHAWICA[1] and MARZENA WYGANOWSKA-SWIATKOWSKA[1]

[1]Department of Dental Surgery and Periodontology, Poznan University of Medicinal Sciences, 60-812 Poznań, Greater Poland, Poland; [2]Earth and Life Institute, University Catholique of Louvain, B-1348 Louvain-la-Neuve, Ottignies-Louvain-la-Neuve, Belgium

Abstract. Sjögren's syndrome (SS) is an autoimmune disorder that affects the salivary glands, leading to reduced secretory functions and oral and ocular dryness. The salivary glands are composed of acinar cells that are responsible for the secretion and production of secretory granules, which contain salivary components, such as amylase, mucins and immunoglobulins. This secretion process involves secretory vesicle trafficking, docking, priming and membrane fusion. A failure during any of the steps in exocytosis in the salivary glands results in the altered secretion of saliva. Soluble N-ethylmaleimide-sensitive-factor attachment protein receptors, actin, tight junctions and aquaporin 5 all serve an important role in the trafficking regulation of secretory vesicles in the secretion of saliva via exocytosis. Alterations in the expression and distribution of these selected proteins leads to salivary gland dysfunction, including SS. Several studies have demonstrated that green tea polyphenols, most notably Epigallocatechin gallate (EGCG), possess both anti-inflammatory and anti-apoptotic properties in normal human cells. Molecular, cellular and animal studies have indicated that EGCG can provide protective effects against autoimmune and inflammatory reactions in salivary glands in diseases such as SS. The aim of the present article is to provide a comprehensive and up-to-date review on the possible therapeutic interactions between EGCG and the selected molecular mechanisms associated with SS.

Contents

1. Introduction
2. Literature review
3. SNAREs
4. Aquaporins
5. Actin
6. Tight junctions
7. GTPs
8. Discussion and conclusions

1. Introduction

The quantity and composition of the daily salivary production of the major (submandibular, sublingual and parotid) and minor salivary glands is important for oral health. In a healthy individual the salivary glands produce 0.5-1.5 l saliva per a day, which is composed of 99.5% water 0.3% protein and 0.2% organic and inorganic substances (1-3). The salivary glands are composed of acinar cells, which are responsible for the secretion and production of secretory granules (3-5). These granules contain amylase, mucins and immunoglobulins, which are essential for the maintenance of a stable oral environment, lubrication, digestion and in the immunity of the oral mucosa (6).

There are two types of acinar cells, mucinous and serous acinar cells, and each plays a different role in the proper functioning of the salivary glands. Mucinous acinar cells are characterized by an accumulation of a large number of mucinous granules in the apical cytoplasmic region (7). These cells are responsible for the production of mucins, which are essential for lubrication and oral health (8). Serous acinar cells also accumulate secretory granules, but are responsible for the secretion of digestive amylase α, which aids primary food digestion (7,8).

Sjögren's syndrome (SS) is an autoimmune disease characterized by a dysfunction of the salivary glands. The primary symptom is dryness of the mouth and eyes due to impaired salivary and lacrimal gland function (9,10). It has been reported that several factors may be responsible for this, such

Correspondence to: Professor Marzena Wyganowska-Świątkowska, Department of Dental Surgery and Periodontology, Poznan University of Medicinal Sciences, ul. Bukowska 70, 60-812 Poznań, Greater Poland, Poland
E-mail: marzena.wyganowska@periona.pl

as acinar cell atrophy and apoptosis (9,11), glandular denervation (12), inhibition of cytokine neurotransmitter release (13), Acetylcholine (ACh) depletion and increased secretion of cholinesterase (14), presence of anti-muscarinic autoantibodies blocking muscarinic M3 receptors (14), decreased nitric oxide production [which in turn can disrupt calcium release induced by altered levels of cyclic adenosine diphosphate-ribose (15)], altered calcium tunneling (16), and aberrant aquaporin (AQP)5 expression and localization (17).

Tea and its polyphenols, a product of the dried leaves of *Camellia sinensis*, have been suggested to possess several pharmacological properties (18). The green tea polyphenols (GTPs) are useful in delaying or managing SS-like autoimmune disorders that can affect multiple tissues or organs (19,20). The prevalence of SS seems to be higher in the US population compared to the Japanese and Chinese population (21,22). It was reported that apoptosis, cytotoxicity and autoantigen expression (23), as well as oxidative stress (24), all of which are involved in SS pathogenesis and the primary cellular mechanisms underlying its development, are inhibited by GTPs (19,25,26).

The major GTP, Epigallocatechin-3-gallate (EGCG), inhibits autoantigen expression in normal human keratinocytes and immortalized normal human salivary acinar cells (26). Another study demonstrated that EGCG can protect normal human salivary acinar cells from tumor necrosis factor-α (TNF-α)-induced cytotoxicity, and the phosphorylation of p38 mitogen-activated protein kinase (MAPK) serves a major role in this protection (27).

EGCG undergoes modification by gut microbes following oral administration (28). Multiple resultant gut soluble metabolites have been reported to possess pharmacological properties (29). EGCG reaches systemic circulation at low concentrations, and excretion is complete within several hours (30). Identification of the true active component of EGCG, and conversely the true effect of EGCG on the body, is a contentious topic (31). Nonetheless, whether the activity of EGCG is carried out by the unaltered molecule, its microbial metabolites, or its cellular metabolites, in the framework of SS and the selected proteins, this activity may be beneficial.

The dysregulation of secretory granules in SS by selected families of molecules, including soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), AQP5, actin, and tight junctions, are comprehensively discussed. Furthermore, the possible mechanism of protection by GTPs against the dysregulation of these molecules is reviewed.

2. Literature review

PRISMA guidelines were used to plan this comprehensive review (32). Fig. 1 shows a flowchart summarizing the results of the search strategy carried out for this review. The review was split in to two bouts of data gathering, the molecular action of selected molecules in SS covered by search key 1, and the effect of EGCG on selected molecules covered by search key 2. Key 1: Sjogren AND salivary gland AND (aquaporin OR SNARE OR actin OR tight junction); and Key 2: EGCG AND (aquaporin OR SNARE OR actin OR tight junction).

The global inclusion criteria were: Published after 1998, the full text available, the article was written in the English
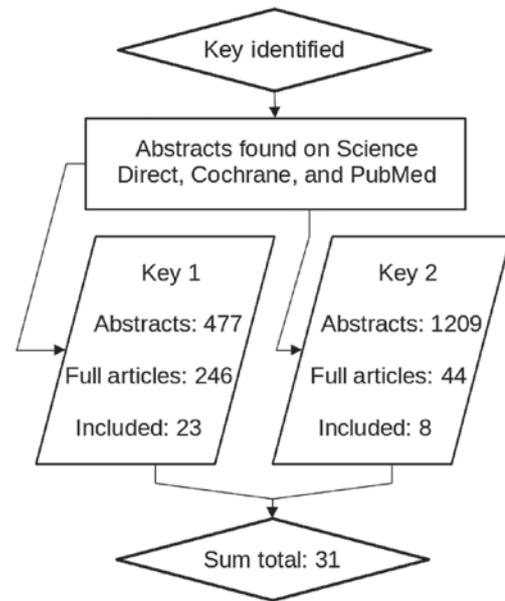

Figure 1. Flowchart summarizing the results of the search strategy carried out for the present review. VAMP, vesicle-associated membrane proteins; STX, syntaxin; SNAP, soluble N-ethylmaleimide-sensitive factor attachment protein; AQP, aquaporin; ZO-1, Zonula occludens-1; SS, Sjögren's syndrome.

language and it described non-trivial involvement of selected molecules. The PubMed, Science Direct and Cochrane databases were searched, and Medical Subject Headings Terms used where permitted by the search engine. Abstracts were screened for the selection criteria, and the chosen full text articles were screened again. Applicable results are summarized in Tables I-IV, and a pictorial representation is shown in Figs. 2 and 3.

3. SNAREs

Physiological roles of SNAREs. SNARE proteins have been shown to serve a major role in the regulation of exocytosis (33). Vesicle-associated membrane proteins (VAMPs) are SNAREs that play a crucial role in the regulation of secretory vesicle trafficking (34). Indeed, these vesicles are transported from the trans-Golgi network to the plasma membrane via the cytoskeleton (35). Once these vesicles reach the plasma membrane, interactions between Ras-related protein (RAB), mammalian uncoordinated-18 (MUNC18) and SNARE proteins promote the formation of trans-SNARE (t-SNARE) (36). During fusion between the plasma membrane and the secretory vesicles, a small opening is created at the point of contact between the two membranes (vesicles and plasma membrane), which gradually increases in size until exocytosis (37,38).

In the salivary gland, mucous acinar cells contain a large number of mucin granules aggregated in the apical cytoplasm (39,40). Several studies have shown that the exocytosis of mucins required precise localization of SNAREs and other components (41). In acini cells, the secretory pathway synthesizes, processes and exocytoses mucins (42). Several families of proteins, such as SNAREs, RAB and MUNC-18-bound proteins, are involved in the assembly of the membrane fusion complex (42-44). The localization of each unit of this molecular machinery is essential for exocytosis (43).

Table I. Known distribution of protein involved in the mucosecretory mechanism between the salivary acini and ducts, in healthy individuals and patients with SS.

Location in gland	Subject	Aquaporins	SNARE	Tight Junctions	Actin
Acinus	Healthy	AQP1 (EC, MEC); AQP3 (AC on the BM); AQP4 (NA); AQP5 (AC on the AM); AQP8 (NA)	SNAP23 (AC at the AM and BM); VAMP8 (AC at the AM); Syntaxin-4 (AC at the AM and BM); Syntaxin-3 (AC at the AM)	Claudins 1,3,4 and 5 (AC at the AM); Occludin (AC at the AM); ZO-1 (AC in the C)	F-actin (AC at the AM) actin-α1/α2 (MEC)
	SS	AQP1 (MEC); AQP5 (at the AM and BM)	SNAP 23 (AC at the LM); VAMP8 (AC in the C); Syntaxin-4 (AC in the C); Syntaxin-3 (AC in the C and at the BM)	Claudins 3-5 (AC at the BM); Occludin (AC at the AM); ZO-1 (AC in the C)	Cofilin-1, α-enolase (NA) actin-α1/ α2 (NA)
Duct	Healthy	AQP5 (NA); AQP3 (NA)	VAMP8 (NA); Syntaxin-4 (NA)	Claudins 1,3 and 4 (DC, NA); Occludin (DC, NA); ZO-1 (DC, NA)	NA
	SS	NA	NA	NA	NA

AC, acinar cells; AM, apical membrane; BM, basolateral membrane; EC, endothelial cell; MEC, myoepithelial cell; C, cytoplasm, LM, lateral membrane; DC, ductal cells; NA, Not available.

VAMP8 in patients with SS. Studies of endosomal compartments, notably VAMP8 (42), have indicated that it is an important component in exocytosis (44). In SS, the expression and localization of the VAMP8 protein in acinar cells has been shown to be associated with dysfunction of the salivary labial glands. In healthy individuals, the localization of VAMP8 is cytoplasmic in the apical region of acinar cells. In contrast, in patients with SS, it was observed that the expression of this protein is decreased and localized throughout the entire cytoplasm. This aberration in VAMP8 distribution is considered to be related to ectopic mucin secretion (45).

Other studies in VAMP8-deficient (VAMP8-/-) mice (normal at birth) have shown that the absence of interaction between VAMP8 with t-SNARE, Syntaxin (STX)4, and soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP)23 induces abnormal accumulation of secretory granules, and increased production of amylases and carbonic anhydrase VI in acinar cells of the salivary glands (46,47). This suggests that the expression and distribution of SNARE proteins serve an essential role in the secretion of salivary gland proteins.

STX in patients with SS. Studies of STX in rat parotid glands have shown that the localization of STX4 is abundantly expressed across the entire plasma membrane, whereas STX2 and STX3 are expressed only at the apical level of the plasma membrane (48,49). In contrast, in the submandibular glands of humans, STX4 is expressed in the apical and basolateral plasma membranes, and STX2 is localized in both the apical plasma membrane and the cytoplasmic vesicles (50).

In the labial salivary glands in patients with primary SS, the pattern of expression and/or localization of STX3 and STX4 exhibits several differences compared with healthy individuals. The localization of STX4 does not change compared with healthy individuals, whereas its expression is decreased in patients with SS. In contrast, whereas STX3 is normally localized only at the cytoplasmic level in the apical region of acinar cells, its expression was drastically increased in the entire cytoplasm in patients (45). It has been reported that increased STX3 expression in patients is related to the fusion of secretory granules that were previously described as large pleomorphic granules (51).

STX4-VAMP8 and STX3-SNAP23 complexes are over-abundant in acinar cells of the salivary labial glands in patients with SS (45). It is interesting to note that the interactions between STX4 and VAMP8 are observed only in the basolateral membrane of patients, suggesting a major role of this complex in secretory vesicle trafficking dysfunction and exocytosis (45). Additionally, it has been reported that the co-localization of STX4 and RAB3D in mature secretory vesicles in the subapical region is altered from the apical to basolateral plasma membrane in patients with SS. This perturbation of SNARE complexes results in altered interactions, distribution and co-localization, which in-turn induce alterations in the distribution of secretory mucins in patients with SS (45,51,52).

Altered expression and localization of SNARE complexes with aberrant mucin exocytosis in the basal region could induce mucin accumulation in the extracellular matrix. It has also been suggested that the STX3/SNAP23/VAMP8 complex may serve a major role in homotypic granule-granule fusion in Labial Salivary Gland (LSG) acinar cells in patients with SS, as described in the pancreas (44).

SNAPs in patients with SS. SNAP23 belongs to the SNAP25 (also known as Q-SNARE) family and is anchored and

Table II. Known distribution of proteins involved in the muco-secretory mechanism between the salivary acini and ducts, in healthy individuals and patients with Sjögren's syndrome.

Main protein group	Examples	(Refs.)
Aquaporins	AQP1	(54,55)
	AQP3	(54,55)
	AQP4	(54,55)
	AQP5	(54-56,90,137)
	AQP8	(138)
SNAREs	VAMP8	(45)
	STX3	(45)
	STX4	(45)
	SNAP-23	(45)
Tight Junctions	Claudin-1	(80,83)
	Claudin-3	(80,139)
	Claudin-4	(80)
	Claudin-5	(140)
	Occludin	(80,85)
	ZO-1	(80)
	JAM-1	(83)
Actins	Moesin	(77)
	Cofilin-1	(76)
	α-enolase	(76)
	Actin α1/α2	(138)
	RGI2	(76)

AQP, aquaporin; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; VAMP, vesicle-associated membrane proteins; STX, syntaxin; SNAP, soluble N-ethylmaleimide-sensitive factor attachment protein; ZO-1, Zonula occludens-1; JAM-1, Junctional adhesion molecule 1; RGI2, respiratory growth induced protein 2.

localized in the plasma membrane (53). Whilst SNAP25 is expressed in the plasma membrane of neuronal and endocrine cells, SNAP23 which is a non-neuronal homologue of SNAP25, is specifically expressed in the apical and basolateral membranes of human submandibular glands (50). In contrast, in rat parotid glands, SNAP23 has been detected in the apical plasma membrane and intracellular membranes, and forms complexes with STX3, STX4, VAMP2 and VAMP3 (49).

Several differences have been noted in the comparison of the expression and localization of SNAP23 in acinar cells of the labial salivary glands between healthy individuals and patients with SS. Whilst the expression of SNAP23 was detected throughout the plasma membrane in healthy individuals, its expression was absent in the apical plasma membrane and decreased in the lateral plasma membrane in patients with SS. In contrast, no changes were detected in the basal plasma membrane (45).

4. Aquaporins

Physiological roles of AQP5. In human salivary glands, it is hypothesized that AQP5 is the only AQP involved in salivary secretion (54). In the human parotid, submandibular, sublingual and labial glands, AQP5 labelling was localized to the apical membrane of acinar cells, and its expression was not detectable in duct cells (55,56). Proper expression and subcellular localization of AQP5 are required to maintain homeostasis (56,57). Parasympathetic innervation is essential for the expression and distribution of AQP5 in the salivary gland (58,59). Acetylcholine (ACh) acts on M3 muscarinic acetylcholine receptors to induce translocation of AQP5 in the rat parotid glands (60), whereas in the rat submandibular gland, cholinergic denervation reduces AQP5 expression (61).

AQP5 in patients with SS. Studies have shown defective function of AQP5 in patients with SS (56,62). Abnormal localization of AQP5 has been reported in patients with SS compared with patients without SS but with xerostomia. AQP5 was shown to be expressed at both the apical and basolateral membranes in the acinar cells of minor salivary glands in patients with SS compared to healthy individuals, in whom AQP5 was restricted to the apical membrane of acinar cells (56).

Analysis of the saliva of transgenic mice lacking AQP5 showed that it is viscous and hypertonic (63). Indirect immunofluorescence tests performed in patients with SS detected the anti-AQP5 Immunoglobulin G (IgG), which may explain the low rate of resting salivary flow (64). The same authors demonstrated in another study that these anti-AQP5 antibodies found in patients with SS recognize different epitopes of AQP5, suggesting their role in salivary gland dysfunction (65). Treatments of SS AQP5 expression with plant extracts, such as *Dendrobium candidum*, has been shown to yield positive results when crude materials or the isolated phenolic active compound chrystoxine (66,67).

5. Actin

Physiological role of actin. Cytoskeletal components, such as actin filaments, play a major role in the proliferation and differentiation of cells (68). They are also involved in salivary gland protein secretion (69). It has been reported that stimuli which promote salivation can regulate F-actin activity within acinar cells of the salivary glands. Indeed, when salivatory stimuli are absent, F-actin, which is located under the plasma membrane and separates the secretory granules from the luminal membrane, prevents the secretory granules from reaching their exocytotic destination in the human parotid and submandibular gland (70).

Following stimulation, the actin-cytoskeleton is rearranged and disassembled, consequently allowing secretory granules to reach their destination for exocytosis (71). F-actins are not only involved in the regulation of the secretion granules trafficking, but also regulate the formation of these vesicles and their movement to the cell membrane (72). Similarly, it has been reported that depolymerization of F-actin in the rat submandibular gland prevents amylase release and exocytosis (73,74).

It has been suggested in other studies that proteins, such as cofilin, a protein necessary for the depolymerization of actin and for controlling the renewal and branching of microfilaments, may play a role in secretory vesicle trafficking (75,76). In adrenal chromaffin cells, cofilin is indispensable in the reorganization of the cortical actin cytoskeleton, which is necessary to allow the movement of secretory granules not yet attached to the plasma membrane (75).

Table III. Comparison of physiological and pathological distribution and function of selected proteins involved in SS.

Primary protein group	Healthy individuals	Patients with SS	Most significant dysregulation in patients with SS
AQPs	Expressed in the apical membrane of acinar cells (parotid, submandibular, sublingual and labial glands) (53,54).	Expressed in the apical and basolateral membranes of acinar cells (minor salivary glands) (54,102,103).	Presence of anti-AQP5 IgG (62). Glandular hypofunction (63).
SNARE	VAMP8: Expressed in the apical cytoplasm (labial salivary glands) (38). STX4: Expressed in the apical and basolateral plasma membranes (submandibular glands) (38). STX3: Expressed in the apical region of acinar cells (labial salivary glands) (38). SNAP23: Expressed in the apical membrane and in the basolateral plasma membrane (submandibular glands, labial salivary glands) (38,43).	VAMP8: Expression at the gene and protein level is decreased and is localized throughout the entire cytoplasm (labial salivary glands) (38). STX3: Expression is increased and localized throughout the entire cytoplasm and the basolateral plasma membrane in patients (labial salivary glands) (38). STX4: Expression is decreased and localized at the basal plasma membrane (labial salivary glands) (38). SNAP23: Expression is absent in the apical plasma membrane and decreased in the lateral plasma membrane (labial salivary glands) (38).	Ectopic mucin secretion (38). Fusion of secretory granules (44).
Tight junctions	Claudins: Expressed in the apical plasma membrane (92). Occludin: Expressed in the apical plasma membrane (90,91). ZO-1: Cytosolic expression (90,91)	Claudin-1, 4 and 5: Expression id increased (92,106). Claudin-3, 4 and 5: Localized in the basal plasma membrane (92,105,106). Occludin: Expression is decreased (92). ZO-1: Expression is decreased (92).	Alteration of paracellular permeability in the salivary gland (92,105). Presence of exosomes containing autoantigens from salivary gland epithelial cell lines (99).
Actin	F-actin: Located under the plasma membrane (parotid and subman dibular gland) (75). Actin-α1/α2: Present in myoepithe lial cells around the acini (104).	Expression of (cofilin-1, α-enolase and RGI2) increased (81). Decrease in actin-α1/ α2 levels (104).	Presence of anti-Moesin (82). The expression of anti-cofilin-1, α-enolase and RGI2 increased (81).

AQP, aquaporin; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; VAMP, vesicle-associated membrane proteins; STX, syntaxin; SNAP, soluble N-ethylmaleimide-sensitive factor attachment protein; ZO-1, Zonula occludens-1; RGI2, respiratory growth induced protein 2.

Table IV. Summary of interactions of EGCG on selected proteins in salivary glands.

Protein group	Interaction with EGCG	(Refs.)
SNAREs	Stimulated lysosomal activity	(108)
Aquaporins	Upregulated AQP5 and AQP3 expression	(105,116)
Actin	Maintenance of apical F-actin structure and organization	(119)
Tight Junctions	Stimulation of Occludin and ZO-1 expression	(127-129)

SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; EGCG, Epigallocatechin-3-gallate; AQP, aquaporin; ZO-1, Zonula occludens-1.

Actin in patients with SS. F-actin in the human parotid and submandibular glands serves to separate the secretory granules from the luminal membrane, and to regulate the trafficking of secretory vesicles to reach the sites of exocytosis (50,70).
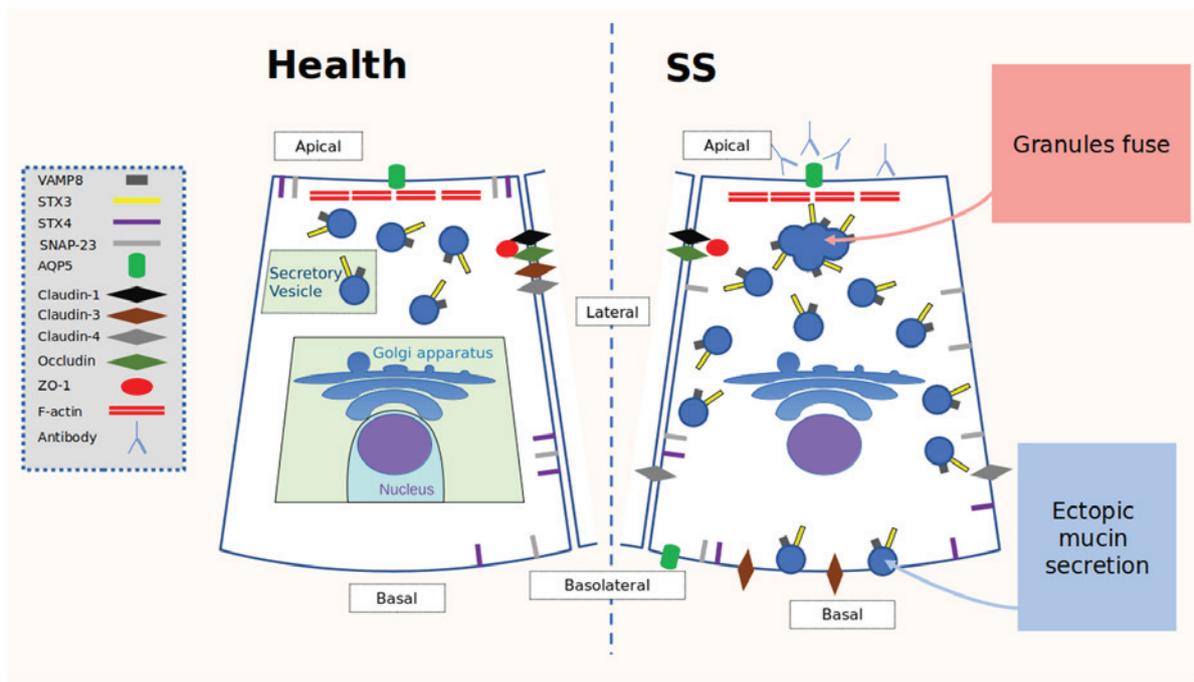
Figure 2. Model of distribution of AQP5, SNARE, ACTIN and tight junctions in patients with SS. The aberrant localization of SNARE proteins and the disruption to tight junctions induces the redistribution of AQP5 and alterations to the trafficking of secretory granules patients with SS. The alterations to the distribution of SNARE complexes in the basolateral plasma membrane under unstimulated conditions, may explain the ectopic mucin secretion, as these mucins normally undergo exocytosis at the apical pole of acinar cells. AQP, aquaporin; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptors; SS, Sjögren's syndrome.
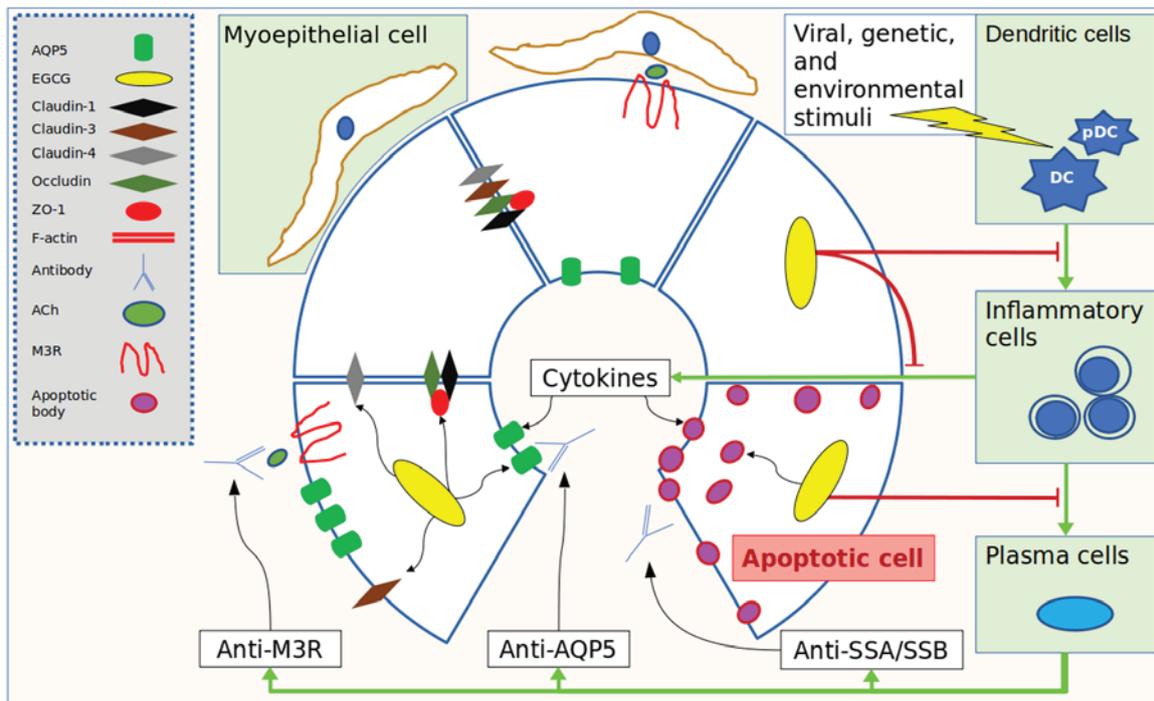

Figure 3. Summary of the impact of EGCG on the selected proteins in terms of the acinus of a salivary gland. AQP, aquaporin; EGCG, Epigallocatechin gallate; ZO-1, Zonula occludens-1; SSA, Sjögren's syndrome-related antigen A autoantibodies; SSB, Sjögren's syndrome-related antigen B autoantibodies; ACh, acetylcholine; M3R, muscarinic ACh receptor M3.

Several molecules have been reported to play a major role in this regulation.

Recently, a study on moesin, a structural protein involved in cytoskeletal organization and signaling pathways, showed the presence of anti-moesin antibodies by ELISA and western blotting in patients with SS (77).

Gland tissue samples from patients with primary-SS and primary SS/mucosal associated lymphoid tissue (MALT)

lymphoma exhibited significantly upregulated expression of cofilin 1, α-enolase and Rho GDP-dissociation inhibitor 2 compared to non-SS controls. ELISA tests that were used to detect autoantibodies against these proteins showed that three autoantibodies were upregulated in patients with primary-SS/MALT lymphoma compared with patients with primary-SS and the healthy controls, and that patients with primary-SS also had higher levels compared with the healthy controls (76). This suggests that these autoantibodies may affect the functional role of F-actin and may be indirectly involved in altered secretory vesicle trafficking and exocytosis dysfunction. However, these results require further research to understand the molecular mechanism of the immune reaction against these proteins *in vivo*.

6. Tight junctions

Physiological role of tight junctions. Tight junctions are protein complexes that are localized in the plasma membrane, such as claudin and occludin, or in the cytosol, such as zonula occludens 1 (ZO1). The functional role of tight junctions is to facilitate the transcellular epithelial flow of ions and water (78,79).

Tight junctions in patients with SS. Comparison of the expression and localization of claudin-1, claudin-3, claudin-4, ZO1 and occludin in labial salivary gland between patients with SS and healthy individuals showed several differences. Whereas the expression of claudin-1 and claudin-4 was significantly increased in patients with SS, the levels of ZO1 and occludin were decreased compared to the healthy individuals. In contrast, no changes were observed in the expression of claudin-3, although a relocation of claudin-3 and claudin-4 was observed in these patients, which may alter the transcellular permeability of the salivary gland (80).

It has been shown in patients with SS that TNF and interferon (IFN) levels are elevated, resulting in the alteration of tight junctions and the dysfunction of the salivary epithelium (41,81). In these patients, the alteration of tight junctions induced by increased TNF and IFN may cause cell alterations, increasing paracellular permeability (82), upregulated expression of claudin-1 and claudin-4, redistribution of claudin-3 and claudin-4 from the apical to the basal plasma membrane, and a strong negative regulation of occludin and ZO1 expression (80). The molecular mechanisms proposed for TNF and IFN action on tight junctions (83,84) are endocytosis of occluding (85), claudin-1 and junctional adhesion molecule 1 by activation of Ras homologue family member A (RhoA)/RhoA kinase (83), and promotion of accumulation of myosin II-dependent vesicles in the apical region and reorganization of the cytoskeleton (86). Other mechanisms have also been suggested, such as an indirect effect of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB), either by modulating ZO1 mRNA translation or ZO1 protein degradation via the proteasome (80).

It has been reported that exosomes that are released by epithelial cells in salivary glands contain autoantigens [anti-SS-related antigen A autoantibodies (SSA), SS type B antigen (SSB) and Anti-Smith], which may induce an immune response in these glands (87). Furthermore, a study of the microvilli that are localized in the apical zone of the acinar cells of labial salivary gland from patients with SS has shown that they are disorganized, possibly due to alterations of the tight junctions and modifications of the actin cytoskeleton (88).

A recent study on AQP-null mice suggested that AQP5 and tight junctions are functionally related (89). Indeed, this previous study demonstrated that alterations of the tight junctions were also detected in these mice. Conversely, water transport was only partially altered (51,89), suggesting that alteration of AQP5 allows water to pass through other routes.

As previously mentioned, AQP5 in patients with SS is detected at both the apical and basolateral plasma membranes of acinar cells, whereas normally these proteins are localized only to the apical membrane (89,90). This indicates that the alteration in AQP5 distribution may be related to the loss of integrity of tight junction proteins. All proteins and their distribution in healthy individuals and patients with SS are described in Tables II and III, and Fig. 2.

7. GTPs

EGCG in autoimmune disorders. EGCG, the ester of epigallocatechin and gallic acid, is the most abundant catechin in green tea. Its potential effects on human health and disease have been widely examined (91,92). Targets for EGCG include phosphoinositide 3-kinase/protein kinase B, Janus kinase/signal transducer and activator of transcription proteins, MAPK, as well as proteases, such as metalloproteinases and urokinase (93).

Autoantigen expression and apoptosis are the most common factors that lead to salivary gland damage (19,27). It has been shown that the administration of EGCG in drinking water in non-obese diabetic mice had a protective effect against autoimmune reactions in the salivary glands, as well as inhibiting apoptosis and cell proliferation (27).

Inhibition of apoptosis (94), a cellular process in which cells undergo controlled cell death, may be achieved through dampening of proteolytic polymerases. This activity is dissimilar to the pro-apoptotic results seen in cancer studies, and this difference in behavior could be due to EGCG binding to sugars (95), such as ADP-ribose, is necessary for the intrinsic metabolic apoptotic pathway (96) inhibiting their ability to bind to Poly-(ADP-ribose) polymerases and carry out apoptosis in the salivary glands. Additionally, EGCG itself can be an agonist of receptor mediated apoptosis, particularly in cell lines were expression of the TNF superfamily of receptors are upregulated (97). EGCG also inhibits the cellular process of proliferation (98). Both apoptosis and cellular proliferation require intracellular remodeling, especially of components such as actin filaments (99,100). Inhibition of both of these processes may result in a suspended state of the tissue in cell cycle arrest (101).

Other studies have shown that green tea extract can also reduce the levels of autoantibodies in animal serum and that EGCG affects TNF-α levels by preventing cytotoxicity in salivary gland cells *ex vivo* (102,103). EGCG can exert an anti-inflammatory effect by inhibiting interleukin-1β, suppressing dendritic cell maturation and reducing T cell activation (104). A summary of EGCG interactions with the selected proteins is shown in Table IV.

EGCG and the SS autoimmune response. The study of the expression of autoantigens genes [SSA, SSB, fodrin, centromere protein C, golgin-67, coilin and poly (ADP-ribose) polymerase] in normal human epidermal keratinocytes and immortalized salivary gland acinar cells has shown that exposure to EGCG inhibits the expression of these genes (26), which may explain the low levels of autoantibodies and salivary lymphocyte infiltration following GTP administration in an accepted mouse model of SS [Non-Obese Diabetic (NOD) mice] and in a mouse model of lupus erythematosus [Murphy Roths Large (MRL) mice] (25).

An autoimmune sialadenitis model of MRL-Fas-lpr mice demonstrated that AQP5 expression is reduced in the salivary glands damaged by apoptosis of cells, and that EGCG was able to restore AQP5 expression and improve the functionality of the glands. The molecular mechanism of EGCG action has been suggested to involve inactivating both the NF-κB and the N-terminal c-Jun kinase, as well as preserving the activity of protein kinase A (105).

Anti-inflammatory activity of EGCG. EGCG affects the submandibular gland in a NOD animal by reducing the size of the SS foci (25). By using a MTT viability assay on the human salivary gland cell line NS-SV-AC, it was shown that EGCG could ameliorate the effects of TNF-α produced by inflammatory cells, via the attenuation of the cytotoxic effect at the target acinar cells. Similarly, the phosphorylation of p38 was shown to be induced by EGCG via modulation of the MAPK signal transduction pathways, suggesting the presence of another mechanism by which GTPs can attenuate SS pathogenesis (27). p38 is a key modulator of inflammation, regulating TNF-α and interleukin mediator secretion (106).

In a dextran sulphate sodium mouse model of colitis, EGCG has been found to be effective at reducing the severity of inflammation and symptoms both preventatively and therapeutically (107).

SNAREs and EGCG. EGCG, but not its metabolites, has been found to marginally increase the lysosomal proteolysis and autophagy (108-110). Lysosomal function is dependent on cathepsins and inhibition of Cathepsin S has been found to induce autophagy through reactive oxygen species-mediated phosphatidylinositol 3-kinase and c-Jun N-terminal kinase signaling pathways (111). Similarly, Cathepsin S activity was found to be elevated in the lacrimal glands of the SS murine model (112).

AQPs and EGCG. EGCG significantly inhibits the expression levels of AQP4 in a traumatic brain injury model (113), as well as a rat spinal injury model (114), reducing the associated oedema. EGCG prevention of vasogenic oedema associated with status epilepticus was also mediated through regulation of AQP4 expression, however this time via its upregulation (115).

AQP mediated moisture retention is increased with EGCG; however, whilst moisturizing cream containing the additive was found to cause cellular retention by increasing AQP3 mRNA expression (116), a murine model of EGCG induced liver failure found that the associated cellular turgidity was concomitant to inhibition of AQP2 (117).

Cellular apoptosis stimulated by EGCG in ovarian cancer was also associated with inhibition of AQP5 production (118), whereas a murine model of sialadenitis exhibited upregulated secretin levels due to the EGCG-mediated increase in AQP5 expression (105).

Actin and EGCG. Extracellular expression of α-enolase was inhibited in a kidney model of calcium oxalate monohydrate (COM) crystal binding, resulting in improved outcomes and reduced extracellular crystallization (119). Furthermore, EGCG was found to maintain and protect the apical microvillar structure and F-actin organization of tubular epithelial cells in a COM induced injury model (120).

Whilst EGCG can prevent IFN-γ mediated disorganization and inhibition of moesin binding (121), at high enough concentrations, it can begin to disrupt F-actin organization, which is associated with reduced rates of proliferation (119).

Otherwise, commonly in the literature EGCG is found to have an anti-fibrotic effect, associated with an inhibition of α-smooth muscle actin expression (122-124).

Tight junctions and EGCG. Reports have shown that whilst EGCG is unable to easily cross the intestinal or blood-brain barrier, there is some mechanism involving tight junctions, which, *in vivo* allows it (125,126). On a molecular level, EGCG was associated with stimulated secretion of tight junction proteins zonula occludens-1 (ZO-1) and occluding (127-129) in response to bacterial and TNF-α stimulation.

The protective role of EGCG against inflammation stimulated by cytokine release extends to inhibition of tight junction dysfunction induced by IFN-γ (130,131). These findings have been replicated in a colitis mouse model, showing a significant increase in expression of occludin, claudin-1 and ZO-1, whilst inhibiting claudin-4, all of which were associated with EGCG mediated inhibition of IL-6 and 12, as well as IFN-γ (107).

8. Discussion and conclusions

Selecting publications on EGCG with a narrow pharmacokinetic focus is challenging. Epidemiological and longitudinal studies on the health benefits in large populations are far outnumbered by cellular, histological and pharmacological investigations. This problem is further exacerbated by the complex interactions EGCG can have, which depend on its route of administration, the gut microflora and its cellular metabolism, which appears to be organ specific. Nonetheless, some signs of structured analysis of EGCG are appearing in the literature, allowing a comprehensive look at its role in salivary physiology.

It is striking to observe the action of the autoimmune response on AQP5, actin and tight junction directly or indirectly. It seems that the alteration of the localization and expression of AQP5, SNAREs, actin and tight junctions can lead to salivary gland dysfunction in SS (Table I; Fig. 1). An attractive hypothesis is that SNAREs act as a regulator of the distribution and movement of all these components through the cell, and the autoimmune reaction is the result of the alterations of these proteins. However, as mentioned above GTPs, such as EGCG, have multiple protective effects for autoimmune disease. They maintain total serum autoantibody production at moderate

levels due to an inhibitory effect on antigen expression (26) and suppress the increase in TNF-α-induced apoptotic activity in human salivary gland acinar cells *in vitro* (27). However, the mechanisms for such multi-level protection by polyphenols are still poorly understood and warrant further investigation of this unique phytochemical with regard to its potential human benefits. The apical plasma membrane water channel AQP5 plays an important role in transporting water across the apical surface of the salivary gland epithelia (58,59).

Multiple studies have demonstrated the presence of anti-AQP5 IgG antibodies in patients with primary-SS (132,133). Others have demonstrated that EGCG (592 μg/mouse in drinking water, for 57 days) was able to restore AQP5 expression levels and improve gland functionality (105). These findings suggest a central role of AQP5 in SS, without excluding the importance of SNAREs (134,135), actin (136) and tight junctions (51,89), in the regulation of AQP trafficking, activity and distribution. It is also necessary to underline the involvement of these molecules in the regulation of secretory vesicles and exocytosis. Alterations of the molecules involved in the regulation of AQP5 trafficking and activity may lead to dysfunction in secretion of vesicles. However, the direct link between the alterations of the trafficking activity of AQP5, the presence of the anti-AQP5 antibodies and the impact on the secretory granule trafficking dysfunction requires further investigation.

Acknowledgements

Funding

Availability of data and materials

Not applicable.

Authors' contributions

MWS and AE conceived the topic of study. AE performed the methodology. MWS validated the content. MWS, AE and MN performed the investigation. AE curated the data. AE drafted the manuscript. AE and MWS edited the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Liu J and Duan Y: Saliva: A potential media for disease diagnostics and monitoring. Oral Oncol 48: 569-577, 2012.
2. Chiappin S, Antonelli G, Gatti R and De Palo EF: Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 383: 30-40, 2007.
3. Karnati R, Laurie DE and Laurie GW: Lacritin and the tear proteome as natural replacement therapy for dry eye. Exp Eye Res 117: 39-52, 2013.
4. Sitaramamma T, Shivaji S and Rao GN: Effect of storage on protein concentration of tear samples. Curr Eye Res 17: 1027-1035, 1998.
5. Wilmarth PA, Riviere MA, Rustvold DL, Lauten JD, Madden TE and David LL: Two-dimensional liquid chromatography study of the human whole saliva proteome. J Proteome Res 3: 1017-1023, 2004.
6. Humphrey SP and Williamson RT: A review of saliva: Normal composition, flow, and function. J Prosthet Dent 85: 162-169, 2001.
7. Tucker AS: Salivary gland development. Semin Cell Dev Biol 18: 237-244, 2007.
8. Br H and Mp H: Regulatory mechanisms driving salivary gland organogenesis. Curr Top Dev Biol 115: 111-130, 2015.
9. Voulgarelis M and Tzioufas AG: Pathogenetic mechanisms in the initiation and perpetuation of Sjögren's syndrome. Nat Rev Rheumatol 6: 529-537, 2010.
10. Mavragani CP and Moutsopoulos HM: The geoepidemiology of Sjögren's syndrome. Autoimmun Rev 9: A305-A310, 2010.
11. Busamia B, Gonzalez-Moles MA, Ruiz-Avila I, Brunotto M, Gil-Montoya JA, Bravo M, Gobbi C and Finkelberg A: Cell apoptosis and proliferation in salivary glands of Sjögren's syndrome. J Oral Pathol Med 40: 721-725, 2011.
12. Pedersen AM, Dissing S, Fahrenkrug J, Hannibal J, Reibel J and Nauntofte B: Innervation pattern and Ca2+ signalling in labial salivary glands of healthy individuals and patients with primary Sjögren's syndrome (pSS). J Oral Pathol Med 29: 97-109, 2000.
13. Zoukhri D and Kublin CL: Impaired neurotransmitter release from lacrimal and salivary gland nerves of a murine model of Sjögren's syndrome. Invest Ophthalmol Vis Sci 42: 925-932, 2001.
14. Dawson LJ, Stanbury J, Venn N, Hasdimir B, Rogers SN and Smith PM: Antimuscarinic antibodies in primary Sjögren's syndrome reversibly inhibit the mechanism of fluid secretion by human submandibular salivary acinar cells. Arthritis Rheum 54: 1165-1173, 2006.
15. Caulfield VL, Balmer C, Dawson LJ and Smith PM: A role for nitric oxide-mediated glandular hypofunction in a non-apoptotic model for Sjogren's syndrome. Rheumatology (Oxford) 48: 727-733, 2009.
16. Dawson LJ, Fox PC and Smith PM: Sjogrens syndrome-the non-apoptotic model of glandular hypofunction. Rheumatology (Oxford) 45: 792-798, 2006.
17. Soyfoo MS, Vriese CD, Debaix H, Martin-Martinez MD, Mathieu C, Devuyst O, Steinfeld SD and Delporte C: Modified aquaporin 5 expression and distribution in submandibular glands from NOD mice displaying autoimmune exocrinopathy. Arthritis Rheum 56: 2566-2574, 2007.
18. Aktas O, Prozorovski T, Smorodchenko A, Savaskan NE, Lauster R, Kloetzel PM, Infante-Duarte C, Brocke S and Zipp F: Green tea epigallocatechin-3-gallate mediates T cellular NF-kappa B inhibition and exerts neuroprotection in autoimmune encephalomyelitis. J Immunol 173: 5794-5800, 2004.
19. Gillespie K, Kodani I, Dickinson DP, Ogbureke KU, Camba AM, Wu M, Looney S, Chu TC, Qin H, Bisch F, et al: Effects of oral consumption of the green tea polyphenol EGCG in a murine model for human Sjogren's syndrome, an autoimmune disease. Life Sci 83: 581-588, 2008.
20. Dickinson D, Yu H, Ohno S, Thomas C, Derossi S, Ma YH, Yates N, Hahn E, Bisch F, Yamamoto T and Hsu S: Epigallocatechin-3-gallate prevents autoimmune-associated down- regulation of p21 in salivary gland cells through a p53-independent pathway. Inflamm Allergy Drug Targets 13: 15-24, 2014.
21. Carsons S: A review and update of Sjögren's syndrome: Manifestations, diagnosis, and treatment. Am J Manag Care 7: S433-443, 2001.
22. Zhang NZ, Shi CS, Yao QP, Pan GX, Wang LL, Wen ZX, Li XC and Dong Y: Prevalence of primary Sjögren's syndrome in China. J Rheumatol 22: 659-661, 1995.

23. Ohno S, Yu H, Dickinson D, Chu TC, Ogbureke K, Derossi S, Yamamoto T and Hsu S: Epigallocatechin-3-gallate modulates antioxidant and DNA repair-related proteins in exocrine glands of a primary Sjogren's syndrome mouse model prior to disease onset. Autoimmunity 45: 540-546, 2012.

24. Saito K, Mori S, Date F and Ono M: Epigallocatechin gallate inhibits oxidative stress-induced DNA damage and apoptosis in MRL-Fas(lpr) mice with autoimmune sialadenitis via upregulation of heme oxygenase-1 and Bcl-2. Autoimmunity 47: 13-22, 2014.

25. Hsu S and Dickinson D: A new approach to managing oral manifestations of Sjogren's syndrome and skin manifestations of lupus. J Biochem Mol Biol 39: 229-239, 2006.

26. Hsu S, Dickinson DP, Qin H, Lapp C, Lapp D, Borke J, Walsh DS, Bollag WB, Stöppler H, Yamamoto T, *et al*: Inhibition of autoantigen expression by (-)-epigallocatechin-3-gallate (the major constituent of green tea) in normal human cells. J Pharmacol Exp Ther 315: 805-811, 2005.

27. Hsu SD, Dickinson DP, Qin H, Borke J, Ogbureke KU, Winger JN, Camba AM, Bollag WB, Stöppler HJ, Sharawy MM and Schuster GS: Green tea polyphenols reduce autoimmune symptoms in a murine model for human Sjogren's syndrome and protect human salivary acinar cells from TNF-alpha-induced cytotoxicity. Autoimmunity 40: 138-147, 2007.

28. Guo T, Song D, Cheng L and Zhang X: Interactions of tea catechins with intestinal microbiota and their implication for human health. Food Sci Biotechnol 28: 1617-1625, 2019.

29. Chiou YS, Wu JC, Huang Q, Shahidi F, Wang YJ, Ho CT and Pan MH: Metabolic and colonic microbiota transformation may enhance the bioactivities of dietary polyphenols. J Funct Foods 7: 3-25, 2014.

30. Pervin M, Unno K, Takagaki A, Isemura M and Nakamura Y: Function of green tea catechins in the brain: Epigallocatechin gallate and its metabolites. Int J Mol Sci 20: 3630, 2019.

31. Kim HS, Quon MJ and Kim J: New insights into the mechanisms of polyphenols beyond antioxidant properties; lessons from the green tea polyphenol, epigallocatechin 3-gallate. Redox Biology 2: 187-195, 2014.

32. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, Shamseer L, Tetzlaff JM, Akl EA, Brennan SE, *et al*: The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ 372: n71, 2021.

33. Hong W: SNAREs and traffic. Biochim Biophys Acta 1744: 120-144, 2005.

34. Grote E, Hao JC, Bennett MK and Kelly RB: A targeting signal in VAMP regulating transport to synaptic vesicles. Cell 81: 581-589, 1995.

35. Alberts B, Johnson A, Lewis J, Raff M, Roberts K and Walter P: Transport from the ER through the Golgi Apparatus. Molecular Biology of the Cell 4th edition, 2002.

36. Whyte JRC and Munro S: Vesicle tethering complexes in membrane traffic. J Cell Sci 115: 2627-2637, 2002.

37. Chen YA and Scheller RH: SNARE-mediated membrane fusion. Nat Rev Mol Cell Biol 2: 98-106, 2001.

38. Han J, Pluhackova K and Böckmann RA: The multifaceted role of SNARE proteins in membrane fusion. Front Physiol 8: 5, 2017.

39. Srivanitchapoom P, Pandey S and Hallett M: Drooling in Parkinson's Disease: A review. Parkinsonism Relat Disord 20: 1109-1118, 2014.

40. Yu GY, Zhu ZH, Mao C, Cai ZG, Zou LH, Lu L, Zhang L, Peng X, Li N and Huang Z: Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca. Int J Oral Maxillofac Surg 33: 235-239, 2004.

41. Ewert P, Aguilera S, Alliende C, Kwon YJ, Albornoz A, Molina C, Urzúa U, Quest AF, Olea N, Pérez P, *et al*: Disruption of tight junction structure in salivary glands from Sjögren's syndrome patients is linked to proinflammatory cytokine exposure. Arthritis Rheum 62: 1280-1289, 2010.

42. Wong SH, Zhang T, Xu Y, Subramaniam VN, Griffiths G and Hong W: Endobrevin, a novel synaptobrevin/VAMP-like protein preferentially associated with the early endosome. Mol Biol Cell 9: 1549-1563, 1998.

43. Lang T and Jahn R: Core proteins of the secretory machinery. Handb Exp Pharmacol: 107-127, 2008.

44. Cosen-Binker LI, Binker MG, Wang CC, Hong W and Gaisano HY: VAMP8 is the v-SNARE that mediates basolateral exocytosis in a mouse model of alcoholic pancreatitis. J Clin Invest 118: 2535-2551, 2008.

45. Barrera MJ, Sánchez M, Aguilera S, Alliende C, Bahamondes V, Molina C, Quest AF, Urzúa U, Castro I, González S, *et al*: Aberrant localization of fusion receptors involved in regulated exocytosis in salivary glands of Sjögren's syndrome patients is linked to ectopic mucin secretion. J Autoimmun 39: 83-92, 2012.

46. Wang CC, Shi H, Guo K, Ng CP, Li J, Gan BQ, Chien Liew H, Leinonen J, Rajaniemi H, *et al*: VAMP8/endobrevin as a general vesicular SNARE for regulated exocytosis of the exocrine system. Mol Biol Cell 18: 1056-1063, 2007.

47. Wang CC, Ng CP, Lu L, Atlashkin V, Zhang W, Seet LF and Hong W: A role of VAMP8/endobrevin in regulated exocytosis of pancreatic acinar cells. Dev Cell 7: 359-371, 2004.

48. Takuma T, Arakawa T and Tajima Y: Interaction of SNARE proteins in rat parotid acinar cells. Arch Oral Biol 45: 369-375, 2000.

49. Imai A, Nashida T, Yoshie S and Shimomura H: Intracellular localisation of SNARE proteins in rat parotid acinar cells: SNARE complexes on the apical plasma membrane. Arch Oral Biol 48: 597-604, 2003.

50. Stoeckelhuber M, Scherer EQ, Janssen KP, Slotta-Huspenina J, Loeffelbein DJ, Rohleder NH, Nieberler M, Hasler R and Kesting MR: The human submandibular gland: Immunohistochemical analysis of SNAREs and cytoskeletal proteins. J Histochem Cytochem 60: 110-120, 2012.

51. Goicovich E, Molina C, Pérez P, Aguilera S, Fernández J, Olea N, Alliende C, Leyton C, Romo R, Leyton L and González MJ: Enhanced degradation of proteins of the basal lamina and stroma by matrix metalloproteinases from the salivary glands of Sjögren's syndrome patients: Correlation with reduced structural integrity of acini and ducts. Arthritis Rheum 48: 2573-2584, 2003.

52. Coursey TG, Tukler Henriksson J, Barbosa FL, de Paiva CS and Pflugfelder SC: Interferon-γ-induced unfolded protein response in conjunctival goblet cells as a cause of mucin deficiency in Sjögren syndrome. Am J Pathol 186: 1547-1558, 2016.

53. Holt M, Varoqueaux F, Wiederhold K, Takamori S, Urlaub H, Fasshauer D and Jahn R: Identification of SNAP-47, a novel Qbc-SNARE with ubiquitous expression. J Biol Chem 281: 17076-17083, 2006.

54. Wang W, Hart PS, Piesco NP, Lu X, Gorry MC and Hart TC: Aquaporin expression in developing human teeth and selected orofacial tissues. Calcif Tissue Int 72: 222-227, 2003.

55. Gresz V, Kwon TH, Hurley PT, Varga G, Zelles T, Nielsen S, Case RM and Steward MC: Identification and localization of aquaporin water channels in human salivary glands. Am J Physiol Gastrointest Liver Physiol 281: G247-G254, 2001.

56. Steinfeld S, Cogan E, King LS, Agre P, Kiss R and Delporte C: Abnormal distribution of aquaporin-5 water channel protein in salivary glands from Sjögren's syndrome patients. Lab Invest 81: 143-148, 2001.

57. Krane CM, Melvin JE, Nguyen HV, Richardson L, Towne JE, Doetschman T and Menon AG: Salivary acinar cells from aquaporin 5-deficient mice have decreased membrane water permeability and altered cell volume regulation. J Biol Chem 276: 23413-23420, 2001.

58. Ishikawa Y, Cho G, Yuan Z, Inoue N and Nakae Y: Aquaporin-5 water channel in lipid rafts of rat parotid glands. Biochim Biophys Acta 1758: 1053-1060, 2006.

59. Ishikawa Y, Cho G, Yuan Z, Skowronski MT, Pan Y and Ishida H: Water channels and zymogen granules in salivary glands. J Pharmacol Sci 100: 495-512, 2006.

60. Ishikawa Y, Eguchi T, Skowronski MT and Ishida H: Acetylcholine acts on M3 muscarinic receptors and induces the translocation of aquaporin5 water channel via cytosolic Ca2+ elevation in rat parotid glands. Biochem Biophys Res Commun 245: 835-840, 1998.

61. Xiang B, Zhang Y, Li YM, Zhang K, Zhang YY, Wu LL and Yu GY: Effects of phenylephrine on transplanted submandibular gland. J Dent Res 85: 1106-1111, 2006.

62. Tsubota K, Hirai S, King LS, Agre P and Ishida N: Defective cellular trafficking of lacrimal gland aquaporin-5 in Sjögren's syndrome. Lancet 357: 688-689, 2001.

63. Ma T, Song Y, Gillespie A, Carlson EJ, Epstein CJ and Verkman AS: Defective secretion of saliva in transgenic mice lacking aquaporin-5 water channels. J Biol Chem 274: 20071-20074, 1999.

64. Alam J, Koh JH, Kim N, Kwok SK, Park SH, Song YW, Park K and Choi Y: Detection of autoantibodies against aquaporin-5 in the sera of patients with primary Sjögren's syndrome. Immunol Res 64: 848-856, 2016.

65. Alam J, Koh JH, Kwok SK, Park SH, Park K and Choi Y: Functional Epitopes for Anti-Aquaporin 5 Antibodies in Sjögren Syndrome. J Dent Res 96: 1414-1421, 2017.
66. Xiao L, Ng TB, Feng YB, Yao T, Wong JH, Yao RM, Li L, Mo FZ, Xiao Y, Shaw PC, *et al*: Dendrobium candidum extract increases the expression of aquaporin-5 in labial glands from patients with Sjögren's syndrome. Phytomedicine 18: 194-198, 2011.
67. Lin X, Shaw PC, Sze SCW, Tong Y and Zhang Y: Dendrobium officinale polysaccharides ameliorate the abnormality of aquaporin 5, pro-inflammatory cytokines and inhibit apoptosis in the experimental Sjögren's syndrome mice. Int Immunopharmacol 11: 2025-2032, 2011.
68. Sart S, Errachid A, Schneider YJ and Agathos SN: Modulation of mesenchymal stem cell actin organization on conventional microcarriers for proliferation and differentiation in stirred bioreactors. J Tissue Eng Regen Med 7: 537-551, 2013.
69. Nashida T, Yoshie S, Imai A and Shimomura H: Presence of cytoskeleton proteins in parotid glands and their roles during secretion. Arch Oral Biol 49: 975-982, 2004.
70. Segawa A, Riva A, Loffredo F, Congiu T, Yamashina S and Testa Riva F: Cytoskeletal regulation of human salivary secretion studied by high resolution electron microscopy and confocal laser microscopy. Eur J Morphol 36 (Suppl): S41-S45, 1998.
71. Perrin D, Möller K, Hanke K and Söling HD: cAMP and Ca(2+)-mediated secretion in parotid acinar cells is associated with reversible changes in the organization of the cytoskeleton. J Cell Biol 116: 127-134, 1992.
72. Valentijn KM, Gumkowski FD and Jamieson JD: The subapical actin cytoskeleton regulates secretion and membrane retrieval in pancreatic acinar cells. J Cell Sci 112: 81-96, 1999.
73. Muallem S, Kwiatkowska K, Xu X and Yin HL: Actin filament disassembly is a sufficient final trigger for exocytosis in nonexcitable cells. J Cell Biol 128: 589-598, 1995.
74. Busch L, Sterin-Borda L and Borda E: Differences in the regulatory mechanism of amylase release by rat parotid and submandibular glands. Arch Oral Biol 47: 717-722, 2002.
75. Birkenfeld J, Kartmann B, Betz H and Roth D: Cofilin activation during Ca(2+)-triggered secretion from adrenal chromaffin cells. Biochem Biophys Res Commun 286: 493-498, 2001.
76. Cui L, Elzakra N, Xu S, Xiao GG, Yang Y and Hu S: Investigation of three potential autoantibodies in Sjogren's syndrome and associated MALT lymphoma. Oncotarget 8: 30039-30049, 2017.
77. Zhang Y, Hussain M, Yang X, Chen P, Yang C, Xun Y, Tian Y and Du H: Identification of moesin as a novel autoantigen in patients with Sjögren's syndrome. Protein Pept Lett 25: 350-355, 2018.
78. Mitic LL, Van Itallie CM and Anderson JM: Molecular physiology and pathophysiology of tight junctions I. Tight junction structure and function: Lessons from mutant animals and proteins. Am J Physiol Gastrointest Liver Physiol 279: G250-G254, 2000.
79. Beguin P, Errachid A, Larondelle Y and Schneider YJ: Effect of polyunsaturated fatty acids on tight junctions in a model of the human intestinal epithelium under normal and inflammatory conditions. Food Funct 4: 923-931, 2013.
80. Flynn AN, Itani OA, Moninger TO and Welsh MJ: Acute regulation of tight junction ion selectivity in human airway epithelia. Proc Natl Acad Sci USA 106: 3591-3596, 2009.
81. Fox RI, Kang HI, Ando D, Abrams J and Pisa E: Cytokine mRNA expression in salivary gland biopsies of Sjögren's syndrome. J Immunol 152: 5532-5539, 1994.
82. Fox PC, Grisius MM, Bermudez DK and Sun D: Cytokine mRNA expression in labial salivary glands and cytokine secretion in parotid saliva in Sjögren's syndrome. Adv Exp Med Biol 438: 909-915, 1998.
83. Baker OJ, Camden JM, Redman RS, Jones JE, Seye CI, Erb L and Weisman GA: Proinflammatory cytokines tumor necrosis factor-alpha and interferon-gamma alter tight junction structure and function in the rat parotid gland Par-C10 cell line. Am J Physiol Cell Physiol 295: C1191-C1201, 2008.
84. Youakim A and Ahdieh M: Interferon-gamma decreases barrier function in T84 cells by reducing ZO-1 levels and disrupting apical actin. Am J Physiol 276: G1279-G1288, 1999.
85. Ma TY, Iwamoto GK, Hoa NT, Akotia V, Pedram A, Boivin MA and Said HM: TNF-alpha-induced increase in intestinal epithelial tight junction permeability requires NF-kappa B activation. Am J Physiol Gastrointest Liver Physiol 286: G367-376, 2004.
86. Mankertz J, Tavalali S, Schmitz H, Mankertz A, Riecken EO, Fromm M and Schulzke JD: Expression from the human occludin promoter is affected by tumor necrosis factor alpha and interferon gamma. J Cell Sci 113: 2085-2090, 2000.
87. Utech M, Ivanov AI, Samarin SN, Bruewer M, Turner JR, Mrsny RJ, Parkos CA and Nusrat A: Mechanism of IFN-gamma-induced endocytosis of tight junction proteins: Myosin II-dependent vacuolarization of the apical plasma membrane. Mol Biol Cell 16: 5040-5052, 2005.
88. Manoussakis MN and Kapsogeorgou EK: The role of epithelial cells in the pathogenesis of Sjögren's syndrome. Clin Rev Allergy Immunol 32: 225-230, 2007.
89. Kawedia JD, Nieman ML, Boivin GP, Melvin JE, Kikuchi K, Hand AR, Lorenz JN and Menon AG: Interaction between transcellular and paracellular water transport pathways through Aquaporin 5 and the tight junction complex. Proc Natl Acad Sci USA 104: 3621-3626, 2007.
90. Ichiyama T, Nakatani E, Tatsumi K, Hideshima K, Urano T, Nariai Y and Sekine J: Expression of aquaporin 3 and 5 as a potential marker for distinguishing dry mouth from Sjögren's syndrome. J Oral Sci 60: 212-220, 2018.
91. Chow HH, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, Dorr RT, Hara Y and Alberts DS: Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and polyphenon E in healthy individuals. Clin Cancer Res 9: 3312-3319, 2003.
92. Fürst R and Zündorf I: Plant-derived anti-inflammatory compounds: Hopes and disappointments regarding the translation of preclinical knowledge into clinical progress. Mediators Inflamm 2014: 146832, 2014.
93. Wyganowska-Świątkowska M, Matthews-Kozanecka M, Matthews-Brzozowska T, Skrzypczak-Jankun E and Jankun J: Can EGCG alleviate symptoms of down syndrome by altering proteolytic activity? Int J Mol Sci 19: 248, 2018.
94. Yan X, Li Y, Yu H, Wang W, Wu C, Yang Y, Hu Y, Shi X and Li J: Epigallocatechin-3-gallate inhibits H_2O_2-induced apoptosis in mouse vascular smooth muscle cells via 67kD laminin receptor. Sci Rep 7: 7774, 2017.
95. Wyganowska-Swiatkowska M, Nohawica M, Grocholewicz K and Nowak G: Influence of herbal medicines on HMGB1 release, SARS-CoV-2 viral attachment, acute respiratory failure, and sepsis. A literature review. Int J Mol Sci 21: 4639, 2020.
96. Soldatenkov VA and Smulson M: Poly(ADP-ribose) polymerase in DNA damage-response pathway: Implications for radiation oncology. Int J Cancer 90: 59-67, 2000.
97. Zhang Y, Duan W, Owusu L, Wu D and Xin Y: Epigallocatechin-3-gallate induces the apoptosis of hepatocellular carcinoma LM6 cells but not non-cancerous liver cells. Int J Mol Med 35: 117-124, 2015.
98. Harakeh S, Abu-El-Ardat K, Diab-Assaf M, Niedzwiecki A, El-Sabban M and Rath M: Epigallocatechin-3-gallate induces apoptosis and cell cycle arrest in HTLV-1-positive and -negative leukemia cells. Med Oncol 25: 30-39, 2008.
99. Lancaster OM and Baum B: Shaping up to divide: Coordinating actin and microtubule cytoskeletal remodelling during mitosis. Semin Cell Dev Biol 34: 109-115, 2014.
100. Desouza M, Gunning PW and Stehn JR: The actin cytoskeleton as a sensor and mediator of apoptosis. Bioarchitecture 2: 75-87, 2012.
101. Mayr C, Wagner A, Neureiter D, Pichler M, Jakab M, Illig R, Berr F and Kiesslich T: The green tea catechin epigallocatechin gallate induces cell cycle arrest and shows potential synergism with cisplatin in biliary tract cancer cells. BMC Complement Altern Med 15: 194, 2015.
102. Yang CS, Lee MJ and Chen L: Human salivary tea catechin levels and catechin esterase activities: Implication in human cancer prevention studies. Cancer Epidemiol Biomarkers Prev 8: 83-89, 1999.
103. Wheeler DS, Catravas JD, Odoms K, Denenberg A, Malhotra V and Wong HR: Epigallocatechin-3-gallate, a green tea-derived polyphenol, inhibits IL-1 beta-dependent proinflammatory signal transduction in cultured respiratory epithelial cells. J Nutr 134: 1039-1044, 2004.
104. Ahn SC, Kim GY, Kim JH, Baik SW, Han MK, Lee HJ, Moon DO, Lee CM, Kang JH, Kim BH, *et al*: Epigallocatechin-3-gallate, constituent of green tea, suppresses the LPS-induced phenotypic and functional maturation of murine dendritic cells through inhibition of mitogen-activated protein kinases and NF-kappaB. Biochem Biophys Res Commun 313: 148-155, 2004.
105. Saito K, Mori S, Date F and Hong G: Epigallocatechin gallate stimulates the neuroreactive salivary secretomotor system in autoimmune sialadenitis of MRL-Fas(lpr) mice via activation of cAMP-dependent protein kinase A and inactivation of nuclear factor κB. Autoimmunity 48: 379-388, 2015.
106. Schieven GL: The biology of p38 kinase: A central role in inflammation. Curr Top Med Chem 5: 921-928, 2005.

107. Stillman A, Connors M, Miller M, Qazzaz H and Dryden G: P-145 oral administration of EGCG, a green tea polyphenol, both suppresses and rescues mice from DSS-induced colitis. Inflamm Bowel Dis 22: S54-S54, 2016.

108. Sakai M, Ohnishi K, Masuda M, Ohminami H, Yamanaka-Okumura H, Hara T and Taketani Y: Isorhamnetin, a 3'-methoxylated flavonol, enhances the lysosomal proteolysis in J774.1 murine macrophages in a TFEB-independent manner. Biosci Biotechnol Biochem 84: 1221-1231, 2020.

109. Holczer M, Besze B, Zámbó V, Csala M, Bánhegyi G and Kapuy O: Epigallocatechin-3-Gallate (EGCG) promotes autophagy-dependent survival via influencing the balance of mTOR-AMPK pathways upon endoplasmic reticulum stress. Oxid Med Cell Longev 2018: e6721530, 2018.

110. Zhang S, Cao M and Fang F: The role of Epigallocatechin-3-Gallate in autophagy and endoplasmic reticulum stress (ERS)-induced apoptosis of human diseases. Med Sci Monit 26: e924558, 2020.

111. Zhang L, Wang H, Xu J, Zhu J and Ding K: Inhibition of cathepsin S induces autophagy and apoptosis in human glioblastoma cell lines through ROS-mediated PI3K/AKT/mTOR/p70S6K and JNK signaling pathways. Toxicol Lett 228: 248-259, 2014.

112. Hamm-Alvarez SF, Janga SR, Edman MC, Madrigal S, Shah M, Frousiakis SE, Renduchintala K, Zhu J, Bricel S, Silka K, *et al*: Tear cathepsin S as a candidate biomarker for Sjögren's syndrome. Arthritis Rheumatol 66: 1872-1881, 2014.

113. Zhang B, Wang B, Cao S and Wang Y: Epigallocatechin-3-Gallate (EGCG) attenuates traumatic brain injury by inhibition of edema formation and oxidative stress. Korean J Physiol Pharmacol 19: 491-497, 2015.

114. Ge R, Zhu Y, Diao Y, Tao L, Yuan W and Xiong X: Anti-edema effect of epigallocatechin gallate on spinal cord injury in rats. Brain Res 1527: 40-46, 2013.

115. Kim JE, Park H, Jeong MJ and Kang TC: Epigallocatechin-3-Gallate and PEDF 335 peptide, 67LR activators, attenuate vasogenic edema, and astroglial degeneration following status epilepticus. Antioxidants (Basel) 9: 854, 2020.

116. Nakamura Y, Tsuchiya T, Hara-Chikuma M, Yasui M and Fukui Y: Identification of compounds in red wine that effectively upregulate aquaporin-3 as a potential mechanism of enhancement of skin moisturizing. Biochem Biophys Rep 24: 100864, 2020.

117. Wang X, Yang L, Wang J, Zhang Y, Dong R, Wu X, Yang CS, Zhang Z and Zhang J: A mouse model of subacute liver failure with ascites induced by step-wise increased doses of (-)-epigallocatechin-3-gallate. Sci Rep 9: 18102, 2019.

118. Yan C, Yang J, Shen L and Chen X: Inhibitory effect of Epigallocatechin gallate on ovarian cancer cell proliferation associated with aquaporin 5 expression. Arch Gynecol Obstet 285: 459-467, 2012.

119. Tepedelen BE, Soya E and Korkmaz M: Epigallocatechin-3-gallate reduces the proliferation of benign prostatic hyperplasia cells via regulation of focal adhesions. Life Sci 191: 74-81, 2017.

120. Fong-Ngern K, Vinaiphat A and Thongboonkerd V: Microvillar injury in renal tubular epithelial cells induced by calcium oxalate crystal and the protective role of epigallocatechin-3-gallate. FASEB J 31: 120-131, 2017.

121. Magro F, Fraga S and Soares-da-Silva P: Interferon-gamma-induced STAT1-mediated membrane retention of NHE1 and associated proteins ezrin, radixin and moesin in HT-29 cells. Biochem Pharmacol 70: 1312-1319, 2005.

122. Meng M, Li YQ, Yan MX, Kou Y and Ren HB: Effects of epigallocatechin gallate on diethyldithiocarbamate-induced pancreatic fibrosis in rats. Biol Pharm Bull 30: 1091-1096, 2007.

123. Higashi N, Kohjima M, Fukushima M, Ohta S, Kotoh K, Enjoji M, Kobayashi N and Nakamuta M: Epigallocatechin-3-gallate, a green-tea polyphenol, suppresses Rho signaling in TWNT-4 human hepatic stellate cells. J Lab Clin Med 145: 316-322, 2005.

124. Asaumi H, Watanabe S, Taguchi M, Tashiro M, Nagashio Y, Nomiyama Y, Nakamura H and Otsuki M: Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits ethanol-induced activation of pancreatic stellate cells. Eur J Clin Invest 36: 113-122, 2006.

125. Cano A, Ettcheto M, Chang JH, Barroso E, Espina M, Kühne BA, Barenys M, Auladell C, Folch J, Souto EB, *et al*: Dual-drug loaded nanoparticles of Epigallocatechin-3-gallate (EGCG)/Ascorbic acid enhance therapeutic efficacy of EGCG in a APPswe/PS1dE9 Alzheimer's disease mice model. J Control Release 301: 62-75, 2019.

126. Qiu J, Kitamura Y, Miyata Y, Tamaru S, Tanaka K, Tanaka T and Matsui T: Transepithelial transport of theasinensins through Caco-2 cell monolayers and their absorption in Sprague-Dawley rats after oral administration. J Agric Food Chem 60: 8036-8043, 2012.

127. Lagha AB and Grenier D: Tea polyphenols protect gingival keratinocytes against TNF-α-induced tight junction barrier dysfunction and attenuate the inflammatory response of monocytes/macrophages. Cytokine 115: 64-75, 2019.

128. Li J, Ye L, Wang X, Liu J, Wang Y, Zhou Y and Ho W: (-)-Epigallocatechin gallate inhibits endotoxin-induced expression of inflammatory cytokines in human cerebral microvascular endothelial cells. J Neuroinflammation 9: 161, 2012.

129. Lagha AB, Groeger S, Meyle J and Grenier D: Green tea polyphenols enhance gingival keratinocyte integrity and protect against invasion by Porphyromonas gingivalis. Pathog Dis 76, 2018.

130. Watson JL, Ansari S, Cameron H, Wang A, Akhtar M and McKay DM: Green tea polyphenol (-)-epigallocatechin gallate blocks epithelial barrier dysfunction provoked by IFN-gamma but not by IL-4. Am J Physiol Gastrointest Liver Physiol 287: G954-961, 2004.

131. Suzuki T and Hara H: Role of flavonoids in intestinal tight junction regulation. J Nutr Biochem 22: 401-408, 2011.

132. Amerongen AV, Bolscher JG and Veerman EC: Salivary mucins: Protective functions in relation to their diversity. Glycobiology 5: 733-740, 1995.

133. Alliende C, Kwon YJ, Brito M, Molina C, Aguilera S, Pérez P, Leyton L, Quest AF, Mandel U, Veerman E, *et al*: Reduced sulfation of muc5b is linked to xerostomia in patients with Sjögren syndrome. Ann Rheum Dis 67: 1480-1487, 2008.

134. Xu H, Shan XF, Cong X, Yang NY, Wu LL, Yu GY, Zhang Y and Cai ZG: Pre- and post-synaptic effects of botulinum toxin A on submandibular glands. J Dent Res 94: 1454-1462, 2015.

135. Besserer A, Burnotte E, Bienert GP, Chevalier AS, Errachid A, Grefen C, Blatt MR and Chaumont F: Selective regulation of maize plasma membrane aquaporin trafficking and activity by the SNARE SYP121. Plant Cell 24: 3463-3481, 2012.

136. Noda Y, Horikawa S, Kanda E, Yamashita M, Meng H, Eto K, Li Y, Kuwahara M, Hirai K, Pack C, *et al*: Reciprocal interaction with G-actin and tropomyosin is essential for aquaporin-2 trafficking. J Cell Biol 182: 587-601, 2008.

137. Beroukas D, Hiscock J, Jonsson R, Waterman SA and Gordon TP: Subcellular distribution of aquaporin 5 in salivary glands in primary Sjögren's syndrome. Lancet 358: 1875-1876, 2001.

138. Nashida T, Yoshie S, Haga-Tsujimura M, Imai A and Shimomura H: Atrophy of myoepithelial cells in parotid glands of diabetic mice; detection using skeletal muscle actin, a novel marker. FEBS Open Bio 3: 130-134, 2013.

139. Mei M, Xiang RL, Cong X, Zhang Y, Li J, Yi X, Park K, Han JY, Wu LL and Yu GY: Claudin-3 is required for modulation of paracellular permeability by TNF-α through ERK1/2/slug signaling axis in submandibular gland. Cell Signal 27: 1915-1927, 2015.

140. Cong X, Zhang XM, Zhang Y, Wei T, He QH, Zhang LW, Hua H, Lee SW, Park K, Yu GY and Wu LL: Disruption of endothelial barrier function is linked with hyposecretion and lymphocytic infiltration in salivary glands of Sjögren's syndrome. Biochim Biophys Acta Mol Basis Dis 1864: 3154-3163, 2018.