Received: 9 October 2023 | Revised: 9 November 2023 | Accepted: 4 December 2023

DOI: 10.1111/joa.13996

Journal of Anatomy ANATOMICAL SOCIETY WILEY

ORIGINAL ARTICLE

Prostate-specific antigen: An unfamiliar protein in the human salivary glands

Michela Isola[1] | Cristina Maxia[1] | Daniela Murtas[1] | Jörgen Ekström[2] | Raffaella Isola[1] | Francesco Loy[1]

[1]Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

[2]Division of Pharmacology, Institute of Neuroscience and Physiology, Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden

Correspondence
Michela Isola, Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042 Monserrato (CA), Italy.
Email: misola@unica.it

Abstract

Objectives: The presence of prostate-specific antigen (PSA) in saliva and salivary glands has been reported. Nevertheless, its release pathway in these glands remains to be elucidated. Here, we showed PSA subcellular distribution focusing on its plausible route in human salivary parenchyma.

Materials and Methods: Sections of parotid and submandibular glands were subjected to the immunohistochemical demonstration of PSA by the streptavidin–biotin method revealed by alkaline phosphatase. Moreover, ultrathin sections were collected on nickel grids and processed for immunocytochemical analysis, to visualize the intracellular distribution pattern of PSA through the observation by transmission electron microscopy.

Results: By immunohistochemistry, in both parotid and submandibular glands PSA expression was detected in serous secretory acini and striated ducts. By immunocytochemistry, immunoreactivity was retrieved in the cytoplasmic compartment of acinar and ductal cells, often associated with small cytoplasmic vesicles. PSA labeling appeared also on rough endoplasmic reticulum and in the acini's lumen. A negligible PSA labeling appeared in most of the secretory granules of both glands.

Conclusions: Our findings clearly support that human parotid and submandibular glands are involved in PSA secretion. Moreover, based on the immunoreactivity pattern, its release in oral cavity would probably occur by minor regulated secretory or constitutive-like secretory pathways.

KEYWORDS
immunocytochemistry, immunohistochemistry, light microscopy, PSA, salivary glands, transmission electron microscopy

1 | INTRODUCTION

The prostate-specific antigen (PSA) was believed to be produced exclusively by prostatic epithelial cells, and it was considered the biochemical marker of prostatic cancer (Carlsson & Vickers, 2020). However, circumstantial evidence confirmed that, in both women and men, other tissues and organs express PSA (Dash, 2015; Eklund et al., 2017; Musrap & Diamandis, 2016; Pérez-Ibave et al., 2018).

Raffaella Isola and Francesco Loy contributed equally to this work.

Now, it is widely accepted that PSA is not prostate-specific (Mashkoor et al., 2013); hence, its screening utility has been questioned (Filella, 2020; Gandaglia et al., 2019).

Immunohistochemically, PSA was detected in some human exocrine glands, as mammary gland, acinar pancreatic cells, thyroid gland, uroepithelial cells, periurethral glands, and Skene glands, known as the female prostate (Andrews et al., 2020; James et al., 1996; Tazawa et al., 1999; Van Krieken, 1993), as well as in various tumors (Olsson et al., 2005). The presence of PSA was also described in several body fluids, such as serum, urine, and milk (Diamandis & Yu, 1997; Yu & Diamandis, 1995). It remains to be ascertained whether non-prostatic PSA is enzymatically active and further its physiologic role in tissues and body fluids. It was speculated that PSA might be involved in the regulation of steroid hormones, and itself could possibly act as a growth factor (Yousef & Diamandis, 2001).

Furthermore, PSA was detected in salivary glands and saliva (Aksoy et al., 2002; Khan et al., 2018), where a proteolytic- and digestion-related function was presumed (Elgamal et al., 1996). In the past, it was believed that salivary PSA was derived exclusively from prostatic secretion since the salivary PSA level reflected that of serum. However, Breul and Colleagues detected a high concentration of total PSA in saliva which did not correlate with the serum concentration (Breul et al., 1994). On the other hand, Ayatollahi and Colleagues reported that there was a significant correlation between the ratio of free/total PSA in saliva and that of the serum of men with a normal prostate, but no correlation was found between serum and salivary total PSA or free PSA (Ayatollahi et al., 2007). Similar data were obtained by Turan and Colleagues (Turan et al., 2000). Although serum and salivary PSA were positively related in women during the menstrual cycle (Aksoy et al., 2002), higher salivary PSA levels were observed compared with those in serum in women taking oral contraceptives (Mannello et al., 1996). The authors attributed this increase to the stimulation of PSA synthesis in the salivary glands by the content of progesterone and estrogens in the contraceptive pills, suggesting that the salivary glands themselves may be considered an extra-prostatic source of PSA (Mannello et al., 1996). This assumption was strengthened by the detection of the PSA gene expression in salivary glands (Ishikawa et al., 1998; Yousef et al., 1999) and by the occurrence of PSA reactivity in small-sized duct epithelial cells of human parotid and submandibular glands (Elgamal et al., 1996; Musrap & Diamandis, 2016; Olsson et al., 2005; Tazawa et al., 1999).

The parotid and submandibular glands exert, by their different types of saliva, digestive, protective, and trophic functions. Parotid glands show only serous acini, while submandibular glands show serous and mucous tubules capped by serous cells called Giannuzzi's demilunes. Numerous ducts modify the composition of the saliva before it reaches the oral cavity. The glands are mostly regulated by the autonomic nervous system, but are also under endocrine influence, as recently exemplified by some gastrointestinal hormones (Ekström et al., 2017; Isola et al., 2018; Loy et al., 2012).

The present manuscript is intended to verify the presence of PSA in parotid and submandibular glands, focusing on its plausible route in human salivary parenchyma. To accomplish this, we performed an immunohistochemical and an immunocytochemical analysis on salivary gland (parotid and submandibular) samples, not compromised by tumors or other oral pathologies, using the streptavidin–biotin–alkaline phosphatase method on paraffined sections and post-embedding immunogold staining technique. This precise investigation method allows to elucidate in detail the subcellular localization of PSA, a method earlier applied by us to demonstrate a number of biological molecules (Isola et al., 2012, 2019).

2 | MATERIALS AND METHODS

2.1 | Patients and study design

Fragments of salivary glands were obtained from 9 male patients (aged 30–50 years) undergoing surgery for the removal of tumors of the mouth and neck regions at the Otorhinolaryngology Clinic, University of Cagliari. Only glandular fragments not compromised by tumors or other oral pathologies were selected. Gland eligibility was confirmed by light (LM) and transmission electron microscopy (TEM), based on an accurate macroscopic and histological evaluation. Patients were neither habitual smokers, alcohol consumers, nor obese. Moreover, they had not been subjected to chemotherapy or radiotherapy before surgery. Demographic and clinical information on patients was available in all cases.

The study was approved by the local Institutional Ethic Committee for human experimentation at the Azienda Sanitaria Locale 8 (ASL 8), Cagliari (Italy), and written consent was obtained from all patients before the beginning of the study, according to the World Medical Association Declaration of Helsinki.

2.2 | Immunohistochemical analysis

Among the total number of samples, 5 specimens of parotid and 5 of submandibular glands were fixed in 10% formalin and processed for paraffin embedding. Three microtome histological sections (6–7 μm thick) per sample were subjected to immunohistochemistry (IHC) for the demonstration of PSA by the streptavidin–biotin–alkaline phosphatase method, as previously described (Maxia et al., 2018, 2023; Murtas et al., 2017). They were dewaxed, rehydrated, and rinsed in phosphate-buffered saline (PBS), pH 7.4. Proteolytic antigen retrieval was obtained by immersion in a solution containing 0.1% Trypsin (Difco, Becton, Dickinson and Company, Sparks, MD), at 37°C for 5 min. Furthermore, sections were treated with 10% non-immune serum for 45 min at room temperature (RT) to block non-specific binding. Anti-human PSA mouse monoclonal antibody (1:100, A67-B/E3; Abcam, Cambridge, UK), raised against the 30 kDa protein fraction prepared from human seminal plasma, was used as primary antiserum for 1 h at RT. Biotinylated anti-mouse secondary antiserum (1:200; Vector Laboratories, Burlingame, CA, USA) was incubated for 30 min at RT; then, the sections were treated with

alkaline phosphatase–streptavidin (1:1000; Vector Laboratories) for 30 min at RT and were reacted with SIGMA FAST™ Fast Red substrate–chromogen system (SIGMA, St. Louis, MO, USA). All sections were carefully rinsed in PBS after each step, finally counterstained by Carazzi hematoxylin, and mounted in glycerol gelatin (SIGMA). Table 1 includes reported source, dilution, time of incubation, and the details of the primary and secondary antibodies used for the IHC staining, according to the Resource Identification Initiative (Bandrowski et al., 2015).

Positive and negative controls were run simultaneously to evaluate reaction specificity. Archival sections of normal human prostate were used as positive controls. Negative controls were carried out by omitting the primary antibody or by replacing the primary antibody with an isotype-matched antibody.

A Zeiss Axioplan2 microscope (Carl Zeiss Vision, Hallbergmoos, Germany), equipped with the following objectives: 20×/0.45 Zeiss Achroplan; 40×/0.75 Zeiss Plan-Neofluar, was used for the analysis of immunolabeled slides, while image capture was performed by a Lumenera Infinity 3-1URC camera (1.4 megapixels; Lumenera Corporation, Ontario, Canada) and Infinity Capture 6.3.0 software (Lumenera Corporation). The figure panels building and a slight adjustment of brightness and contrast were carried out by Adobe Photoshop CS3 Extended (ver. 10.0, Adobe Systems Incorporated, CA, USA).

2.3 | Immunocytochemistry

Among the total number of samples, 4 specimens of parotid and 4 of submandibular glands were cut into small pieces and fixed for 2 h with a mixture of 3% paraformaldehyde and 0.1% glutaraldehyde in 0.1 M cacodylate buffer at RT. Then, they were rinsed in cacodylate buffer added with 3.5% sucrose, dehydrated, and embedded in Epon Resin (Glycide Ether100, Merck, Darmstadt, Germany). To preserve the antigenicity of the tissue, treatment with osmium tetroxide was omitted. Semithin sections (2 μm), stained with toluidine blue, were examined by LM to check the histological appearance. Ultrathin sections (80 nm) were collected on nickel grids and processed for immunocytochemical analysis (Isola et al., 2023). The grids were treated with 1% bovine serum albumin (BSA) and 5% normal goat serum (NGS) in PBS solution to block non-specific binding. Then, they were incubated overnight at 4°C with a mouse monoclonal antibody specific for PSA (A67-B/E3; Abcam, Cambridge, UK) diluted 1:30 in PBS 1% BSA and 5% NGS. The grids were then incubated for 1 h at RT with the secondary antiserum, a goat anti-mouse IgG conjugated to 15 nm gold particles (GE Healthcare, UK), diluted 1:50 in PBS 1% BSA (Table 1). After washing, they were stained with uranyl acetate and bismuth subnitrate and finally observed and photographed with a JEOL JEM 1400 Plus (CeSAR core facilities) and with a JEOL 100S transmission electron microscopes (Isola et al., 2018).

Negative control sections were incubated with a non-immune serum or without the primary antibody.

3 | RESULTS

PSA immunoreactivity was detected in parotid and submandibular glands both by immunohistochemistry and immunocytochemistry, that is the immunogold technique.

3.1 | Immunohistochemistry

PSA immunohistochemical labeling of salivary glands resulted in a varying impression in terms of distribution and intensity of staining. In both glands, a discontinuous distribution of staining in acinar serous cells was observed (Figure 1a,b).

Moreover, an evident PSA immunostaining in Giannuzzi's demilunes of submandibular glands was observable (Figure 1b), while mucous acini were totally unreactive (Figure 1b).

Furthermore, strong immunoreaction in intercalated and striated duct cells of both parotid and submandibular glands was observable (Figure 1a,b). In addition, a strong staining in Wharton's duct was also appreciable (Figure 1c).

In acinar and ductal cells of both glands, PSA reactivity appeared distributed homogeneously in the cytoplasmic compartment (Figure 1a,b); however, in submandibular glands scattered immunoreactive nuclei were noticed (Figure 1b).

TABLE 1 Characteristics of the antibodies.

	Primary antibody							
Target	Method	Vendor	Origin	Dilution	Incubation	Antigen retrieval	RRID*	Secondary antibody
PSA	LM	Abcam	Mouse (mc)	1:100	1 h RT	Proteolytic[a]	AB_2134249*	Horse anti-mouse IgG[b] (biotinylated)
	TEM			1:30	ON	-		Goat anti-mouse IgG[c] (conjugated to 15 nm gold particles)

Abbreviations: mc, monoclonal; ON, overnight; RT, room temperature.

[a]Proteolytic pre-treatment with Trypsin (Difco, Becton, Dickson and Company, MD, USA), 0.1% 37°C for 5 min.

[b]Vector Laboratories, Burlingame, CA, USA, dilution 1:200 for 30 min RT.

[c]GE Healthcare, UK, dilution 1:50 for 1 h RT.

*The Resource Identification Initiative (Bandrowski et al., 2015); Resource Identification Portal (https://scicrunch.org/resources).

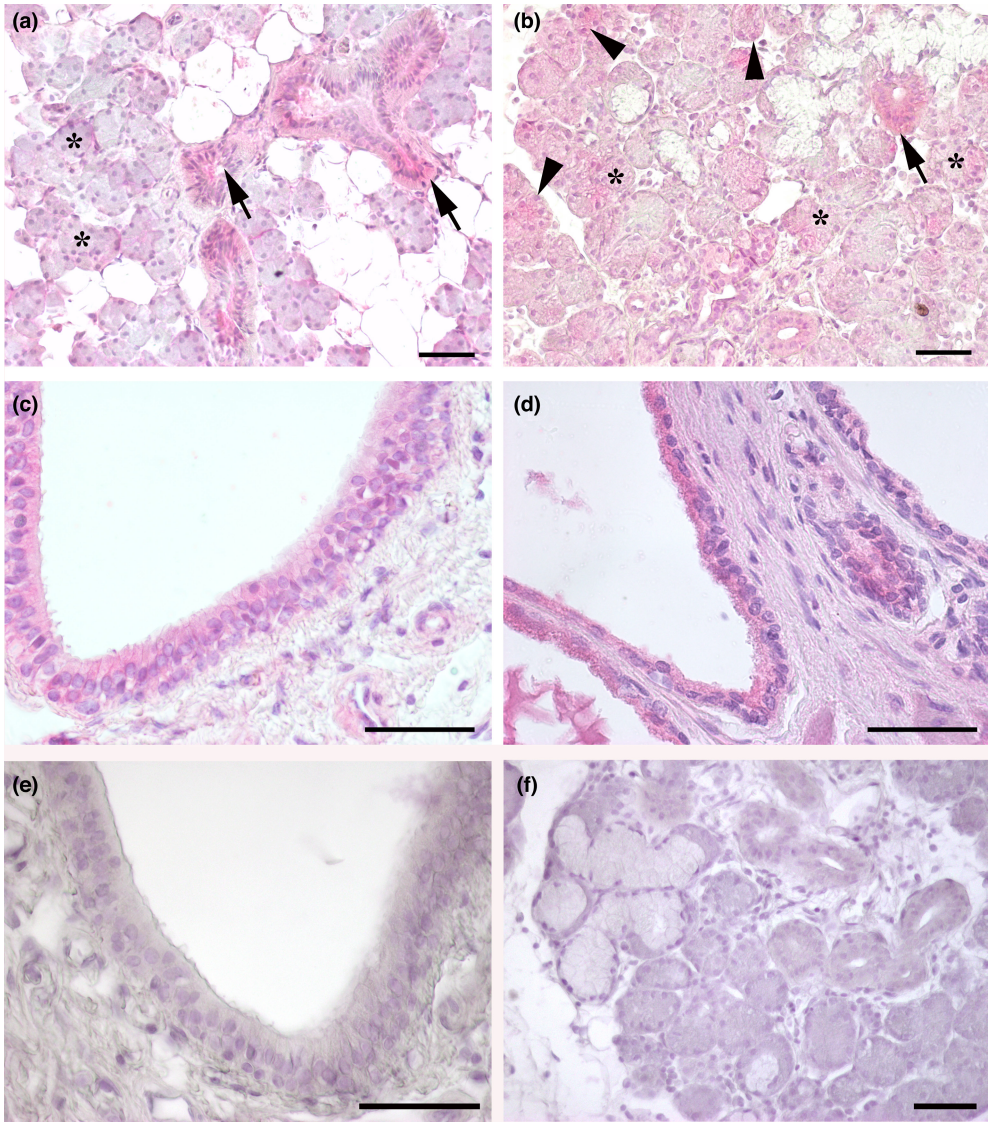

FIGURE 1 Light microscopy. Immunohistochemical staining for PSA. (a) Human parotid gland; (b, c) human submandibular gland; (d) positive control; (e, f) negative controls. A rather variable staining in terms of distribution and intensity in both parotid and submandibular glands was observable (a, b). It is noteworthy the evident PSA immunostaining in some serous acini (asterisks, a, b) and in Giannuzzi's demilunes (b). A conspicuous reactivity in intercalated and striated duct cells (arrows) of both parotid and submandibular glands was recorded (a, b). In addition, a marked staining in Wharton's duct was also noticed (c). In both acinar and ductal cells, PSA staining was localized in the cytoplasmic compartment (a, b); some positive nuclei in submandibular glands were easily appreciable (b, arrowheads). (d) Prostate tissue, as positive control section. (e, f) Negative controls of Wharton's duct and submandibular gland. Counterstained with Carazzi Hematoxylin. (a–f) bar 25 μm.

Human prostate gland, used as positive control for PSA, showed a rather strong reactivity in the secretory parenchyma (Figure 1d). Negative controls did not show any immunoreactivity (Figure 1e,f).

3.2 | Immunocytochemistry

At the mere observation, the reactivity of PSA, with respect to the acinar serous cells of parotid and submandibular glands, seemed to match both in distribution pattern and in strength of staining (Figures 2 and 3). Both glands expressed a PSA reactivity homogeneously scattered in the cytoplasmic compartment (Figures 2 and 3). Specifically, gold particles were noticed next to the perinuclear zone (Figures 2 and 3) and among the secretory granules (Figure 3). PSA reactivity decorated basal and lateral membranes of secretory cells, as well (Figures 2 and 3). Often, staining was associated with cytoplasmic vesicles, spread among secretory granules (Figure 4).

Moreover, rough endoplasmic reticulum (Figure 5) and nuclei (Figures 1–3) appeared marked for PSA.

Most of the serous granules, which are the typical secreting organelles of acinar cells, appeared negative for PSA. A negligible positivity was observed in few serous granules of both glands. (Figures 2–4).

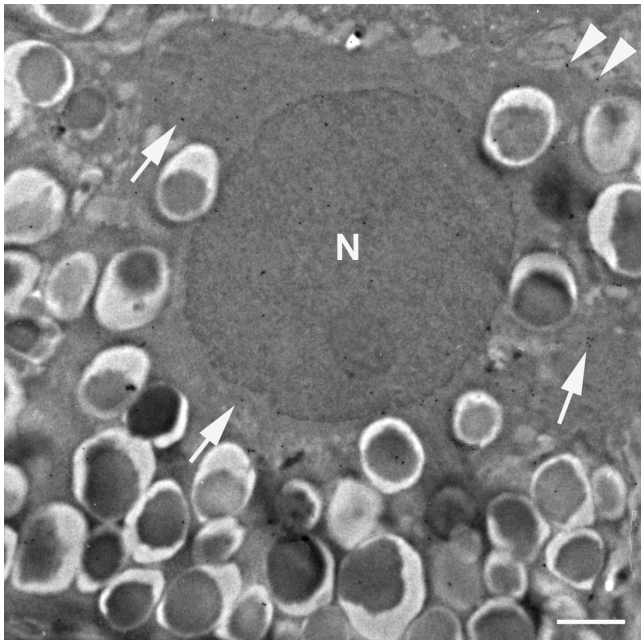

FIGURE 2 Parotid gland. Panoramic view of an acinar serous cell by TEM. PSA reactivity was homogeneously scattered in the cytoplasmic compartment, with gold particles located in the perinuclear zone and among the secretory granules (arrows). Few serous granules were weakly marked. The basal and lateral membranes of these secretory cells were marked (arrowheads). N: nucleus. Bar 1 μm.

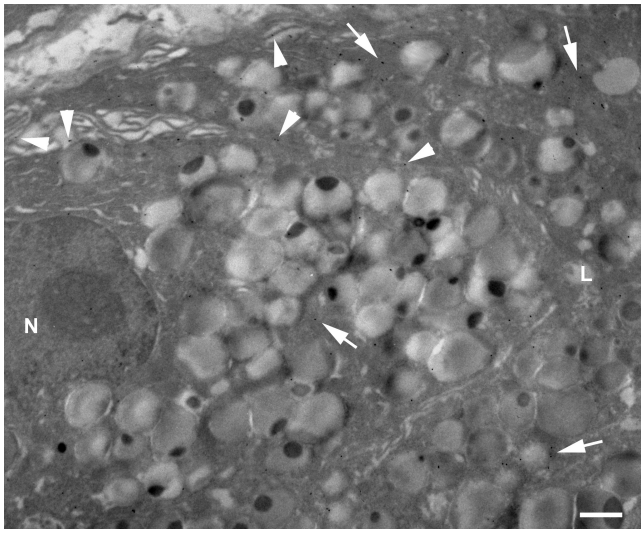

FIGURE 3 Submandibular gland. Electron transmission image of serous cells. Reactivity for PSA was mainly exhibited in the cytoplasmic area and among secretory granules (arrows). Plasma membranes showed reactivity for PSA (arrowheads). L: lumen. N: nucleus. Bar 1 μm.

In mucous cells of submandibular glands, a weak reactivity was observed exclusively in the thin cytoplasm surrounding the nucleus and mucous granules (Figure 6) which were totally unreactive to PSA (Figures 1b and 2–4).

In intercalated duct cells, numerous gold particles were equally dispersed into the cytoplasmic compartment. PSA reactivity was observed next to the lumen and in the apical membranes. Moreover, PSA was highlighted in the luminal content. Small secretory granules of these ductal cells were always unstained (Figure 7).

In striated duct cells, gold particles were preferentially spread among mitochondria, which were unstained. Basal membrane folds showed reactivity for PSA (Figure 8).

Specimens used as control samples, where the primary antibody was omitted, did not exhibit any immunoreaction for PSA (Figure 9).

4 | DISCUSSION

This study revealed the expression pattern and subcellular localization of PSA in human salivary parenchyma, showing its presence in secretory serous, mucous, and ductal cells in the two types of examined glands. Our study utilized various methods to gain a comprehensive understanding of the PSA presence in the secretory salivary cells. Through LM, we obtained a global view of the parenchyma, while TEM allowed for a fine ultrastructural localization. By LM, the results showed that PSA immunostaining was rather variable in terms of distribution and intensity. Instead, by TEM, PSA immunoreactivity with respect to the secretory and ductal cells of parotid and submandibular glands seemed to match both in distribution pattern and in staining intensity. In the secretory cells, PSA was mainly expressed in the cytoplasmic compartment. As opposite, most of serous and mucous secretory granules were unstained. The distinct staining pattern of PSA provided useful information regarding its presence in salivary gland cells, and in turn, its plausible traffic aimed at its release in the oral cavity. The immunopositivity of rough endoplasmic reticulum in serous cells suggests that major salivary glands have the capability to produce PSA themselves, beyond carrying it from plasma.

The mechanism of secretion implies that several salivary proteins are stored in large secretory granules and are then released into the saliva in response to extracellular stimuli. This pathway, called major regulated secretion, is mainly activated by vigorous stimuli and it occurs during eating, making available large quantities of salivary proteins in support of the initial phase of digestive process (Huang et al., 2001). Other proteins, not stored in secretory granules, are secreted by either the minor regulated secretory pathway or the constitutive-like secretory pathway (Gorr et al., 2005), which are considered as sources of resting (or basal) salivary secretion to sustain the physiology of the oral cavity (Huang et al., 2001). Both minor regulated secretory and constitutive-like secretory pathways originate from small vesicles budding from immature granules. They differ because the former needs a weak stimulus to be discharged, while the later spontaneously releases its cargo at the apical plasma membrane (Gorr et al., 2005). Moreover, it seems that the salivary proteins secreted via the minor regulated secretory or the constitutive-like secretory pathway, are different from those that are stored

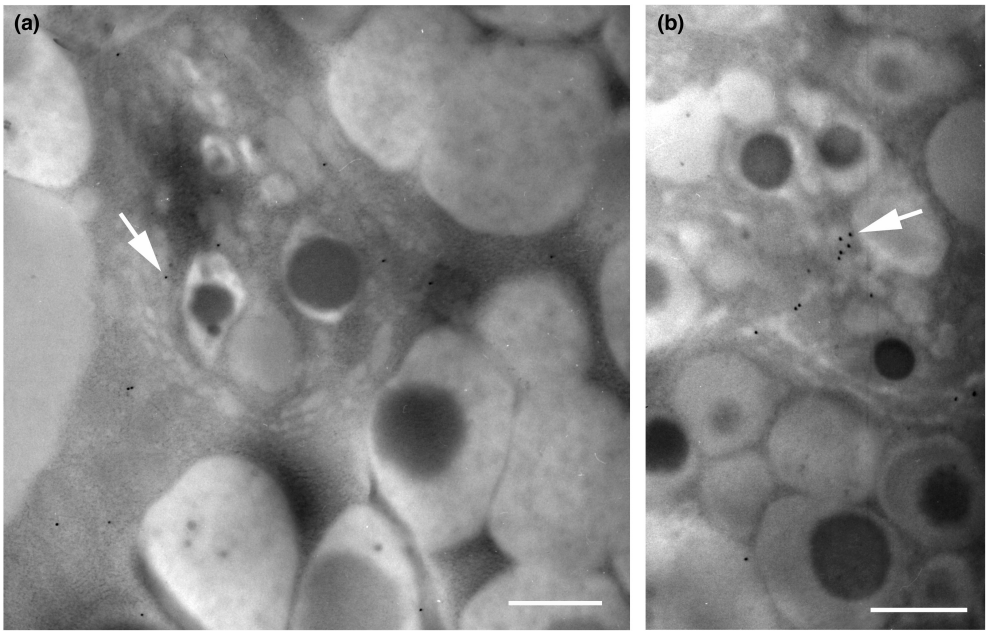

FIGURE 4 High magnification of submandibular serous cell portions. (a, b): Gold particles associated with cytoplasmic vesicles (arrows), dispersed among secretory granules, were observed. (a, b): Bar 1 μm.

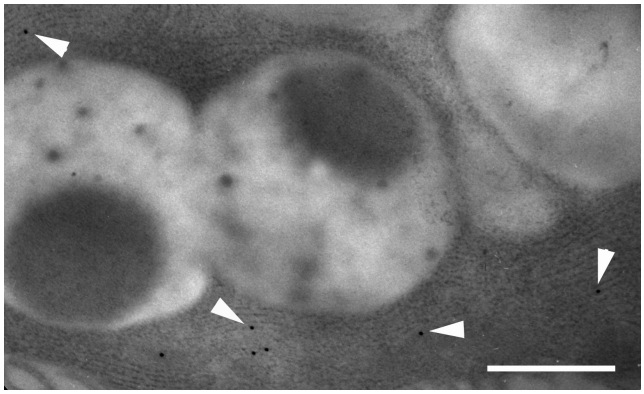

FIGURE 5 High magnification of submandibular serous cell portion. PSA labeling was appreciated in rough endoplasmic reticulum (arrowheads). Bar: 1 μm.

in large secretory granules (Arvan & Castle, 1998). Our data suggest that the PSA found in oral cavity would originate or move from glandular parenchyma. Indeed, PSA immunoreactivity was associated with the rough endoplasmic reticulum in both glands. However, its release into the glandular lumen would not occur by major regulated secretion, as seen for other salivary proteins (Isola et al., 2010, 2019; Isola & Lilliu, 2016), but, based on the distinct distribution pattern of PSA immunoreactivity, its secretion would preferentially follow an alternative secretory pathway. The minor regulated secretory or constitutive-like secretory pathways would seem the more plausible pathways. If so, PSA would be, at least in part, continuously discharged into the oral cavity. These mechanisms would be additive to the simple passage of non-protein-bound PSA to saliva through the paracellular pathway.

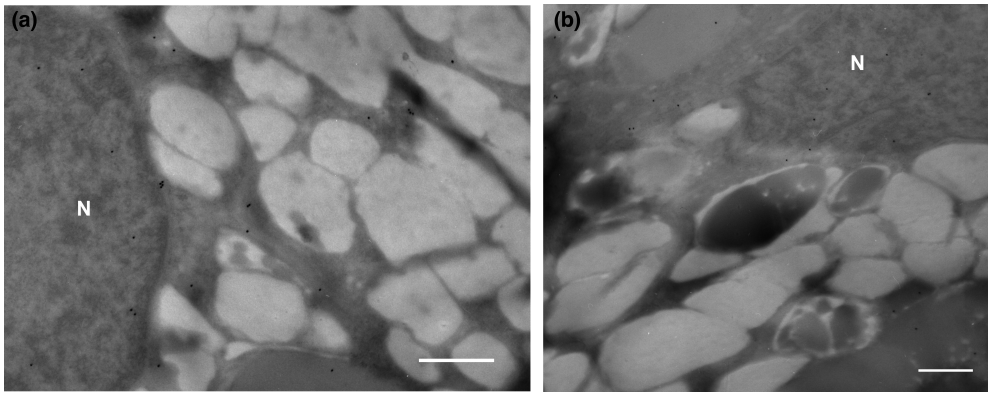

FIGURE 6 Submandibular gland mucous cell. (a, b): PSA labeling was weakly expressed in the cytoplasmic portion of mucous cells. The mucous granules were unreactive to PSA. N: nucleus. (a, b); bar 1 μm.

In the literature, some data related to the presence of PSA and its expression in salivary glands of normal tissues were provided (Ayatollahi et al., 2007; Ishikawa et al., 1998; Olsson et al., 2005; Yousef et al., 1999). Moreover, a consistent PSA reactivity in small-sized duct epithelial cells of major salivary glands was previously reported (Elgamal et al., 1996; Tazawa et al., 1999). The cytoplasmic localization of PSA, observed in striated and intercalated ductal cells, could mean that it would be discharged into ductal lumen with a secretion mechanism similar to the one that we proposed in acinar cells. Moreover, since our study demonstrated, by LM, a strong immunoreaction to PSA antibody in Wharton's duct, also major ducts might share this mechanism as well, although further investigation is needed.

The PSA expression in ductal cells observed in our study partially confirmed the results of Tazawa and Colleagues who detected immunoreactivity in the apical cytoplasm of ductal cells by paraffin-embedded samples and the immunoperoxidase method (Tazawa et al., 1999). However, Tazawa and Colleagues did not observe PSA reactivity in the cytoplasm of the acinar cells (Tazawa et al., 1999). This discrepancy might be due to the different approach used for protein localization. The precise information provided by electron microscopy makes it easier to visualize where all the gold particles are localized, even if their number is limited (Griffiths & Lucocq, 2014). Moreover, the preferentially cytoplasmic localization of PSA, observed in acinar and ductal cells, is in agreement with the subcellular distribution of PSA previously found in prostate secreting cells (Ben Jemaa et al., 2013; Sinha et al., 1987).

Introductory, attention was drawn to the fact that the levels of PSA in saliva did not always correspond to the levels in serum (Breul et al., 1994; Mannello et al., 1996). For instance, the women on oral contraceptives showed higher levels of PSA in saliva than in serum

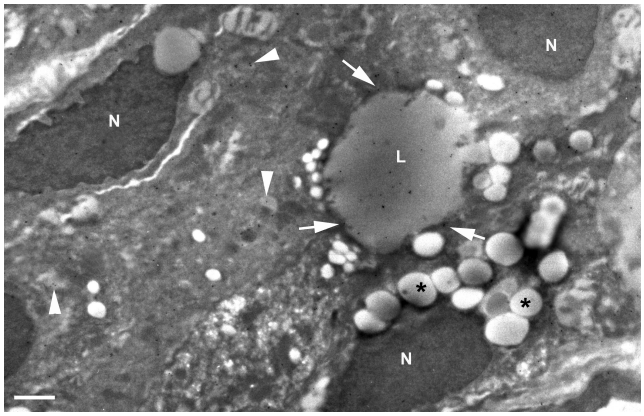

FIGURE 7 Parotid intercalated ductal cells. Reactivity was scattered in the cytoplasmic portion. Some small vesicles positive for PSA (arrowheads) were appreciable. Large and small secretory granules were negative (asterisks). Arrows indicated PSA localization in the apical membranes. L: lumen. N: nucleus. Bar: 1 μm.

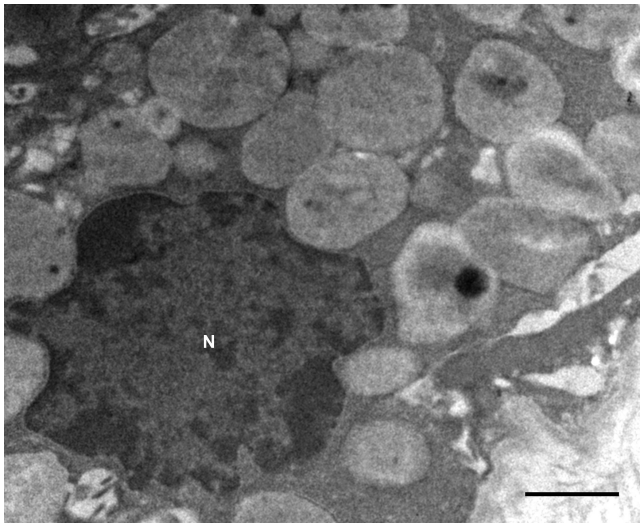

FIGURE 9 Parotid gland control image. Portion of secretory cells. Incubation of the sections without the primary antiserum did not show any labeling for PSA. N: nucleus. Bar 1 μm.

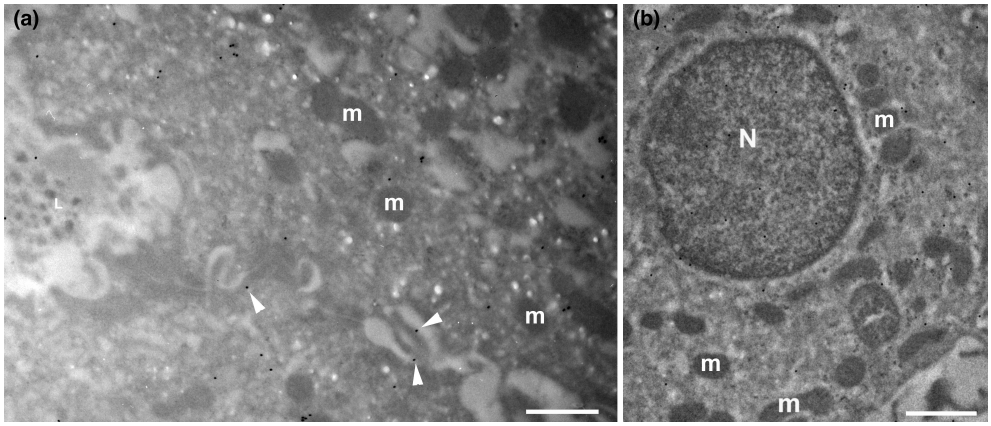

FIGURE 8 Submandibular striated duct cells. (a, b): Gold particles were preferentially spread among mitochondria, which were always unstained. To note that luminal membranes of these ductal cells were unstained, while an opposite labeling was detectable in lateral membrane (arrowheads). L: lumen. m: mitochondria. N: nucleus. (a, b): bar 1 μm.

(Mannello et al., 1996). Observations that make us to consider/hypothesize that the salivary glands could be the additional source of PSA in saliva.

The secretion, pathways, and biological role of PSA in salivary parenchyma and oral cavity are open to speculation. Salivary PSA could act as a growth factor in normal tissues, or it could be involved in regulating the function of insulin-like growth factor binding proteins (IGFBPs) (Cohen et al., 1992; Nagar & Msalati, 2013). Also, it might have a proteolytic- and digestion-related function (Elgamal et al., 1996). PSA could be also secreted into the interstitial space to mediate, by a paracrine pathway, the interaction between epithelial and stromal cells, as proposed for other salivary proteins (Cossu et al., 2011; Isola et al., 2010; Loy et al., 2015) or into the circulation, as suggested for PSA released from other tissues (Olsson et al., 2005). However, given the low levels detected in those sites, it seems unlikely that non-prostatic PSA, under normal circumstances, would interfere with PSA tests in use for prostate cancer.

In summary, our findings clearly support that human major (parotid and submandibular) salivary glands are involved in PSA secretion. Moreover, the distinct immunoreactive pattern displayed in the glands may suggest a PSA release into the oral cavity by the minor regulatory pathway and/or the constitutive-like secretory pathway. The present study may offer basic information for further translational studies, considering pathological conditions such as salivary gland inflammation and salivary gland tumors and possible ultrastructural changes associated with synthesis, trafficking, and release of PSA.

AUTHOR CONTRIBUTIONS

MI, RI, FL, CM, and DM performed experiments. MI, FL, JE, and RI drafted manuscript. MI, FL, CM, DM, and RI elaborated the images. All authors edited, revised, and approved final version of manuscript.

ACKNOWLEDGMENTS

We acknowledge the CeSAR (Centro Servizi Ricerca d'Ateneo) core facility of the University of Cagliari, Italy, and Dr. Andrea Ardu for assistance with the generation of transmission electron microscopy images. Furthermore, the authors are thankful to Mrs. Maria Itala Mosso for her skillful technical assistance.

FUNDING INFORMATION

The authors' research is supported by grants from the "Fondo Integrativo per la Ricerca" (FIR) of the University of Cagliari, Italy.

CONFLICT OF INTEREST STATEMENT

No conflicts of interest, financial or otherwise, are declared by the authors.

DATA AVAILABILITY STATEMENT

The corresponding author agrees to make available all data generated or analysed during this study on reasonable request.

ORCID

Michela Isola https://orcid.org/0000-0001-6347-4682
Cristina Maxia https://orcid.org/0000-0002-3490-1793
Daniela Murtas https://orcid.org/0000-0002-1357-2492
Raffaella Isola https://orcid.org/0000-0002-8211-6191
Francesco Loy https://orcid.org/0000-0002-6600-3883

REFERENCES

Aksoy, H., Akçay, F., Umudum, Z., Yildirim, A.K. & Memisogullari, R. (2002) Changes of PSA concentrations in serum and saliva of healthy women during the menstrual cycle. *Annals of Clinical and Laboratory Science*, 32(1), 31–36.

Andrews, J.R., Ahmed, M.E., Motterle, G., Albadri, S.T., Haloi, R., Karnes, R.J. et al. (2020) A rare case of prostate-specific antigen-producing metastatic parotid adenocarcinoma developing androgen receptor resistance. *Mayo Clinic Proceedings: Innovations, Quality & Outcomes*, 4(5), 601–607.

Arvan, P. & Castle, D. (1998) Sorting and storage during secretory granule biogenesis: looking backward and looking forward. *The Biochemical Journal*, 332(Pt3), 593–610.

Ayatollahi, H., Darabi Mahboub, M.R., Mohammadian, N., Parizadeh, M.R., Kianoosh, T., Khabbaz Khoob, M. et al. (2007) Ratios of free to total prostate-specific antigen and total prostate specific antigen to protein concentrations in saliva and serum of healthy men. *Urology Journal*, 4(4), 238–241.

Bandrowski, A., Brush, M., Grethe, J.S., Haendel, M.A., Kennedy, D.N., Hill, S. et al. (2015) The resource identification initiative: a cultural shift in publishing. *Brain and Behavior: A Cognitive Neuroscience Perspective*, 6(1), e00417. Available from: https://doi.org/10.1002/brb3.417

Ben Jemaa, A., Bouraoui, Y., Sallami, S., Banasr, A., Nouira, Y., Horchani, A. et al. (2013) Cellular distribution and heterogeneity of PSA and PSMA expression in normal, hyperplasia and human prostate cancer. *La Tunisie Médicale*, 91(7), 458–463.

Breul, J., Pickl, U. & Hartung, R. (1994) Prostate-specific antigen in urine. *European Urology*, 26(1), 18–21.

Carlsson, S.V. & Vickers, A.J. (2020) Screening for prostate cancer. *The Medical Clinics of North America*, 104(6), 1051–1062.

Cohen, P., Graves, H.C., Peehl, D.M., Kamarei, M., Giudice, L.C. & Rosenfeld, R.G. (1992) Prostate-specific antigen (PSA) is an insulin-like growth factor binding protein-3 protease found in seminal plasma. *The Journal of Clinical Endocrinology and Metabolism*, 75(4), 1046–1053.

Cossu, M., Isola, M., Solinas, P., De Lisa, A., Massa, D. & Lantini, M.S. (2011) Immunoreactivity of the salivary protein statherin in human male accessory sex glands. *Prostate*, 71(6), 671–674.

Dash, P. (2015) Reconnoitring the status of prostate specific antigen and its role in women. *Indian Journal of Clinical Biochemistry*, 30(2), 124–133.

Diamandis, E.P. & Yu, H. (1997) Nonprostatic sources of prostate-specific antigen. *The Urologic Clinics of North America*, 24(2), 275–282.

Eklund, E., Diamandis, E.P., Muytjens, C., Wheeler, S., Mathew, A., Stengelin, M. et al. (2017) Serum complexed and free prostate specific antigen levels are lower in female elite athletes in comparison to control women. *F1000Res*, 6, 1131.

Ekström, J., Khosravani, N., Castagnola, M. & Messana, I. (2017) Saliva and the control of its secretion. In: Ekberg, O. (Ed.) *Dysphagia*. Medical Radiology: Springer.

Elgamal, A.A., Ectors, N.L., Sunardhi-Widyaputra, S., Van Poppel, H.P., Van Damme, B.J. & Baert, L.V. (1996) Detection of prostate specific antigen in pancreas and salivary glands: a potential impact on prostate cancer overestimation. *The Journal of Urology*, 156, 464–468.

Filella, X. (2020) Towards personalized prostate cancer screening. *Advances in Laboratory Medicine*, 1(1), 20190027.

Gandaglia, G., Albers, P., Abrahamsson, P.A., Briganti, A., Catto, J.W.F., Chapple, C.R. et al. (2019) Structured population-based prostate-specific antigen screening for prostate cancer: the European Association of Urology position in 2019. *European Urology*, 76(2), 142–150.

Gorr, S.U., Venkatesh, S.G. & Darling, D.S. (2005) Parotid secretory granules: crossroads of secretory pathways and protein storage. *Journal of Dental Research*, 84(6), 500–509.

Griffiths, G. & Lucocq, J.M. (2014) Antibodies for immunolabeling by light and electron microscopy: not for the faint hearted. *Histochemistry and Cell Biology*, 142(4), 347–360.

Huang, A.Y., Castle, A.M., Hinton, B.T. & Castle, J.D. (2001) Resting (basal) secretion of proteins is provided by the minor regulated and constitutive-like pathways and not granule exocytosis in parotid acinar cells. *The Journal of Biological Chemistry*, 276(25), 22296–22306.

Ishikawa, T., Kashiwagi, H., Iwakami, Y., Hirai, M., Kawamura, T., Aiyoshi, Y. et al. (1998) Expression of alpha-fetoprotein and prostate-specific antigen genes in several tissues and detection of mRNAs in normal circulating blood by reverse transcriptase-polymerase chain reaction. *Japanese Journal of Clinical Oncology*, 28(12), 723–728.

Isola, M., Cossu, M., Diana, M., Isola, R., Loy, F., Solinas, P. et al. (2012) Diabetes reduces statherin in human parotid: immunogold study and comparison with submandibular gland. *Oral Diseases*, 18(4), 360–364.

Isola, M., Cossu, M., Massa, D., Casti, A., Solinas, P. & Lantini, M.S. (2010) Electron microscopic immunogold localization of statherin in human minor salivary glands. *Journal of Anatomy*, 216(5), 572–576.

Isola, M., Ekström, J., Isola, R. & Loy, F. (2019) Melatonin release by exocytosis in the rat parotid gland. *Journal of Anatomy*, 234(3), 338–345.

Isola, M. & Lilliu, M.A. (2016) Melatonin localization in human salivary glands. *Journal of Oral Pathology & Medicine*, 45(7), 510–515.

Isola, M., Lilliu, M.A., Loy, F. & Isola, R. (2018) Diabetic status influences the storage of melatonin in human salivary glands. *Anat Rec (Hoboken)*, 301(4), 711–716.

Isola, R., Lai, Y., Noli, R., Masala, C., Isola, M. & Loy, F. (2023) Melatonin ultrastructural localization in mitochondria of human salivary glands. *Journal of Anatomy*, 242(2), 146–152.

James, G.K., Pudek, M., Berean, K.W., Diamandis, E.P. & Archibald, B.L. (1996) Salivary duct carcinoma secreting prostate-specific antigen. *American Journal of Clinical Pathology*, 106(2), 242–247.

Khan, M.S., Dighe, K., Wang, Z., Srivastava, I., Daza, E., Schwartz-Dual, A.S. et al. (2018) Detection of prostate specific antigen (PSA) in human saliva using an ultra-sensitive nanocomposite of graphene nanoplatelets with diblock-co-polymers and Au electrodes. *Analyst*, 143(5), 1094–1103.

Loy, F., Diana, M., Isola, R., Solinas, P., Isola, M., Conti, G. et al. (2012) Morphological evidence that pentagastrin regulates secretion in the human parotid gland. *Journal of Anatomy*, 220(5), 447–453.

Loy, F., Isola, M., Isola, R., Solinas, P., Lilliu, M.A., Puxeddu, R. et al. (2015) Ultrastructural evidence of a secretory role for melatonin in the human parotid gland. *Journal of Physiology and Pharmacology*, 66(6), 847–853.

Mannello, F., Bianchi, G. & Gazzanelli, G. (1996) Immunoreactivity of prostate-specific antigen in plasma and saliva of healthy women. *Clinical Chemistry*, 42(7), 1110–1111.

Mashkoor, F.C., Al-Asadi, J.N. & Al-Naama, L.M. (2013) Serum level of prostate-specific antigen (PSA) in women with breast cancer. *Cancer Epidemiology*, 37(5), 613–618.

Maxia, C., Isola, M., Grecu, E., Cuccu, A., Scano, A., Orrù, G. et al. (2023) Synergic action of insulin-like growth Factor-2 and miRNA-483 in Pterygium pathogenesis. *International Journal of Molecular Sciences*, 24(5), 4329.

Maxia, C., Murtas, D., Isola, M., Tamma, R., Zucca, I., Piras, F. et al. (2018) Immunophenotypic characterization of telocyte-like cells in pterygium. *Molecular Vision*, 24, 853–866.

Murtas, D., Maxia, C., Diana, A., Pilloni, L., Corda, C., Minerba, L. et al. (2017) Role of epithelial-mesenchymal transition involved molecules in the progression of cutaneous melanoma. *Histochemistry and Cell Biology*, 148(6), 639–649.

Musrap, N. & Diamandis, E.P. (2016) Prostate-specific antigen as a marker of hyperandrogenism in women and its implications for antidoping. *Clinical Chemistry*, 62(8), 1066–1074.

Nagar, R. & Msalati, A.A. (2013) Changes in serum PSA during normal menstrual cycle. *Indian Journal of Clinical Biochemistry*, 28(1), 84–89.

Olsson, A.Y., Bjartell, A., Lilja, H. & Lundwall, A. (2005) Expression of prostate-specific antigen (PSA) and human glandular kallikrein 2 (hK2) in ileum and other extraprostatic tissues. *International Journal of Cancer*, 113(2), 290–297.

Pérez-Ibave, D.C., Burciaga-Flores, C.H. & Elizondo-Riojas, M.Á. (2018) Prostate-specific antigen (PSA) as a possible biomarker in non-prostatic cancer: a review. *Cancer Epidemiology*, 54, 48–55.

Sinha, A.A., Wilson, M.J. & Gleason, D.F. (1987) Immunoelectron microscopic localization of prostatic-specific antigen in human prostate by the protein A-gold complex. *Cancer*, 60(6), 1288–1293.

Tazawa, K., Kurihara, Y., Kamoshida, S., Tsukada, K. & Tsutsumi, Y. (1999) Localization of prostate-specific antigen-like immunoreactivity in human salivary gland and salivary gland tumors. *Pathology International*, 49(6), 500–505.

Turan, T., Demir, S., Aybek, H., Atahan, O., Tuncay, O.L. & Aybek, Z. (2000) Free and total prostate-specific antigen levels in saliva and the comparison with serum levels in men. *European Urology*, 38(5), 550–554.

van Krieken, J.H. (1993) Prostate marker immunoreactivity in salivary gland neoplasms. A rare pitfall in immunohistochemistry. *The American Journal of Surgical Pathology*, 17(4), 410–414.

Yousef, G.M. & Diamandis, E.P. (2001) The new human tissue kallikrein gene family: structure, function, and association to disease. *Endocrine Reviews*, 22(2), 184–204.

Yousef, G.M., Obiezu, C.V., Luo, L.Y., Black, M.H. & Diamandis, E.P. (1999) Prostase/KLK-L1 is a new member of the human kallikrein gene family, is expressed in prostate and breast tissues, and is hormonally regulated. *Cancer Research*, 59(17), 4252–4256.

Yu, H. & Diamandis, E.P. (1995) Prostate-specific antigen in milk of lactating women. *Clinical Chemistry*, 41(1), 54–58.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Isola, M., Maxia, C., Murtas, D., Ekström, J., Isola, R. & Loy, F. (2024) Prostate-specific antigen: An unfamiliar protein in the human salivary glands. *Journal of Anatomy*, 244, 873–881. Available from: https://doi.org/10.1111/joa.13996