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Chapter XVII

EXPECTED FUNCTIONS OF SALIVARY HSP70 IN THE ORAL CAVITY (REVIEW ARTICLE)

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ABSTRACT

Saliva is a major determinant of the environment on all oral surfaces in both healthy and pathological conditions. It is a body fluid, secreted by three pairs of major salivary glands and by many of minor salivary glands supplemented with several constituents originated from intact or destroyed mucosal cells, immune cells, oral microorganism and blood. *Salivary Hsp70* is an important constituent of saliva, originating from several sources including salivary glands, mucosal cells, gingival crevicular fluid, blood and oral microbes. Salivary Hsp70 seems to be excreted constitutively, but its level can be increased significantly with psychological stress conditions, and also with chewing and taste stimulation. Several physiotherapeutic procedures like massage and local heat stimulation of major salivary glands also increases the amount. *Expected functions* of salivary Hsp70 include immunological and other antimicrobial defense mechanisms, and also cytoprotective effects on mucosal and immune cells. Consequently, it likely plays a role in healing of mucosal wounds and ulcers; in defense against gingival inflammation; in release of immune answer toward mucosal tumors; and also in allergic and autoimmune mucosal processes. Salivary Hsp70 is also likely participating in the formation of acquired pellicle on tooth and denture surfaces, leading to a possible role in physico-chemical defense of tooth surfaces; in crystal growth homeostasis of the teeth; in bacterial adhesion to tooth/denture surfaces; and in prevention and healing of denture

induced mucosal irritations. However our knowledge related to salivary Hsp70 is still rather limited. Consequently the expectations about several functions detailed in this chapter are partially based on data related to extracellular but not particularly salivary Hsp70.

INTRODUCTION

Saliva and the Oral Cavity

Saliva is a body fluid, secreted by three pairs of major salivary glands (parotid, submandibular and sublingual) and by many of minor salivary glands [28]. Primary saliva is secreted in secretory endpieces (acini) of salivary glands. Primary saliva is modified in the intercalated, striated and excretory (collecting) ducts leading from the acini to the mouth [116]. In the intercalated duct water and electrolyte transport into the saliva is supposed. Striated duct is responsible for both electrolyte secretion and reabsorption. Probably there is a transport of proteins like IgA, lysozyme, kallikrein and Hsp70 [28,29] in the striated duct as well. Some electrolyte transport in the excretory (collecting) duct is also suspected [116]. Primary saliva is also modified by serum exudates via tight junctions between several glandular cells (ultrafiltration) and via transcellular diffusion through these cells [37,51]. Entering the mouth, ductal saliva of several salivary glands are blended, and supplemented with many constituents originated from intact or destroyed mucosal cells, immune cells, and oral microorganism. Blood constituents also enter the oral cavity via gingival crevicular fluid, via the mucosa as mucosal transudate, and via intraoral bleeding [28]. Consequently a complex mixture of a high variety of molecules is resulted in the oral cavity, frequently called as "mixed saliva" and/or "whole saliva" in the scientific literature [28].

Main Functions of Saliva

Saliva is a major determinant of environment on all the oral surfaces. On tooth surfaces saliva plays an important role in acquired pellicle formation, which is a thin (ca. 0.5-1μm) layer of several salivary proteins with calcium hydroxide binding properties [15,48]. Acquired pellicle plays a major role in crystal growth homeostasis of the teeth, and in physico-chemical defense of tooth surfaces. Beside these, acquired pellicle plays a major role in bacterial adhesion on the tooth surfaces [15,48]. Saliva also strongly influences the bacterial biofilm formation following adhesion of bacteria on pellicle [28]. Similarly, saliva plays an important role in physico-chemical defense of the oral (and upper gastro intestinal) soft tissues. Its role is also crucial in antimicrobial defense mechanisms, and in wound (and ulcer) healing pathways of the oral cavity [28]. Saliva is also highly important for appropriate taste-sensation [58].

The Hsp70 Chaperone Family

The human heat shock protein 70 (Hsp70) chaperone family contains at least eight homologous chaperone proteins. Endoplasmic reticulum (ER) and mitochondria have their specific Hsp70 proteins. The remaining six family members reside mainly in the cytosol and nucleus, but some of them are also present in lysosomes [26]. There are also several homologues in bacteria (i.e. DnaK in *E. coli*) and fungi (i.e. Ssa1p/Ssa2p in *C. albicans*). Although there are some significant differences, all known members of human Hsp70 family display highly conserved amino acid sequences, and domain structures. All these proteins consist of an ATPase domain at the N-terminal, followed by a protease sensitive region, a peptide binding domain, and a variable region [26]. Hsp70 molecules present in the cytosol, nucleus and lysosomes also contain a C-terminal region with a certain amino acid motif enabling the proteins to bind co-chaperones and other Hsps. The amino acid sequences of Hsp70 molecules localized in mitochondria and endoplasmic reticulum start with a unique N-terminal localization signal for delivery to the right cellular compartment. Hsp70 molecules in the endoplasmic reticulum have also a C-terminal retention signal sequence, which inhibits their exit from the lumen [26]. Each members of Hsp70 family and their most important intracellular functions are briefly summarized as follows.

Hsp70-1 (Hsp70, Hsp72) is stress inducible protein with two isoforms (Hsp70-1a,b). It can be found in the cytosol, in the nucleus, and also on the luminal side of the lysosomal membrane. Its main function is to cope with harmful aggregations of denatured proteins via chaperoning function [34,45,70], and stabilize lysosomal membranes during stress [26,44]. Hsp70-1 was also showed to influence signal transduction pathways, cell cycle and senescence regulation [80,94,97]. *Hsp70-1t (Hsp70-hom)* is a constitutively expressed protein, localized in the cytosol and nucleus. It is expressed mainly in testis. Its specific role is not yet clear [26]. *Hsp70-2 (Hsp70-3, HspA2)* is also constitutively expressed protein localized in the cytosol and nucleus. It is expressed mainly in testis and brain. It is involved in senescence and cell cycle regulation and inhibition of apoptotic pathways [26,94,97]. *Hsp70-5 (Bip; Grp78)* is a constitutively expressed and roughly endoplasmic reticulum specific protein. Its main function is to facilitate the transport of newly synthesized proteins into the ER lumen and their subsequent folding [110]. It also alleviates ER stress induced apoptotic stimuli via Ca^{2+} binding and preventing Ca^{2+} efflux from the ER [39,79]. *Hsp70-6 (Hsp70B')* is a stress inducible protein which is localized in the cytosol and nucleus with similar functions like Hsp70-1. It is expressed mainly in dendritic cells, monocytes and NK cells [26]. *Hsp70-8 (Hsc70; Hsp73, Hsp70-cognate)* is a constitutively expressed protein in most tissues. It is involved in multiple housekeeping chaperoning functions including protein folding and refolding, protein translocation across membranes, chaperone-mediated autophagy, prevention of protein aggregation and disassemble of clastrin coated vesicles [26,34,45]. *Hsp70-9 (mtHsp70, mortalin, Grp75)* is a constitutively expressed and roughly mitochondria specific protein. Its main function is to assist the incoming proteins in correct folding following transmembrane transport, but outside mitochondria it can also regulate senescence pathways [27,97].

Main Extracellular Functions of Hsp70

In addition to intracellular response, stress also triggers release of Hsp70 into the extracellular space, and Hsp70 is also released from cells undergoing necrosis [17]. Extracellular Hsp70 reveal cytoprotective properties through cell surface association [49], which may be followed by internalization [42,43]. Extracellular Hsp70 is also involved in a number of physiological and pathological events including modulation of cytokine release [2,3], immunity [68,71,89,90,100], and the modulation of neuronal function [16,17,21,43,106]. In addition, Hsp70 is able to enter the blood stream [86,93,117], and possess the ability to act at distant sites of the body as an ancestral danger signal [65,66] triggered by cell injury, immune-inflammatory reactions, and physical [32,57] or behavioral [31,35] stress of the organism. The presence of Hsp70 in human saliva was also demonstrated [30,31] indicating that, premised extracellular functions of Hsp70 may play an important role on the mucosal, periodontal and tooth surfaces too, as detailed in the next sections of this chapter.

ORIGIN AND SUBTYPES OF SALIVARY HSP70

Salivary Gland Origin

Salivary glands are one of the main sources of Hsp70 in the saliva [29,30,31]. Although it has not yet been investigated in detail, it is very likely that, Hsp70 of glandular origin is not a new member of Hsp70 family, but rather a mixture of several known Hsp70-type proteins including stress inducible Hsp70-1 (Hsp72) and constitutive Hsp70-8 (Hsp73) [29,30,31]. The level of salivary Hsp70 shows rather big differences between subjects in both mixed (whole) saliva and cannulated parotid duct saliva (authors' unpublished data). There are also within subject differences may be because salivary Hsp70 is prompt inducible via several stimuli (see also below) [30,31,33]. Hsp70 is not secreted due to the classical secretory exocytotic process of the acinar cells, since the secretory parameters of Hsp70 were independent of those of amylase (a known secretory protein of salivary gland acinar cells) [30,31]. The transport of Hsp70 may involve passive transport via the salivary glands from blood serum [29,30,31], similarly to other blood proteins excreted with saliva [28,37,51]. A small capacity active transport from the striated duct cells of human salivary glands is also possible [29,30,31], since a higher amount of Hsp70 was detected in these cells comparing to other cell types of salivary glands and ducts [109]. Such transport would require either increased cell membrane permeability, or a specific exocytotic pathway [32,57], because Hsp70 family lacks a conventional signal peptide sequence that would allow them to be transported through cytosolic or plasma membranes [41]. An alternative transport of Hsp70 may occur either through lipid rafts [14,20,56], or through exosomes [22,55,56]. Another alternative transport mechanism was reported through lysosomes releasing their content into the extracellular space [17,63].

Mucosal Cell Origin

Mucosal cells are other main source of salivary Hsp70 [29]. Depending on location the oral epithelium can be either keratinized or non-keratinized. Keratinized surfaces (i.e. attached gingiva, hard palate, most dorsal surfaces of tongue) are subjected to strong mechanical loads. The abraded superficial cells of these surfaces lack intracytoplasmic organelles, being filled with keratin. In contrast, mucosal cells with more or less intact cell organelles are continuously released from the surface of moderately stressed non-keratinized epithelium (i.e. labial-, buccal-, alveolar mucosa, floor of the mouth, ventral surface of tongue) [9]. Hence a mixture containing intact and/or fragmented cells, intracellular compartments, membrane particles, and usual cytosolic constituents are released into the saliva from the non-keratinized mucosal surfaces [28]. Consequently all known members of Hsp70 family regularly present in human cells are released into the saliva from this musocal source.

Gingival Crevicular Fluid Origin

Gingival crevicular fluid is also a main source of salivary Hsp70 [29]. From this source serum exudate and some intact and/or destroyed immune cells can reach saliva, also in healthy and particularly in inflamed gingival (and/or periodontal) conditions [28,51]. Consequently Hsp70 family members present in the blood serum and immune cells enters saliva from this source.

Blood Origin

Beside serum exudates originated from the gingival crevicular fluid and salivary glands (see above), serum exudate also reaches saliva through the oral mucosa as mucosal transudate [28,51]. Moreover, each of the cellular and other blood constituents may enter saliva via intraoral bleeding (i.e. bleeding periodontal pockets, wounds, ulcers) [28,51]. Hsp70 family members present in blood serum and blood cells reaches saliva from these sources.

Bacterial (Microbial) Origin

Other main sources of salivary Hsp70 are oral bacteria and other oral microbes [29]. But it should be noted that, bacterial (microbial) homologues of Hsp70 family are originated from this sources only [29]. The amount of microbial Hsp70 homologues in the saliva may be dependent on oral hygiene conditions, properties of bacterial biofilm on tooth surfaces, and microbial flora of the oral cavity and periodontal pockets (if any).

SALIVARY HSP70 AND SURFACE DEFENSE

Defense of Mucosal Surfaces

There are two major facets of mucosal defense mechanisms expected for salivary Hsp70. One of them involves immunological and other antimicrobial defense pathways [29] and will be detailed later (see below). The other is based on the cytoprotective effects of extracellular Hsp70 [42,43,49]. The cytoprotective effects seem to be based on three different mechanisms, such as aspecific binding of Hsp70 on mucosal cell surfaces [49]; a more specific adhesin-type binding to sulfoglycolipid structures of mucosal sells [12]; and surface receptor binding of Hsp70 mostly followed by internalization [17,42,43]. Aspecific binding leads to surface defense against toxins [49] very likely through protection and repair (chaperoning) of mucosal cell surface proteins [30,31]. Adhesin-type binding may prevent bacterial colonization of mucosal surfaces through occupying mucosal binding sites of Hsp70 related bacterial adhesins [12,92]. Surface receptor binding of Hsp70 was proved to several receptors like oxidized low density lipoprotein binding receptor CD91 [5]; tumor necrosis factor receptor CD40 [8]; toll like pattern recognition receptors TLR2 and TLR4 [3]; chemokine receptor CCR5 [36]; and scavenger receptors LOX-1, SREC-1 and FEEL-1/CLEVER-1 [102]. Although premised Hsp70 receptor bindings were studied mostly related to immunological context, it should be considered that, other cell types are also capable of receptor mediated binding and uptaking of Hsp70 [17,21,43]. Up to the present, decrease of the cells' apoptotic and necrotic liability [42] and release of several cytokines [2,3] are the most important proved mechanisms of Hsp70 receptor induced cytoprotection. However, cytoprotective mechanism might be varied, since Hsp70 likely recognize different receptors on different cell types [103], and different members of Hsp70 family releases different effects on the same receptor [17]. The cellular answer may also be influenced by interactions of several Hsp70 receptors of the same cell surface [17] resulting an even more complex picture of possible cytoprotective pathways.

Defense of Tooth Surfaces

Recent data indicated that, Hsp70-1 (inducible Hsp72) binds both Gram-positive (*Streptococcus mutans and mitis*) and Gram-negative (*Escherichia coli*) bacteria [78]. Similarly, salivary Hsp70 also binds *S. mutans, S. mitis* and *E. coli*, moreover binds hydroxyl apatite the major inorganic component of tooth surfaces, (authors unpublished data). Taking together these results, it is likely that, salivary Hsp70 may play a role in the acquired pellicle formation followed by bacterial adhesion on tooth surfaces. The capability to take part in the acquired pellicle formation and to bind bacteria refers to that; salivary Hsp70 in general facilitates bacterial colonization of tooth surfaces. But it should be considered that, the pH optimum of bacterial binding of salivary Hsp70 is rather acidic (in our unpublished data it is around pH 5.0 - 5.5 similarly to recently published data with Hsp70-1 [78]). Such a low pH is not typical for tooth surfaces at the early stage of bacterial colonization, consequently salivary Hsp70 rather inhibits than facilitates bacterial adhesion to the acquired pellicle.

Recent finding also indicated that, ATP partially inhibits bacterial binding of Hsp70-1 [78]. Similarly, binding of salivary Hsp70 to bacteria and also to hydroxyl apatite was found to be partially inhibited by ATP (authors' unpublished data). The meaning of these latter ATP connected data is not jet clear but should be considered in future research of acquired pellicle formation and bacterial adhesion.

Immunological and Antimicrobial Defense Mechanisms

Three major facets of immune activation have been described for Hsp70 [69]. *The first of these* involves the appearance of intracellular Hsp70 on the surface of certain tumor cells leading to lysis of these cells by natural killer (NK) cells [11,72,73]. *The second facet* of immune activation involves released extracellular Hsp70 as an ancestral danger signal [65, 66] of cellular stress, death or lysis. Importantly both, uncomplexed ("free") and membrane bound (lipid rafts, exosomes) Hsp70 was showed to express such danger signal properties [73]. The immune activation here is very similar to that of bacterial lipopolysacharides (LPS) and the effect of LPS and extracellular Hsp70 seems to be additive [19]. Hsp70 as a danger signal induces: release of proinflammatory cytokines from several immune cells (i.e. monocytes, dendritic cells, macrophages, T lymphocytes) [2,13,19,115]; release of NO from macrophages [83]; activation of NK cells [68,71]; activation of complement via an antibody-independent alternative pathway [90]. *The third facet* of immune system activation involves complexes of extracellular Hsp70 and other peptides. Because its chaperoning ability, uncomplexed Hsp70 binds other peptides, and as complex induces receptor-mediated uptake into antigen-presenting cells (i.e. macrophages, Langerhans and dendritic cells) to cross present this complex as an antigen (coupled with MHC-I or MHC-II molecules) to cytotoxic T cells and NK cells [10,101,107]. This mechanism is important in the defense against bacteria (and other microbes) and also as an initiator of immune defense against tumor cells and virus infected cells. From premised three major immunological facets, only the first one is not expected for salivary Hsp70 because in this case the tumor cells express their own Hsp70 on the surface. The other two functions are expected for salivary Hsp70 [29], since oral mucosa is extensively populated by antigen presenting Langerhans and dendritic cells [25]. (The expectation is also confirmed by the interesting finding that, Langerhans cells are properly oriented to "sample" the oral fluids with their dendrites toward the surface [25,47].)

Beside the above most intensely researched immunological functions, there is another immunological mechanism, in which extracellular Hsp70 takes part. Recent data indicate that, Hsp70-1 (inducible Hsp72) exert an opsonizing effect on bacteria, which activate the killing activity of polymorphonuclear neutrophil (PMN) leukocytes [78]. Although there is a considerable flux of neutrophils through the gingival sulcus into the saliva even in health, this function may be especially effective under inflammatory conditions (gingival inflammations, wound healing, fever), because Hsp70 binding of bacteria is facilitated by acidic milieu (pH 5.5) and high temperature (42°C) [78]. It should be noted that, opsonizing effect similar to that of Hsp70-1 [78] is not jet verified with salivary Hsp70. However it is likely because the bacterial binding property of salivary Hsp70 is very similar to that of Hsp70-1: it is also

facilitated by acidic milieu (pH 5.0-5.5) and high (42°C) temperature (authors' unpublished data, see also above).

Beside immunological pathways there are two other hypothesized antimicrobial defense mechanisms of salivary Hsp70 which should be considered. One of them is based on the bacterial binding property of Hsp70 mentioned above. Via this binding salivary Hsp70 may also entrap and agglutinate bacteria. Since Hsp70 is known to be able to form dimers and oligomers, the agglutination could be rather effective [77]. It is also possible that, Hsp70 occur in micelles and/or in smaller homo/heterotypic complexes also known to enhance agglutination in saliva [99]. However it is not jet known whether bacterial binding can occur under such conditions or not. The other hypothesized mechanism is based on the finding that, fungicidal activity of salivary Histatin-5 (Hst5) is initiated by binding to surface Hsp70 (Ssa1p, Ssa2p) of *Candida albicans*, followed by internalization and later to cell death [59,60]. This finding indicates that, Histatin-5 may bind salivary Hsp70 too. Although there is no evidence that, such expected salivary Hsp70/Histatin-5 complex would be able to enter and destroy *C albicans*, but this possibility should rather be considered than simply excluded.

SALIVARY HSP70 AND ORAL DISORDERS

Oral Ulcerations

The role of Hsp70 in the formation and healing of oral ulcers was not jet studied in detail, but possible defense function of Hsp70 against oral ulceration may be expected based on the data related to gastric ulcers. In case of gastric ulcers Hsp70 is markedly overexpressed in cells located at the ulcer base [105], the level decreases with ulcer healing [105], and the extent of Hsp70 induction in mucosal cells is inversely correlates with the severity of newly induced ulcers [95,98]. These data indicate important defense role of intracellular Hsp70 against gastric ulcers. Similar intracellular response against ulcer formation may also be expected in the case of oral mucosa, but in the absence of evidence it remains a hypothesis only.

Beside intracellular defense, a coupled extracellular defense is also likely in the case of gastric ulcers, as indicated by two important findings. In one respect the regulation of gastric intracellular Hsp70 level is mediated by alpha 1A-adrenoceptors [95] the same receptor type which seems to be responsible for extracellular release (increased blood level) of Hsp70 [50]. On the other hand forced intracellular expression of Hsp70 seems to increase the secretion of Hsp70 into the extracellular space [111]. It is not jet known whether such coupling of intra and extracellular surface defense would exist in case of oral ulcers or not. But the possibility of extracellular defense in case of oral ulcers is at least given by the fact that, salivary Hsp70 is present on the ulcer surfaces covered by saliva.

There are three facets of the expected functions of salivary Hsp70 related to oral ulcers [29]. One of them can be recognized as the regular cytoprotective effect of extracellular Hsp70 as detailed above. The second one is based on the protection and repair (chaperoning) [23,24] of other salivary defense proteins (i.e. sIgA, lysozyme, cystatins, hystatins, amylase, etc. [28]). Such repair activity could be especially important in the case of sIgA during

induction of antibody catalyzed ozone formation on the surface of neutrofil PMN cells [76,114]. The third facet is based on the known immunological functions of extracellular Hsp70 (see detailed above). These immune functions could be rather effective in this case, because inflammatory serum exudate containing a high amount of complement system, immunoglobulin, immune/inflammatory mediators, PMN leukocytes, and monocytes/macrophages are usually also present on the surface of oral ulcers [29].

Oral Wound Healing

Although oral tissues were not particularly investigated, based on skin experiments the effect of Hsp70 seems to be rather similar to that of ulcers also in the case of wounds. The level of intracellular Hsp70 positively correlates with the efficiency of healing [67,82], and the level of Hsp70 decreases with the progress of the healing process [67] similarly to ulcers. A coupling of intra- and extracellular Hsp70 response is also likely in this case, because of similar considerations explained in connection with ulcers (see above). The coupling of intra- and extracellular response in case of wound healing is also established by the findings that, extracellular Hsp70 is released by white blood cells in wound fluid [7]; and *in vivo* delivery of Hsp70 increased the efficiency of wound healing by the stimulation of macrophage-mediated phagocytosis of wound debris [54].

Gingival Inflammation

Gingivitis is an inflammation indicative of poor oral hygiene, without destruction of the periodontal tissues [84]. Data in the literature indicate that, the strength of the local immune reaction against microorganisms is crucial to prevent the passing of this condition to periodontitis which is a more severe inflammation with irreversible destruction of the periodontal tissues [62,84]. In case of gingivitis an inflammatory exudate appears in the periodontal sulcus containing molecular constituents such as complement system, immunoglobulin, immune and inflammatory mediators, and immune active cells like PMN leukocytes and monocytes/macrophages [29]. Since there is no pocket formation, salivary molecules are blended with the majority of the inflammatory exudate (gingival crevicular fluid) at the marginal region of the gingival sulcus. Consequently, expected immunological functions of salivary Hsp70 (see above) come into full display because of direct contact with the immune system [29].

Although the role of salivary Hsp70 is likely protective against gingivitis in majority, there are two expected mechanisms by which salivary Hsp70 may induce gingivitis under certain conditions. One of these mechanisms will be discussed later in connection with allergic and autoimmune disorders. The other one is based on the fact that, Hsp70 is secreted mainly in a lipid bound form (i.e. exosomes, lipid rafts). Protein bound lipid molecules are known to form nucleus of calcium-phosphate deposition leading to calculus formation especially in higher pH [28,48]. Since salivary Hsp70 takes part in the acquired pellicle

formation on tooth surfaces (see above), it may play a role in calculus formation leading to gingival inflammation under certain conditions.

Irritation Caused by Dentures

The most frequent reason of denture induced irritation is given by appearance of small mucosal lesions, induced by wearing removable dentures. The larger lesions are usually easily recognized, and will be healed following the correction of the denture. However the small ones (the "micro lesions") are not easily detected. Such lesion may lead to irritative-allergic reactions (see below); because of the appearance of several immune cells and immune molecules on the lesion's surface [29]. Similarly to ulcer- and wound healing (see above) the cytoprotective and immunological effects of salivary Hsp70 may be resulted in advantageously shorter healing time of such lesions. Another effect may be resulted from the fact that, salivary Hsp70 binds acrylic resins (authors unpublished data), which are basic components of removable dentures' base plates. Consequently salivary Hsp70 is likely present on the surface of dentures as constitute of acquired pellicle of dentures. Acquired pellicle of denture surfaces is a major determinant of interaction between denture and fluid-film of saliva between dentures and mucosa. This interaction is rather important in maintaining stability of removable dentures (i.e. "gluing effect" of saliva) especially in full denture cases. Because of the known protein-binding property of Hsp70, it is likely that, salivary Hsp70 plays a role in decreasing mechanical surface irritation by improving the stability of removable dentures.

Allergic and Autoimmune Reactions

There is increasing evidence that, antibodies against human Hsp of the 60 kDa family may serve as autoantigens and could initiate an autoimmune response that contributes to the initiation of gingivitis [96]. Since antibodies against salivary Hsp70 [86] likely also present, it may not be excluded that, salivary Hsp70 also induces such autoimmune mechanisms (although it should be noted that, antibodies against different heat shock proteins are differing from each other in their antigen specificity and complement activating ability [89]). Some data in the literature also suggest that, overexpression of Hsp70 may play a role in the appearance of atopic-type (IgE mediated) allergic reactions [40,61], in autoimmune disorders [91,115], and in haptenation of peptides inducing T cell immunity and sensitization [64]. However it is not jet clear to what extent extracellular Hsp70s are involved in such processes.

Evaluating the role of salivary Hsp70 in allergic and autoimmune reactions it should also be considered that, Hsp70 exerts immune regulatory and anti-inflammatory functions too [85,104,108]. Administration of recombinant Hsp70 may also resulted in attenuation of experimental autoimmune diseases [53,113]. It was also shown that, low-affinity T cells are reactive against autologous heat shock proteins [74], which might lead to generation of Th2 (IL-4- and IL-10-producing), Th3 (transforming growth factor-β-producing), or Tr1 (IL-10-

producing) regulatory T cell responses and consequent release of premised regulatory cytokines [85,104,108].

Taking together these data it is likely that, Hsp70 may take part in both the release and the control of the above allergic and autoimmune reactions under certain conditions. The basis of such "Janus-faced" behavior of Hsp70s may be at least partially rooted in that, these molecules possess both stimulating and inhibitory epitopes toward cytokine production and maturation of dendritic cells [112]. Since the oral mucosa is extensively populated by dendritic cells [25] such epitopes of salivary Hsp70 may play key role in both initiation and regulation of oral mucosal immune reactions including allergic and autoimmune pathologies.

Tumor Formation

As mentioned above a major facet of immune activation caused by Hsp70 involves the appearance of Hsp70 on the surface of certain tumor cells leading to lysis of them by natural killer (NK) cells [11,72,73]. Since in this case the tumor cell expresses its own Hsp70 on the surface, this facet is not expected for salivary Hsp70 in general. However, new findings also indicate that, surface expression of Hsp70 on tumor cells is accompanied by Hsp70 release (via lipid rafts and/or exosomes) leading to activation of NK cells also in the environment [4,6,38]. Similarly, Langerhans and dendritic cells may also be targets of such signaling [18,112], leading to increased uptake and cross presentation of tumor derived Hsp70-peptide complexes for T cell recognition [18,81].

Since there is a high number of Langerhans and dendritic cells present in superficial layer of oral mucosa [25], salivary Hsp70 may act as a confluent stimulator of the immune surveillance of these cells [29], strengthening the effectiveness (increase the perceptibility) of the premised danger signaling of early stage in situ mucosal carcinomas. Since the expression of Hsp70 seems to be positively correlated with the malignancy of oral squamous cell carcinomas [52], this effect may increase with the malignancy of the tumor.

Regulation of Salivary HSP70 Level

Alteration under Psychological Stress Condition

Increase of extracellular Hsp70 level in blood [35] and human saliva [31] in response to psychological stress were demonstrated in previous studies [31,35]. In relation to *blood* a hepatosplanchnic source [32] and also brain [57] is known to be able to release high amount of Hsp70, but it is not yet known if these sources are involved in the elevation of blood level in this case. The increase of Hsp70 in the blood seems to be regulated through α1-adrenerg receptors of not jet known target cells [35,50]. An activation of the sympathetic nervous system and consequent release of norepinephrine is likely one of the major activation pathway [35,50l]. Sympathetic activation seems to be rather important also in the regulation of *salivary* Hsp70 level under stress conditions. Since α1-adrenerg receptors seem to be widespread in both acinar and ductal cells of human submandibular glands [46], it is possible

that, α1-adrenerg stimulation induces secretion of salivary Hsp70 in salivary glands. Further, combined α- plus β-adrenergic stimulation increases permeability of salivary glands to blood molecules in animal model [37]. Such mechanism could also be a reason of increased salivary Hsp70 level under stress conditions accompanied by increased blood Hsp70 level. Finally, elevation of salivary Hsp70 concentration and output values were found to be accompanied by elevation of salivary amylase *concentration* [31]. This finding also indicate strong connections between psychological stress, sympathetic activation and regulation of salivary Hsp70 level, because increased amylase *concentration* is a known indicator of both psychological stress [75] and stimulation of salivary glands via sympathetic nerve [88] and/or circulating catecholamine [1].

Stimulation with Chewing and Taste

Secretion of saliva is dependent upon stimuli from autonomic nerves that are the most important effector arms of reflexes activated by taste and chewing stimuli [87]. Release of proteins can be initiated by both sympathetic and parasympathetic stimuli [1,88]. Animal studies indicated that, fluid secretion is predominantly evoked by parasympathetic nerve-mediated stimuli connected with moderate protein release, whereas sympathetic nerve impulses evoke little fluid secretion with a very high amount of released secretory proteins (i.e. amylase) [1,88]. Using both chewing and taste stimuli together (i.e. mint taste chewing gum) a roughly eight fold increase of secretory rate with nearly constant protein concentration occurred indicating a predominantly parasympathetic character of the stimuli [30]. Under this condition the total Hsp70 output into the saliva increased roughly three times indicating that release of salivary Hsp70 can be stimulated also in this way [30].

Stimulation with Physiotherapy (Massage, Local Heat)

There is high number of stimulations (including mechanical stress and heat) known to induce increased expression of Hsp70 at cellular level. It is also likely that, forced intracellular expression of Hsp70 may increase the secretion of Hsp70 into the extracellular space [111]. Consequently, it is likely that, physiotherapeutic procedures like massage (mechanical stress) and local heat stimulation of major salivary glands may induce Hsp70 release into the saliva. This hypothesis was tested in a pilot study using massage and local heat in the parotid- and submandibular regions [33]. Results indicated that, total output and concentration values of salivary Hsp70 increase in both cases. Increase occurred immediately in both case, however highest values occurred initially in case of massage, whereas with a delay in case of heat stimulation [33]. Above data indicate that, salivary Hsp70 is inducible via such stimulations, however it is not jet clear if local vasodilatation, cellular damage or nervous regulatory pathways of the glands are responsible for the effect [33].

CONCLUSION

Salivary Hsp70 is likely an important factor of the oral defense mechanisms in both healthy and pathological conditions. However our knowledge related to salivary Hsp70 is still rather limited. Consequently the expectations about several functions detailed above are based on data related to exracellular but not particularly salivary Hsp70. The possible effects of special oral environment (including other salivary molecules and certain specificities of the oral mucosa) on the extracellular function of Hsp70 are not jet known. Besides summarizing the possible role of salivary Hsp70 in the oral cavity (still remaining roughly a hypothesis) another goal of the present review was to stimulate research activity leading to clear evidence related to functions of salivary Hsp70.

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