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Epithelial membrane antigen and DOG1 expression in minor salivary gland tumours

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ABSTRACT

Epithelial membrane antigen (EMA) and DOG1 are used as marker of epithelial cells, particularly the luminal cells, of salivary gland tumours. The aim of this study was to compare the EMA and DOG1 expression in tumours of minor salivary glands. Cases of pleomorphic adenoma (PA), basal cell adenoma (BCA), canalicular adenoma (CA), adenoid cystic carcinoma (ACC), polymorphous adenocarcinoma (PAC), mucoepidermoid carcinoma (MEC) and epithelial-myoepithelial carcinoma (EMC) were submitted to immunohistochemistry for EMA and DOG1. In PA and BCA, EMA and DOG1 were observed in luminal cells, while in CA the tumour cells were negative for both proteins. The EMA and DOG1 pattern expression detected in EMC was similar to that one observed in benign tumours. In ACC, both myoepithelial e epithelial expressed EMA and DOG-1. PAC tumour cells were only positive for DOG1, whereas MEC were only positive for EMA. In conclusion, EMA and DOG1 expression in benign salivary gland tumours was similar to normal salivary gland tissue and can be used as good marker of tumoral cells derived from intercalated ducts or its progenitor cells, while in malignant salivary gland tumours EMA expression is, however, better used as an indicator of aggressive behavior than a marker of luminal cells.

Epithelial membrane antigen (EMA; also known as MUC1) [1] and DOG1 are used as marker of epithelial cells in salivary gland tumours. EMA was detected in luminal cells in the early 80s [2], while DOG1 was recently described in apical membranous luminal cells of the intercalated ducts [3].

EMA is the product of the MUC1 gene and is present in various secretory epithelia as a transmembrane glycoprotein. In normal cells, it is restricted to cell membranes and associated microvilli, but in tumours it can be diffusely spread through the cytoplasm [2,4].

DOG1 is a calcium-activated chloride channel, formerly described in gastrointestinal tumours (GIST), the most common mesenchymal tumour of the gastrointestinal tract [5]. Nowadays it has been associated with several lesions that affect endocrine and exocrine secretory cells, including salivary gland intercalated duct cells [3].

DOG1 and EMA have been reported as marker of apical region of ductal cell. In this context, the aim of this study was to compare the DOG1 and EMA expression in 23 cases of tumours derived from minor salivary glands (pleomorphic adenoma [PA], basal cell adenoma [BCA], canalicular adenoma [CA], adenoid cystic carcinoma [ACC], polymorphous adenocarcinoma [PAC], mucoepidermoid carcinoma [MEC] and epithelial-myoepithelial carcinoma [EMC]). Normal minor salivary glands were used as control (NMSG), the diagnosis of the cases was confirmed by hematoxylin and eosin (HE) staining and examination under the light microscope. Tumours were classified according to World Health Organization (WHO) 2017 guidelines. The Ethics Committee of the São Leopoldo Mandic Dental School, Campinas, São Paulo, Brazil, approved this study (protocol number 30222814.8.0000.5374).

The samples were submitted to immunohistochemistry for DOG1 and EMA as described previously [6]. Antigen retrieval was carried out by boiling the slides in a steamer immersed in a citrate buffer (pH 6.0) or Tris/EDTA (pH 8.9) for 30 min. The antibodies used were anti-DOG1 (prediluted, Abcam, Cambridge, MA, USA) and anti-EMA (1:100, E29 clone, Zeta Corporation, Arcadia, CA, USA). Stained sections were photographed using a Zeiss Axioskop 2 microscope (Carl Zeiss MicroImaging GmbH, Germany) equipped with AxioCam HRc (Carl Zeiss). The results are summarized in the Table 1.

NMSG exhibited DOG1 expression in the apical region of the mucous acini, intercalated ducts and partial striated ducts (Fig. 1B). On the

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Table 1
Expression pattern of DOG1 and EMA in minor salivary gland tumours.

Sample	Immunohistochemical positive expression	
	DOG1	EMA
NMSG	Luminal and acinic cells	Luminal cells
BCA	Luminal cells (apical region)	Luminal cells (cytoplasm region)
PA	Luminal and myoepithelial cells	Luminal cells (apical region)
CA	Negative	Negative
EMC	Luminal cells (apical region)	Luminal cells (cytoplasm region)
ACC	Luminal and myoepithelial cells (cytoplasm region)	Luminal and myoepithelial cells (cytoplasm region)
PAC	Luminal cells (apical region)	Negative
MEC	Negative	All cells (cytoplasm region)

Legend: NMSG: normal minor salivary gland; BCA: basal cell adenoma; PA: pleomorphic adenoma; CA: canalicular adenoma; EMC: epithelial-myoepithelial carcinoma; ACC: adenoid cystic carcinoma; PAC: polymorphous adenocarcinoma; MEC: mucoepidermoid carcinoma.

other hand, EMA was detected in luminal cells of ductal structures, while mucous cells were negative for EMA (Fig. 1C).

In benign salivary gland tumours comprised by myoepithelial and luminal cells (BCA and PA) both proteins were detected in ductal structures (Fig. 2B–C and E–F). Moreover, EMA was also expressed in duct content (Fig. 2C), while DOG1 was observed in the cytoplasm of modified myoepithelial cells (Fig. 2E). This pattern of expression of these proteins, mainly DOG1, reinforces the intercalated duct origin of BCA and PA [7]. In CA, EMA and DOG1 expression was negative (Fig. 2H–I), suggesting that this tumour is probably composed by a progenitor cell of excretory duct [8].

In biphasic malignant salivary gland tumours (EMC and ACC), DOG1 and EMA were differentially expressed by myoepithelial and luminal cells. The expression of both proteins in EMC was similar to the one detected in benign salivary tumours, i.e., positivity for luminal cells of duct structures (Fig. 3B–C). Despite its malignancy, it is already known that in EMC both myoepithelial and epithelial show a very well-differentiated phenotype [6,9]. On the other hand, in ACC, DOG1 and

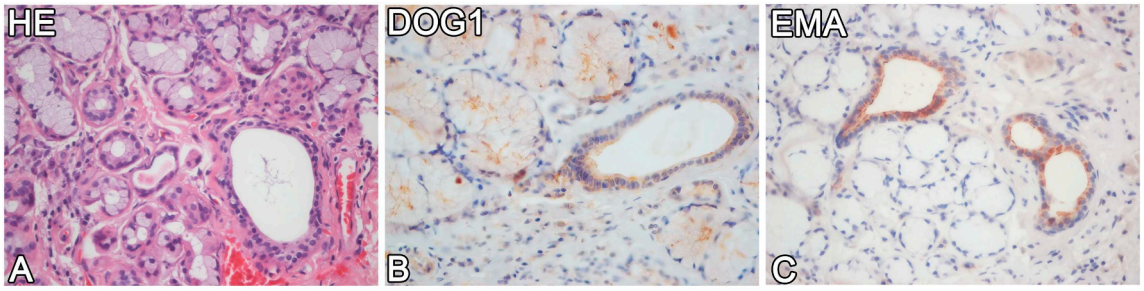

Fig. 1. Histomorphology by HE staining of normal minor salivary gland (A). Immunohistochemistry for DOG1 (B), and EMA (C). Original magnification 40×.

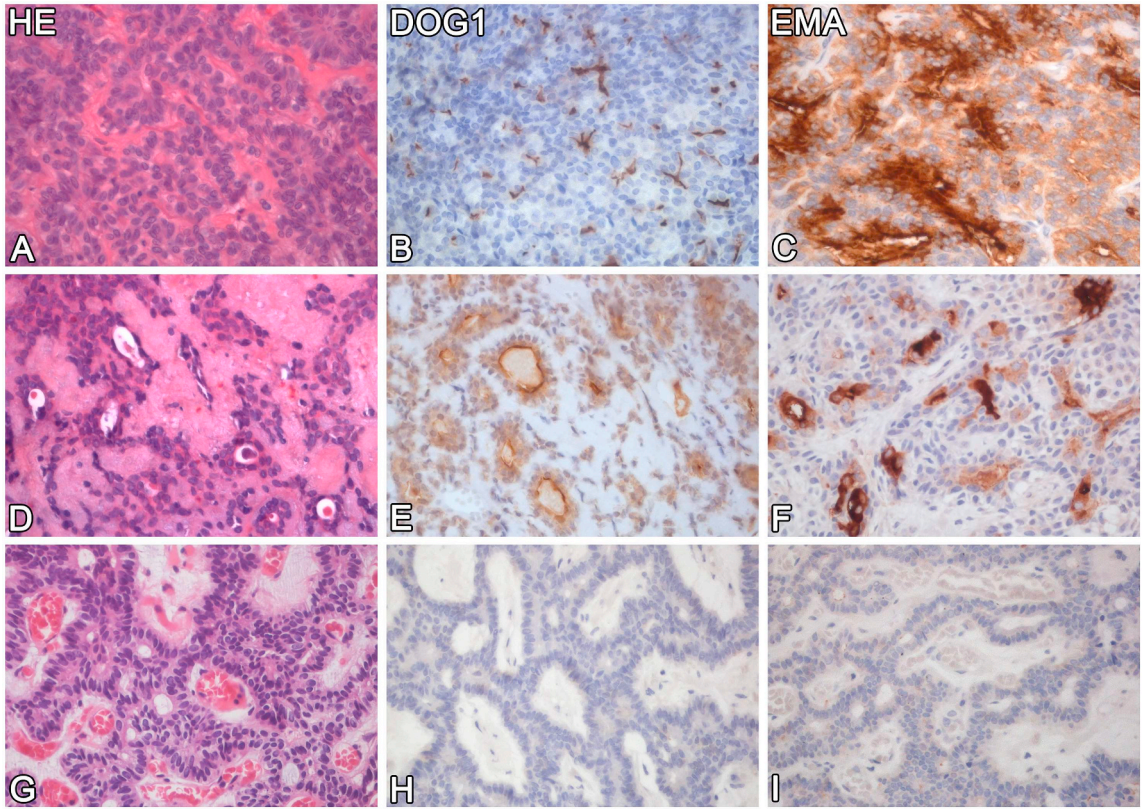

Fig. 2. Histomorphology of BCA (A), PA (D), and CA (G) by HE staining. Immunohistochemistry for DOG1 (B, E, and H), and EMA (C, F, and I) in BCA (B and C), PA (E and F), and CA (H and I), respectively. Original magnification 40×.

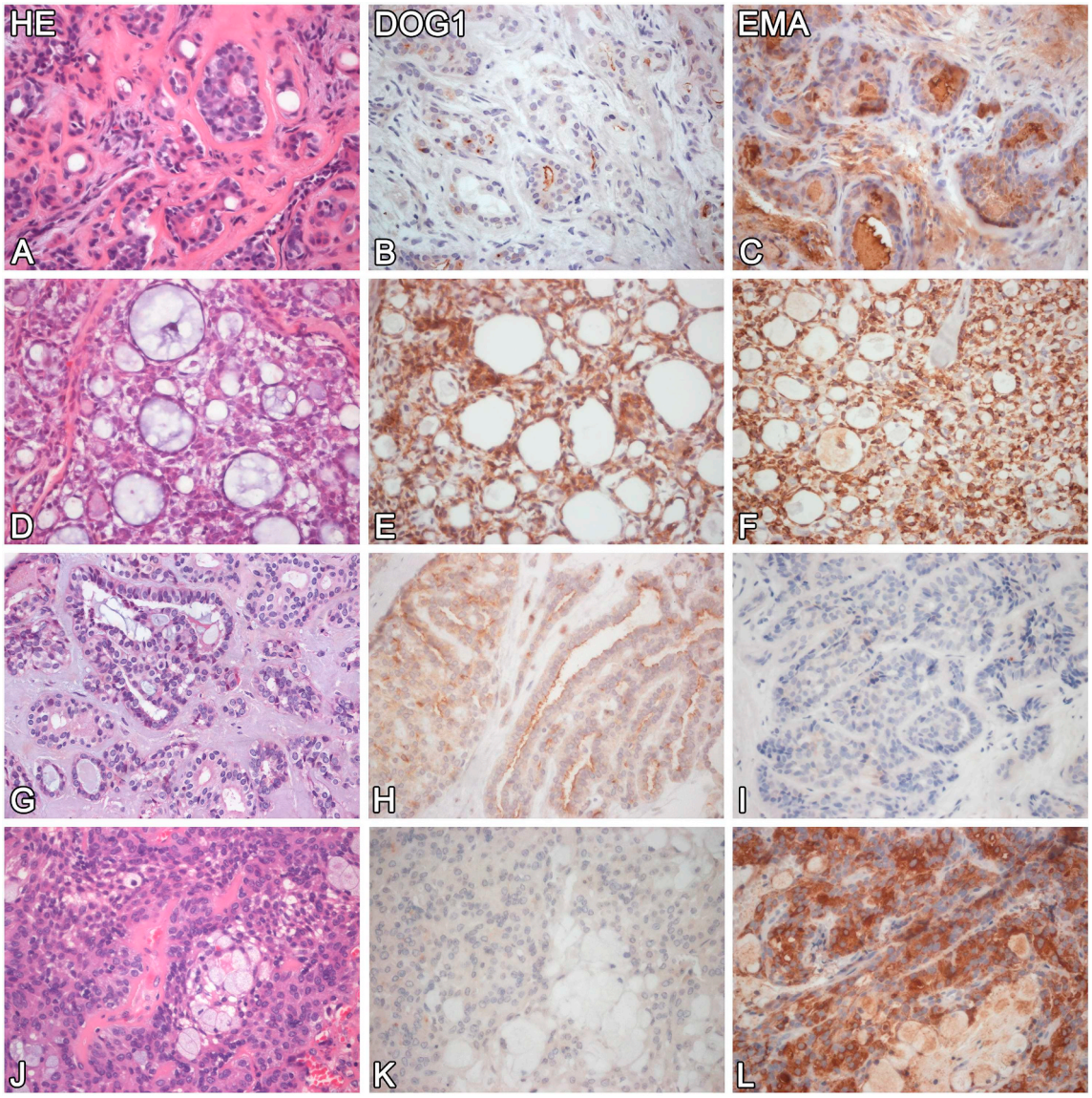

Fig. 3. Histomorphology of EMC (A), ACC (D), PAC (G), and MEC by HE staining. Immunohistochemistry for DOG1 (B, E, H, and K), and EMA (C, F, I, and K) in EMC (B and C), ACC (E and F), PAC (H and I), and MEC (K and L), respectively. Original magnification 40×.

EMA were observed in myoepithelial and luminal cells (Fig. 3E–F). Interestingly, an intense expression of DOG1 was detected in the apical region of luminal cells of ductal structures in tubular pattern of ACC. In another way, EMA was observed in both cells without highlighted the apical portion of duct structures (Fig. 3F). In this case, EMA could be more related to a malignant behavior as have been shown in other malignant tumours [10].

PAC is composed by only one type of cell, which probably represent a progenitor cell that precedes the luminal and myoepithelial differentiation. These cells are also originated from the intercalated duct and showed only DOG1 expression, particularly in sites that lumen was formed as well as in apical region of the cell (Fig. 3H), as previously demonstrated elsewhere [11]. No EMA expression was detected in this salivary gland tumour (Fig. 3I).

During tumorigenesis of some malignant tumours, the expression pattern of EMA is altered: changing the localization from apical region to the cytoplasm of the cell and become stronger. These modifications have been implicated in the inhibition of cell-cell adhesion, favoring detachment, induction of escape from immune system and, consequently, promoting increasing invasion and metastatic potential [12-15]. In MEC, DOG1 was negative (Fig. 3K), while EMA was detected in the cytoplasm of the majority of epithelial cells (Fig. 3L), suggesting that in this tumour EMA could be considered as marker of malignancy, since in MEC the expression of EMA is related with worse prognosis [16].

In conclusion, the expression of DOG1 and EMA in benign salivary gland tumours was similar to that one observed in normal salivary gland tissue, except for the modified myoepithelial cells of PA. Moreover, in minor salivary gland tumours, DOG1 acts as good marker of tumoral cells derived from intercalated ducts or its progenitor cells, excluding the malignant cells of ACC. Interestingly, EMA was not detected in the progenitor cells of the salivary duct system, such as CA and PAC. In salivary gland tumours with high grade malignancy, EMA expression is, however, better used as an indicator of aggressive behavior than a marker of luminal cells.

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Authors' contribution

The design of the present study was conceived by VCA, LNT, EPA, and VAMM, the acquisition of the data was done by EPA, LNT, VAMM, FMG, FPS, and ABS. Data analysis and interpretation, as well as the manuscript elaboration and the final approval of the version submitted were done by all authors.

Declaration of competing interest

None to declare.

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