Mammaglobin protein localization and gene expression in the salivary glands

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Abstract

Purpose: This study aims to delve deeper into the hypothesis that normal salivary gland tissue expresses both protein and mRNA of mammaglobin (MGB).

Methods: Formalin-fixed paraffin-embedded samples of submandibular (10), parotid (5), palatal (5) and labial glands (30) salivary glands were immunohistochemically investigated. The labial samples were used to examine the MGB positive ratio (MGB-PR), and localize MGB by double immunofluorescence staining and quantitative mRNA gene expression. Mann-Whitney U and Kruskal-Wallis rank-sum test for group comparison, and Spearman’s rank correlation coefficient for correlation analysis were used.

Results: The distribution of MGB-positive cells was variable throughout samples with significantly higher MGB-PR of acini than ducts ($P = 0.00376$), and there was no difference when compared based on age ($P = 0.0646$) and gender ($P = 0.245$). Besides acinar cells, a number of myoepithelial cells and ductal cells also demonstrated strong MGB reactivity with varying MGB mRNA expression levels in 6 of the 7 samples (with MGB-PR > 20%) tested.

Conclusion: This novel study shows that unlike aberrant protein expression in some carcinomas, MGB expression in salivary gland neoplasms represents the nature of original cells, giving a better insight into the neoplasms expressing MGB.

Keywords: labial gland, mammaglobin, mRNA expression, salivary gland

Introduction

The term “mammary analogue secretory carcinoma” was first described by Skalova et al. in 2010 [1] and re-classified as secretory carcinoma (SC) in 2017 as per the WHO classification of head and neck tumors [2]. Most commonly affecting the parotid gland, followed by the intra-oral minor salivary glands, this hitherto undescribed and distinctive salivary gland neoplasm with strong similarities to secretory carcinomas of the breast was a surprising revelation for many pathologists, especially oral pathologists, owing to its resemblance with both salivary acinic cell carcinoma and intraductal carcinoma. Salivary and breast SCs not only share histological and immunohistochemical findings but also harbor a specific translocation t(12; 15) (p13; q25) originating fusion of genes ETV6-NTRK3 [1,3]. Although SC can be discriminated from acinic cell carcinoma by its characteristic morphology, both carcinomas sometimes share several morphological subtypes which make diagnosis difficult. An immunohistochemical panel of S100, mammaglobin (MGB), discovered on gastrointestinal stromal tumors (GIST)-1 (DOG1) is considered useful for differential diagnosis [4]. Immunohistochemistry (IHC) for MGB and S100 is highly correlated with the ETV6 gene rearrangement so that molecular conformation is not always required to diagnose SC [5]. Consequently, the importance of MGB-IHC has risen in the field of head and neck pathology.

MGB, a breast-specific protein, belongs to the uroglobulin/Clara cell protein family or secretoglobin superfamily, is encoded at the locus of the MGB gene (including MGB1 and MGB2) that is located at chromosome 11. This gene is frequently amplified in breast carcinomas [6-8]. MGB-IHC serves as a differential marker of metastatic breast carcinomas from primary lung cancer [9]. However, the majority of endometrial adenocarcinomas are also positive for MGB, which is considered as aberrant expression [10]. Similarly, MGB expression can be also detected in certain non-SC salivary gland neoplasms (such as intraductal carcinoma, salivary duct carcinoma, polymorphous adenocarcinoma, mucoepidermoid carcinoma and pleomorphic adenoma) without ETV6-NTRK3 [11,12]. Interestingly, whether MGB expression is caused by any fundamental gene abnormalities or simple up-regulation of the gene with a proliferation of neoplastic cells or others is yet to be established. There are few reports on MGB protein and its gene expression in normal salivary glands, except for a distinctive report by Sjödin et al. [13]. They demonstrated MGB mRNA expression of several tissues including the salivary glands using real-time PCR (qPCR). Surprisingly, the highest MGB mRNA levels were not found in the breast tissue, but rather in the skin, followed by the mammary gland, uterus, and salivary gland [13]. However, they did not show MGB localization in all tissues examined including the salivary glands.

Hence, this study aims to delve deeper into the hypothesis that normal salivary gland tissue expresses both protein and mRNA of MGB. This report provides an overview of the immunohistochemical localization of MGB and a quantitative mRNA expression of the MGB gene, focusing on the labial gland.

Materials and Methods

Ethics Committee Approval

This study was approved by the Ethics Committee of Matsumoto Dental University (approval number 0265) and conducted according to the principles of the Declaration of Helsinki (version 2008). As all samples used in this study were archived paraffin-embedded tissues, informed consents were obtained in the form of opt-out.

Sample selection

Formalin-fixed paraffin-embedded (FFPE) specimens of the parotid, submandibular, palatal and labial salivary glands diagnosed as benign tumors, mucous cyst or xerostomia and surgically removed, were obtained from the archives of the Surgical Pathology Laboratory of Matsumoto Dental University Hospital, Shiojiri, Nagano, Japan. Subsequently, hematoxylin and eosin (HE) sections were prepared from all selected FFPE samples for histopathological review. The following two exclusion criteria were applied: 1) no inflammatory infiltration; 2) no moderate or severe degeneration. The final FFPE samples of 10 submandibular, 5 parotid, 5 palatal and 30 labial glands were used for the study (Table 1). Labial samples were chosen for statistical analysis because xerostomia or mucous cyst might arise from patients of whole generations. For statistical analysis, the 30 samples of labial glands were divided into three groups based on the sub-
ject’s age 20 to 39 years, 40 to 59 years and 60 to 79 years and with a sex ratio of 1: 1. Additionally, a case of myoepithelial cells of the breast as a positive control for IHC and a case of salivary gland SC as a positive control for IHC and polymerase chain reaction (PCR) were used.

**Immunohistochemistry for mammaglobin localization**

Three µm thick sections were prepared from FFPE tissue block followed by deparaffinization and hydration for immunohistochemistry (IHC). The antibodies used were obtained from the following sources: mammaglobin (MGB) (clone 304-1A5, Agilent Technologies, Santa Clara, CA, USA), SOX10 (clone EP268, Abcam, Cambridge, UK), S100 (polyclonal, Leica Biosystems, Wetzlar, Germany), MUC7 (polyclonal, Sigma-Aldrich, St. Louis, MO, USA) and MUC5b (polyclonal, Atras Antibodies, Stockholm, Sweden) for acinar and intercalated duct cells [14], myoepithelial cells [15], mucinous and serous cells [16], respectively. Different antigen retrieval methods were performed, such as using proteinase K for S100 staining and protein block serum-free (Agilent Technologies). All sections were incubated for 1 h at room temperature using the following dilutions: MGB-1:100; MUC5b-1:500; MUC7-1:200; SOX10-1:250; S100-1:400. After washing with PBS, all slides were incubated with Nicholas MAX-PO Multi (Nichirei, Tokyo, Japan) as a secondary antibody for 30 minutes. After visualization with 3′-diamobenzidine tetra hydrochloride (Agilent Technologies), the sections were counter-stained with hematoxilin. For the staining of negative control slides, bovine serum instead of primary antibodies was used.

To localize the MGB expression in the labial gland accurately, co-expression of MGB and other antibodies for constitutional markers such as S100, MUC5b, MUC7, and SOX10 were examined, using Alexa Fluor 488 conjugated F(ab′)2- goat anti-mouse IgG (H+L) secondary antibody and Alexa Fluor 647 conjugated F(ab′)2- goat anti-rabbit IgG (H+L) secondary antibody (Thermo Fisher Scientific, Waltham, MA, USA) for indirect double immunofluorescence. Immunofluorescence images were taken with a fluorescence microscope (BZ-X710, Keyence Corporation, Osaka, Japan).

**Mammaglobin positive ratio (MGB-PR)**

For each MGB-immunostained section of the labial gland, five digital images of 1,360 × 1,024 pixels each and measuring 433 µm in width were captured in hot spots using a microscope imaging system (BX51, Olympus, Tokyo, Japan). All positive and negative cells were counted in the acinar and ductal portions of the salivary gland system, and subsequently the MGB-PR values (all positive cells/all counted cells) for acinar, ductal and the whole areas were calculated. On average, a total of 445.9 salivary gland cells were included in the five digital images.

**Quantitative mRNA-PCR for expression analysis from FFPE**

Before RNA extraction, nine samples of the labial glands with MGP-PR values of over 20% were selected. Two cases (No. 20 and 21) were excluded because the specimens were too small to extract. Finally, RNA was extracted from seven labial gland samples and a positive control sample of salivary SC using the RNeasy FFPE kit (Qiagen, Hilden, Germany). cDNA was synthesized with SuperScript IV VILO Master Mix (Thermo Fisher Scientific Waltham, MA, USA), and MGB mRNA level was analyzed by Eco Real-Time PCR system (Illumina, San Diego, CA, USA) using TB Green Premix Ex Taq II (Tli RNaseH Plus) kit (Takara Bio Inc. Shiga, Japan). Real-time PCR was performed with a final volume of 10 µL; 10 ng cDNA was amplified by 5 µL of TB Green Premix Ex Taq with 10 µM of each primer for the target gene. β-actin was used as a reference gene. Sequences of primer sets were as follows: for MGB, the forward primer was 5′- CTTCTGACAAATGGTTAGGGTG-3′ and the reverse was 5′-GCCTGAGTCTCCTGAGCGCA-3′. Thermal cycling conditions were one cycle at 95°C for 30 s, 40 cycles at 95°C for 30 s followed by 60°C for 30 s, 95°C for 15 s, 60°C for 30 s and 95°C for 15 s.

**Statistical analysis**

The sample’s normality, equality of variances and equality of multiple variances were confirmed by the Shapiro-Wilk normality test, F-test, and Bartlett test, respectively. According to pre-examinations, all values were of unequal distribution. The relative mRNA expression levels were determined as 2^-ΔΔCt in order to detect fold change of the target gene in normal labial glands relative to secretory carcinoma. Mann-Whitney U and Kruskal-Wallis rank-sum tests were used for comparing the groups based on gender and age, respectively. Spearman’s rank correlation coefficient was used to analyze the relationship between MGB-PR values or the number of cells and age. All data were processed using the EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [17], a graphical user interface for R (www.r-project.org). A 5% α-error was considered for statistical significance.

**Results**

**MGB positive cell distribution**

All the salivary gland tissues examined in this study showed positive reactions against MGB antibody in various frequencies, intensities, and localization patterns (Fig. 1). No tissues without a negative reaction were found in this series. The parotid tissues displayed a tendency of positive reactions appearing predominantly in the intralobular ducts, especially in the intercalated ducts (Fig. 1a), while the submandibular gland specimens contained MGB-positive acinar cells and suspected myoepithelial cells presenting as flattened spindle cells (Fig. 1b). In the tested samples of minor salivary gland tissues, both palatal and labial glands demonstrated positive reactions in the serous and mucous acini (Fig. 1c-f) and even in striated and intercalated ducts (Fig. 1f). There were not any detectable trends of immune-positive cell distribution indicating predisposition between the three age groups (Fig. 1d-f). Cell nests strongly positive for MGB were diffusely proliferating in the salivary (Fig. 1g) and breast (Fig. 1h) SCs.

A double immunofluorescence procedure revealed co-expression of MGB and several constituent makers such as SOX10, MUC7, MUS5 and S100 for the salivary gland system (Fig. 2). MGB localization in acini was confirmed by co-expression with SOX10 (Fig. 2a), which were further divided into two types of cells co-expressing Muc7 (Fig. 2b) and Muc5b (Fig. 2c), respectively. In the periphery of the acinar or ductal portions, a few MGB/S100 positive myoepithelial cells were also present (Fig. 2d). The intralobular ductal system demonstrated MGB-positive reactions with (Fig. 2e) or without SOX10 (Fig. 2f).

**MGB positive ratio**

The mean number of MGB positive cells per total glandular cells counted in whole, acinar and ductal areas of labial glands were 72.8 ± 94.5 ± 248.6, 69.2 ± 92.8/421.9 ± 241.0, and 3.6 ± 10.6/27.2 ± 38.3, respectively. The numbers of MGB positive cells were remarkably variable in each gland, acinar and ductal areas of labial glands were 72.8 ± 94.5 ± 248.6, 69.2 ± 92.8/421.9 ± 241.0, and 3.6 ± 10.6/27.2 ± 38.3, respectively. The mean number of MGB positive cells per total glandular cells counted in whole, acinar and ductal areas of labial glands were 72.8 ± 9.2/245.9 ± 248.6, 69.2 ± 92.8/421.9 ± 241.0, and 3.6 ± 10.6/27.2 ± 38.3, respectively. The numbers of MGB positive cells were remarkably variable in each case, ranging from 0 to 373 in whole areas. Only one case of a 79-year-old female showed complete MGB-negative staining in 152 cells counted. Mean values of MGB positive ratio (MGB-PR) in whole, acinar and ductal areas were 17.8 ± 20.5%, 17.2 ± 20.7% and 9.4 ± 19.9%, respectively. The values (positive/negative cell number) of seven cases with an MGP-PR value of over 20% were as follows: 24.7% (110/446) in No.6, 66.8% (373/558) in No. 7, 52.4% (260/496) in No. 8, 49.8% (277/556) in No. 13, 20.4% (123/602) in No. 17, 28.1% (160/570) in No. 18 and 83.3% (30/36) in No. 25.

**MGB-PR of acinar cells was significantly higher than that of ductal cells** (Fig. 3), with median values of 7.7% and 0.0% in acinar and ductal areas, respectively. However, there were no statistical differences between the MGB-PR of both gender and between MGB-PRs of the three age

| Location                  | Number | Mean age (range) | Male/female |
|---------------------------|--------|------------------|-------------|
| Submandibular gland       | 10     | 55.9 (22-76)     | 7/3         |
| Parotid gland             | 5      | 57.4 (43-69)     | 3/2         |
| Palatal gland             | 5      | 69.0 (52-82)     | 1/4         |
| Labial gland              | 30     | 50.2 (21-79)     | 15/15       |
| *Labial 20-39             | 10     | 29.4 (21-38)     | 5/5         |
| *Labial 40-59             | 10     | 51.4 (42-59)     | 5/5         |
| *Labial 60-79             | 10     | 70.9 (60-79)     | 5/5         |

*Subgroups of labial samples based on age*
groups in whole, acinar and ductal areas. Spearman’s rank correlation coefficient test was not able to demonstrate statistical differences between every MGB-PR and age.

Quantitative mRNA expression

MGB mRNA was successfully amplified in 6 out of 7 selected cases and a control case of SC. The fold change of relative gene expression against a salivary SC case was markedly variable ranging from 2.0 to 209.5. However, the relative expression for case No. 25 (Fig. 4) could not be calculated.

Discussion

This study successfully demonstrated immunohistochemical evidence of MGB expression in the major and minor salivary glands and the expression of MGB mRNA in the labial gland tissue. Based on recommendations proposed by Skálová et al. [1] in their study on salivary SC, MGB IHC and fusion gene analysis have become the standard examination procedures for any salivary gland surgical pathology to discriminate SC from its mimickers. However, the fold change of relative gene expression against a salivary SC case was markedly varying from 2.0 to 209.5. However, the relative expression for case No. 25 (Fig. 4) could not be calculated.

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Fig. 1 Immunohistochemical localization of MGB: various intensities and localization patterns are noted in the parotid (a), submandibular (b), palatal (c), and labial glands (d-f). MGB positive reactions are not only in acini but also in the myoepithelial cell (b, arrow) and intercalated (f, arrowheads) and striated duct (f, arrows). No different reactivities between the age groups of the labial glands are seen (d: 20 to 39, e: 40 to 59, and f: 60 to 79-year-old). Both salivary (g) and breast (h) secretory carcinomas are diffusely positive for MGB. Bars: 20 µm.

Fig. 2 Double immunofluorescence of MGB (a1-f1) and constituent markers in the labial gland: SOX10 (a2), MUC7 (b2), MUC5b (c2) in acini, and S100 (d2) and SOX10 (e2) in intercalated ducts and SOX10 (f2) in striated ducts. Figures a3 to f3 have merged images. Arrows highlight positive reactions and bars represent 50 µm.
M-phase endometrium with 17β-estradiol [25]. Classen-Linke et al. also found an alteration in MGB1 mRNA expression of endometrium in the menstrual cycle, confirming the hormone dependency of MGB1 expression in vitro using 17β-estradiol and promegestone [26]. Based on these reports, it was hypothesized that MGB-positive cells may frequently be found in the salivary gland tissues of females. However, the frequency of MGB-positive cells was not associated with gender. Additionally, this study showed no statistically significant association between the frequency of MGB positive cells and age. All samples chosen in this study demonstrated no inflammatory cell infiltration, which was sufficient to deny any inflammation-associated MGB alteration. MGB can be produced at any age and in any gender in the salivary glands, refuting the influence of hormone or inflammation.

The human minor salivary glands have pluripotent mesenchymal stem cells which contribute to a self-renewal system in the salivary glands [27]. Active stem cells located in the intercalated ducts may have implications in self-renewal, but the signaling pathways regulating the fate of these stem cells have not been deciphered [28]. In a self-renewal system, SOX10-positive or negative cells and S100-positive myoepithelial cells are supplied from stem cells. Acinar cells are the site of fluid secretion in the glands responsible for secreting up to 85% of salivary proteins [29]. Therefore, MGB mRNA could be regulated along with salivary protein secretion. MGB is more predominantly produced in acinar cells than in ducts, similar to salivary secretion. The distribution of MGB positive cells was confirmed based on co-expressions of constitutional markers such as SOX10, S100, MUC5B, and MUC7 of the salivary gland system [14-16]. As expected, numerous MGB positive cells distributed in the acini with both MUC7-positive serous and MUC5B-positive mucous cells were observed, accompanied by a significantly higher MGB-PR value in comparison to that of the ductal system. On the other hand, MGB production was noted in both the SOX10-positive intercalated ducts and SOX10-negative striated ducts even though the number of positive cells was less. These results are a striking revelation of the presence of MGB in the salivary gland cells, however detailed explanation of how all components can produce MGB is entailed.

MGB mRNA was not detected from all FFPE samples available in this study, which is a limitation of molecular analysis using FFPE samples. However, another possible cause of amplification failure could be the small number of cells included in the sample. Although MGP-PR of case No. 25 was the highest among all samples, only 30 cells positive cells could be counted from the hot spots. Considering more than three times the positive cell numbers (ranging from 110 to 373 cells) were counted in the other six samples with successful mRNA amplification, it seemed that sample No.25 had insufficient positive cells to amplify. However, the variability in MGB mRNA expression was able to be clearly shown in the labial gland. Considering MGB-positive cell distribution, the MGB-PR vales and MGB qPCR results, it is a very enigmatic phenomenon that expression varies not only among individuals but also among cells in each salivary gland. Further studies need to focus on elucidating the cause of individual differences and the key molecules regulating MGB gene expression in limited cells.

In conclusion, the present study shows that the major and minor salivary glands physiologically secrete MGB protein that is produced from all components of the salivary gland system, except for the excretory duct. In the labial gland, the frequency of MGB-positive cells and mRNA-expression level is markedly variable between individuals. Considering that all intralobular constituent cells can secrete MGB, these probably serve as parental cells for several MGB-positive salivary neoplasms arising from acinar or ductal cells. Also, unlike MGB-positive endometrial carcinoma, MGB production in salivary neoplasms is not an aberrant phenomenon but the nature of original cells. This study provides a novel insight into the understanding of MGB-positive salivary gland neoplasms.

Conflict of Interest
There is no potential conflict of interest.

References
1. Šklíčová A, Vanecek T, Sima R, Laco J, Weirebr J, Perez-Ordono B et al. (2010) Mammary analogue secretory carcinoma of salivary glands, containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumor entity. Am J Surg Pathol 34, 599-608.
2. Seethala RR, Stremman G (2017) Update from the 4th edition of the World Health Organization classification of head and neck tumours: Tumors of the salivary gland. Head Neck Pathol 11, 55-67.
3. Tognon C, Knezevic SR, Huntsman D, Roskelley CD, Melnyk N, Mathers JA, et al. (2002) Expression of the ETV6-NTRK3 gene fusion as a primary event in human secretory breast carcinoma. Cancer Cell 2, 367-376.
4. Hsieh YH, Chiu VH, Yang YJ, Chang YL (2015) Papillary-cystic pattern is characteristic in mammary analogue secretory carcinomas but is rarely observed in acinic cell carcinomas of the salivary gland. Virchows Arch 467, 145-153.
5. Shah AA, Weng BM, Lettiarlo RD, Mills SE, Shelow EB (2015) Morphology in conjunction with immunohistochemistry is sufficient for the diagnosis of mammary analogue secretory carcinoma. Head Neck Pathol 9, 85-95.
6. Becker RM, Darrow C, Zimonic DB, Popescu NC, Watson MA, Fleming TP (1998) Identification of mammaglobin B, a novel member of the urotoglobin gene family. Genomics 54, 70-78.
7. Watson MA, Darrow C, Zimonic DB, Popescu NC, Fleming TP (1998) Structure and transcriptional regulation of the human mammaglobin gene, a breast cancer associated member of the urotoglobin gene family localized to chromosome 11q13. Oncogene 16, 817-824.
8. Leygue E, Snell L, Dotzlau H, Hole K, Troop S, Hiller-Hitchcock T et al. (1999) Mammaglobin, a potential marker of breast cancer nodal metastasis. J Pathol 189, 28-33.
9. Sasaki E, Tsunoda N, Hatanaka Y, Morli N, Iwata H, Yatabe Y (2007) Breast-specific expression of MGB1/mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. Mod Pathol 20, 208-214.
10. Onuma K, Dabbs DJ, Bhargava R (2008) Mammaglobin expression in the female genital tract: immunohistochemical analysis in benign and neoplastic endocervix and endometrium. Int J Gynecol Pathol 27, 418-425.
11. Bishop JA, Yonescu R, Batista D, Begum S, Eisele DW, Westra WH (2013) Utility of mammaglobin immunohistochemistry as a proxy marker for the ETV6-NTRK3 translocation in the diagnosis of salivary mammary analogue secretory carcinoma. Hum Pathol 44, 1982-1988.
12. Montalli VAM, Passador-Santos F, Martinez EF, Furuse C, Aguiar MC, Soares FA et al. (2017) Mammaglobin and DOG-1 expression in polymorphous low-grade adenocarcinoma: an appraisal of its origin and morphology. J Oral Pathol Med 46, 182-187.
13. Sjödin A, Guo D, Hofer P-A, Henriksson R, Hedman H (2003) Mammaglobin in normal

Fig. 3 MGB-PR in acinar and ductal areas of the labial gland
The value of acini is statistically higher than that of ducts (P = 0.00376). Median values are 7.7 versus 6.0, respectively (n = 30).

Fig. 4 Quantitative MGB mRNA expression of the labial gland: The bar graph shows the fold change of expression of mRNA against that of a secretory carcinoma (SC). N/A: not available.
human sweat glands and human sweat gland tumors. J Invest Dermatol 121, 428-429.

14. Ohtomo R, Mori T, Shibata S, Tsuta K, Maeshima AM, Akazawa C et al. (2013) SOX10 is a novel marker of acinar and intercalated duct differentiation in salivary gland tumors: a clue to the histogenesis for tumor diagnosis. Mod Pathol 26, 1041-1050.

15. Ianez RF, Buim ME, Coutinho-Camilo CM, Schultz R, Soares FA, Lourenço SV (2010) Human salivary gland morphogenesis: myoepithelial cell maturation assessed by immunohistochemical markers. Histopathology 57, 410-417.

16. Nielsen PA, Bennett EP, Wandall HH, Therkildsen MH, Hannibal J, Clausen H (1997) Identification of a major human high molecular weight salivary mucin (MG1) as tracheobronchial mucin MUC5B. Glycobiology 7, 413-419.

17. Kanda Y (2013) Investigation of the freely available easy-to-use software “EZR” for medical statistics. Bone Marrow Transplant 48, 452-458.

18. Skalová A, Vaneeck T, Uro-Coste E, Bishop JA, Weinreb I, Thompson LDR et al. (2018) Molecular profiling of salivary gland intraductal carcinoma revealed a subset of tumors harboring NCOA4-RET and novel TRIM27-RET fusions: a report of 17 cases. Am J Surg Pathol 42, 1445-1455.

19. Ni J, Kalf/Suks M, Gentz R, Schageman J, Beato M, Klug J (2000) All human genes of the uteroglobin family are localized on chromosome 1q12.2 and form a dense cluster. Ann N Y Acad Sci 923, 25-42.

20. Mentrükoski MJ, Wick MR (2015) Immunohistochemical distinction of primary sweat gland carcinoma and metastastic breast carcinoma: can it always be accomplished reliably? Am J Clin Pathol 143, 430-436.

21. Hassan EM, Willmore WG, McKay BC, DeRosa MC (2017) In vitro selections of mammaglobin A and mammaglobin B aptamers for the recognition of circulating breast tumor cells. Sci Rep 7, 14487.

22. Choi MS, Ray R, Zhang Z, Mukherjee AB (2004) IFN-gamma stimulates the expression of a novel secretoglobin that regulates chemotactic cell migration and invasion. J Immunol 172, 4245-4252.

23. Lu X, Wang N, Long X-B, You X-J, Cui Y-H, Liu Z (2011) The cytokine-driven regulation of secretoglobins in normal human upper airway and their expression, particularly that of uteroglobin-related protein 1, in chronic rhinosinusitis. Respir Res 12, 28.

24. Jackson BC, Thompson IC, Wright MW, Andrews M, Bernard A, Nebert DW et al. (2011) Update of the human secretoglobin (SCGB) gene superfamily and an example of “evolutionary bloom” of androgen-binding protein genes within the mouse Scgb gene superfamily. Hum Genomics 5, 691-702.

25. Punyadeera C, Dussen H, Klomp J, Dusselman G, Kamps R, Dijcks F et al. (2005) Oestrogen-modulated gene expression in the human endometrium. Cell Mol Life Sci 62, 239-250.

26. Clausen-Linke I, Moss S, Grötting K, Beier HM, Alfer J, Krausche CA (2012) Mammaglobin 1: not only a breast-specific and tumour-specific marker, but also a hormone-responsive endometrial protein. Histopathology 61, 955-965.

27. Lu L, Li Y, Du M, Zhang C, Zhang X, Tong H et al. (2015) Characterization of a self-renewing and multi-potent cell population isolated from human minor salivary glands. Sci Rep 5, 10106.

28. Kwak M, Alston N, Ghazizadeh S (2016) Identification of stem cells in the secretory complex of salivary glands. J Dent Res 95, 776-783.

29. Perez P, Rowzee AM, Zheng C, Adriaansen J, Baum BJ (2010) Salivary epithelial cells: an unassuming target site for gene therapeutics. Int J Biochem Cell Biol 42, 773-777.