African Journal of Advanced Pure and Applied Sciences (AJAPAS)
Online ISSN: 2957-644X
Volume 5, Issue 3, 2026
Page No: 107-115
Website: *https://aaasjournals.com/index.php/ajapas/index*
ISI 2025: 1.126 **SJIFactor 2024: 6.752** **معامل التأثير العربي: 1.62**

Immunohistochemical Expression of Metallothionein as A Biomarker in Salivary Gland Neoplasms

Antisar M. Benamer [1], Ibtisam I. Senussi [2], Ahmed S. Musrati [3*]
[1,2] Department of Oral Surgey, Oral Medicine and Oral Pathology, Misurata University, Musurata, Libya
[3] Department of Oral Medicine and Diagnostics, Libyan Centre for Dental Research, Zliten, Libya

التعبير المناعي النسيجي للكيمياء الحيوية عن بروتين "الميتالوثيونين" كمؤشر حيوي في أورام الغدد اللعابية

انتصار بن عامر [1]، ابتسام السنوسي [2]، أحمد المصراتي [*3]
[2،1] قسم طب وأمراض وجراحة الفم، جامعة مصراتة، مصراتة، ليبيا
[3] قسم طب الفم و التشخيص، المركز الليبي لأبحاث طب الأسنان، زليتن، ليبيا

[*]Corresponding author: ahsamu@utu.fi

Received: April 23, 2026	Accepted: July 04, 2026	Published: July 22, 2026

Abstract

Background: Metallothionein is a cysteine-rich protein present in myoepithelial cells of several benign and malignant neoplasms and as a potential myoepithelial cell marker in breast cancer. The function of metallothionein is associated with DNA protection, oxidative stress and anti-apoptosis.

Aims: The present work was done to assess metallothionein (MT) expression with immunohistochemical localization in benign and malignant salivary gland tumors and to compare metallothionein (MT) expression among the different histological patterns of salivary gland tumors.

Materials/Methods: Forty diagnosed cases of benign and malignant salivary gland tumors were selected, and immunohistochemistry was performed for metallothionein detection.

Results: All cases showed positive metallothionein expression with different intensity. The expression of metallothionein protein is higher in malignant salivary gland tumors than in the benign ones.

Conclusion: Nuclear metallothionein may play a critical role in tumor progression. It can be used as a prognostic marker in salivary gland adenocarcinomas.

Keywords: Metallothionein, Immunohistochemistry, Salivary gland neoplasms.

الملخص

الخلفية: يُعد "الميتالوثيونين" (Metallothionein) بروتيناً غنياً بحمض السيستين الأميني، ويتواجد في الخلايا الظهارية العضلية (myoepithelial cells) ضمن العديد من الأورام الحميدة والخبيثة، كما يُعتبر مؤشراً محتملاً لهذه الخلايا في حالات سرطان الثدي. وترتبط وظيفة الميتالوثيونين بعمليات حماية الحمض النووي (DNA)، والاستجابة للإجهاد التأكسدي، ومقاومة الموت الخلوي المبرمج (apoptosis).

الأهداف: أُجريت هذه الدراسة لتقييم تعبير بروتين الميتالوثيونين (MT) وتحديد توضعه المناعي النسيجي (immunohistochemical localization) في أورام الغدد اللعابية الحميدة والخبيثة، وكذلك لمقارنة مستويات هذا التعبير بين الأنماط النسيجية المختلفة لتلك الأورام.

المواد والطرق: تم اختيار أربعين حالة مُشخَّصة لأورام الغدد اللعابية (الحميدة والخبيثة)، وأُجري فحص الكيمياء النسيجية المناعية للكشف عن وجود الميتالوثيونين.

النتائج: أظهرت جميع الحالات تعبيراً إيجابياً عن الميتالوثيونين بدرجات متفاوتة من الشدة؛ حيث لوحظ أن مستوى تعبير بروتين الميتالوثيونين في أورام الغدد اللعابية الخبيثة أعلى منه في الأورام الحميدة.
الاستنتاج: قد يلعب الميتالوثيونين المتواجد في النواة دوراً جوهرياً في تطور الورم، ويمكن استخدامه كمؤشر إنذاري (prognostic marker) في حالات السرطان الغدي (adenocarcinoma) للغدد اللعابية.

الكلمات المفتاحية: ميتالوثيونين، الكيمياء النسيجية المناعية، أورام الغدد اللعابية.

Introduction

A significant proportion of oral tumors originate in the salivary glands and thus constitute the next most common tumors of the mouth after squamous cell carcinoma [(1)]. They are found in less than 1% of all tumors, and 2-4% of all head and neck tumors [(2)], with an overall global incidence of 0.4-13.5 cases per 100.000 population. The exact etiology of salivary glands tumors, as in other types of tumors, is not fully understood. Implication of several viruses in the causation and pathogenesis of salivary gland tumors has been suggested. There is a strong association between Epstein-Barr Virus (EBV) and lymphoepithelial carcinomas [(3,4)], as well as Warthin's tumor [(5)]. There is also a strong association between smoking and Warthin's tumor, the risk for bilateral Warthin's tumors correlated significantly with the amount of nicotine intake [(6)]. Exposure to ionizing radiation [(7)] as well as to industrial chemicals [(8)] such as asbestos mining and the manufacture of rubber have been documented as critical in the development of salivary gland tumors.

Metallothioneins (MTs) are a group of proteins, first isolated in 1957 as a cadmium (Cd)-binding protein in the horse kidney [(9)]. MTs are proteins characterized by low molecular weight (6–7 kDa), high cysteine content and metal-binding capacity. In mammalian MTs four isoforms have been identified (MT-I–IV) [(10)]. MT-I+II are the best characterized MT proteins, expressed in most tissues and have been localized largely in cell cytoplasm, lysosomes, mitochondria and nuclei [(11)].

There are many functions of MTs, including homeostasis of essential metals, Zinc and copper, which may be required for cell growth and differentiation [(12)], protection against cytotoxicity of cadmium and other toxic metals, inhibition of generation of free radicals in oxidative stress, anti-apoptotic effect, angiogenesis, immune defense responses and cell cycle progression and cell differentiation [(10-13)].

Despite the fact that there is no clear role of MTs in oncogenesis [(9)], some studies have reported increased expression of MT-I+II mRNA and protein in rapidly proliferating normal cells, regenerating cells and in various human tumors such as breast, kidney, lung, nasopharynx, ovary, prostate, salivary gland, as well as in melanoma and leukemia [(9,10)], where in some cases MT-I+II expression correlates with tumor grade/stage, chemotherapy/radiation resistance, and poor prognosis [(14)]. However, downregulation of MT-I and II in certain types of tumors such as hepatocellular, gastric, colorectal, CNS and thyroid cancers has been noticed, where MT-I and II expressions are either unrelated or even inversely correlated to mortality [(9,15,16)]. MTs are highly conserved proteins that first appear in mammalian development [(17)]. These proteins are important in acinar cell morphological differentiation and are the most frequently detected gene induced during laminin-1-dependent differentiation of the human salivary gland cell line [(18,19)].

The number of studies focusing on metallothionein (MT) expression in benign and malignant salivary gland neoplasms is limited; therefore, the present study aims to assess MT expression by immunohistochemical localization in benign and malignant salivary gland tumors and to compare MT expression among the different histological patterns of salivary gland tumors.

Material and methods

This is a cross-sectional study in which immunohistochemistry was applied to assess the presence of the marker metallothionein in sections of salivary gland tumors of the oral cavity. A convenience sample of forty diagnosed cases of benign and malignant salivary gland tumors (8 benign and 32 malignant) were selected from the Cranio-Maxillofacial and Plastic Surgery Department at the Faculty of Dentistry, Alexandria University. The results were recorded and statistically analyzed using student (t) test. Benign salivary gland tumors (8 cases, 20%) included 5 cases (62.5%) pleomorphic adenoma (PL), 1 case for myoepithelioma (Myoep), papillary cystadenoma lymphomatosum (Warthin's tumor) (WT) and basal cell adenoma (BCA) (each 12.5%). Out of the 32 malignant salivary gland tumors (80%), 9 cases (28.1%) were mucoepidermoid carcinoma (MeC) (Fig. 1a and b), while 7 cases (21.9%) were Carcinoma ex pleomorphic adenoma (CXPA), 6 cases (18.5%) were adenoid cystic carcinoma (AdCC), 5 cases (15.6%) were myoepithelial carcinoma (MC) and 2 cases (6.3%) were acinic cell carcinoma

(ACC). Finally, salivary duct carcinoma (SDC), polymorphous low-grade adenocarcinoma (PLGA), and oncocytic carcinoma (OCC) (Fig. 2c and d) were represented by one case each (3.1%) (Table 1).
The median age for patients in this work was 45 years (range: 11-81 years). 25 patients (62.5%) were female, and 15 (37.5%) were male. Regarding tumor location, the parotid gland was the most common site (17 cases), followed by the palate (14 cases). Other sites, such as the cheek (6 cases), the submandibular gland (2 cases), and the maxillary sinus, were represented by only 1 case. No cases were presented with lymph node metastasis.
Biopsies were taken from the tumor tissue and fixed in 10% neutral buffered formalin, processed and embedded in paraffin wax using the conventional procedures. Serial sections of 3-4 µm thick were placed on Figure (1):

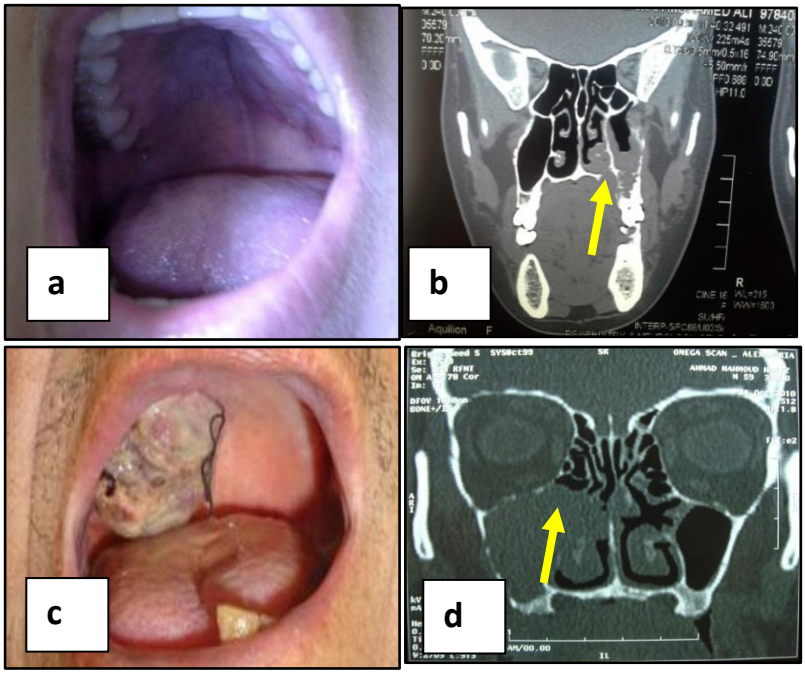

Figure 1: (a) A case of mucoepidermoid carcinoma presenting as an exophytic mass at the lateral wall of the hard palate (arrow). (b) Coronal CT of the previous case exhibiting an ill-defined mass extending into the maxillary sinus with bone destruction (arrow). (c) A Case of oncocytic carcinoma of the maxillary sinus presenting in the oral cavity as an ulcer of the right palatal side. (d) CT of the previous case with the tumor invading the maxillary sinus, reaching the right orbital floor and destroying the right nasal cavity (arrow). glass slides and stained using hematoxylin and eosin (H&E) for routine histopathological examination.

Immunohistochemical staining of metallothionein
An immunohistochemical marker of metallothionein, a primary mouse monoclonal anti-human metallothionein antibody, was used [Cat. #M 3009-10 (1mg/ml)]. The streptavidin-biotin-peroxidase complex method was used. Serial sections 4-5 µm thick were cut from H&E blocks. The slides were mounted on poly-L-lysine coated glass slides. Two sections were obtained for the positive test and one for the negative control, with the latter omitting the primary antibody. The tissue sections were deparaffinized in xylene, rehydrated in graded ethanol and incubated in 0.3% hydrogen peroxide solution to block the endogenous peroxidase. The specimens were incubated with the metallothionein primary antibody. Exposure to biotinylated link antibody and labelled streptavidin-biotin-peroxidase complex was done to bind the primary antibody. Staining was completed by incubation in substrate-chromogen solution and hematoxylin counterstain. Immuno-expression of etallothionein was evaluated by using an image analyser to evaluate both mean area per cent and mean optical density.

Table 1: Types, Numbers and Percentages of the Examined Salivary Gland Tumors.

Tumors	Number	Percentage
Benign salivary gland tumors	8	20
Pleomorphic adenoma	5	62.5
Warthin's tumor	1	12.5
Basal cell adenoma	1	12.5
Myoepithelioma	1	12.5
Malignant salivary gland tumors	32	80
Mucoepidermoid carcinoma	9	28.1
- High-grade MEC	5	15.6
- Low-grade MEC	4	12.5
Adenoid cystic carcinoma	6	18.8
- Cribriform pattern	4	12.5
- Tubular trabecular pattern	1	3.1
- Solid pattern	1	3.1
Carcinoma ex pleomorphic adenoma	7	21.9
Myoepithelial adenoma	5	15.6
Acinic cell carcinoma	2	6.3
Oncocytic carcinoma	1	3.1
Salivary duct carcinoma	1	3.1
Polymorphous low-grade adenocarcinoma	1	3.1

Results and discussion

For the immunohistochemical staining, routinely formalin-fixed, paraffin-embedded 40 salivary gland tumors biopsies were used to detect the anti-metallothionein along with the normal control.

The intensity of immunostaining of metallothionein was calculated in terms of mean area percentage (MA%) and mean optical density (MOD) by the computer image analyzer.

Patterns of Metallothionein Immunostaining in Normal Salivary Gland Tissue

Normal salivary gland tissue showed positive metallothionein immunoreactivity in acinar and ductal epithelial cells.

Patterns of Metallothionein Immunostaining in Benign Salivary Gland Tumors

Metallothionein protein expression was analyzed by immunohistochemistry (IHC) in the 8 benign salivary gland tumors. All 8 cases showed positive expression.

The pleomorphic adenoma cases showed intense nuclear and cytoplasmic metallothionein immunopositivity in luminal, abluminal, and myoepithelial cells (Fig. 2a). The Myoepithelioma exhibited total cell (nuclear and cytoplasmic) metallothionein immunopositivity in myoepithelial cells (Fig. 2b). Papillary cystadenoma lymphomatosum exhibited intense metallothionein immunopositivity in the oncocytic epithelial lining. However, the lymphoid stroma showed no reaction (Fig. 2c). The basal cell adenoma showed intense metallothionein immunoreactivity in the basaloid glandular epithelial cells (Fig. 2d).

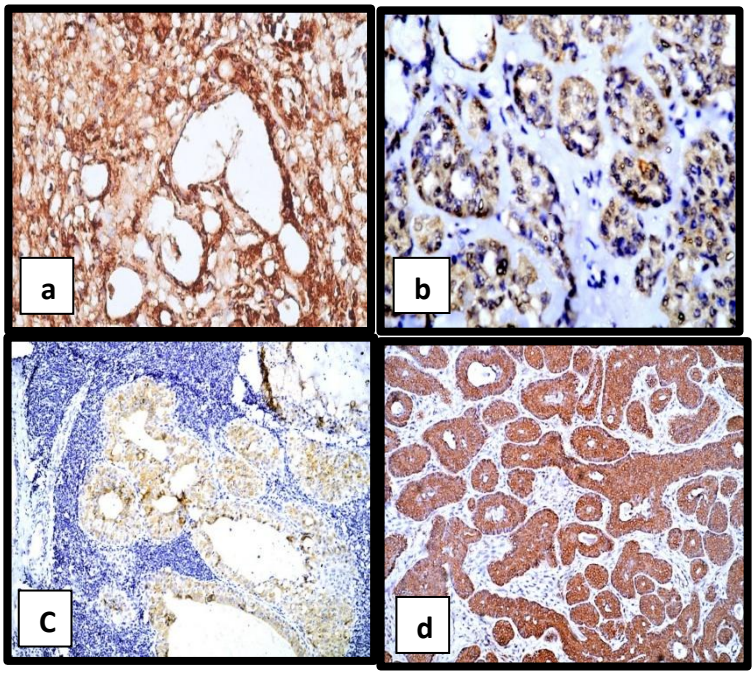

Figure 2: (a) Higher magnification of pleomorphic adenoma revealing intense metallothionein immunoreactivity in abluminal glandular epithelial and myoepithelial cells. (x 400). (b) Higher magnification of the previous case revealing nuclear and cytoplasmic immunopositivity of glandular myoepithelial cells. (x400). (c) Papillary cystadenoma lymphomatosum showing immunoreactivity for metallothionein in the oncocytic epithelial lining. The lymphoid stroma is free from any reaction. (x 100). (d) Basal cell adenoma exhibiting intense immunoreactivity of metallothionein in the basaloid glandular cells. (x100).

Patterns of Metallothionein Immunostaining in Malignant Salivary Gland Tumors

Metallothionein protein expression was analyzed by immunohistochemistry in 32 malignant salivary gland tumors. All tumors analyzed (n=32) showed positive metallothionein expression with different intensities.

Low-grade MEC exhibited intense nuclear and cytoplasmic immunoreactivity in the intermediate and epidermoid cells, whereas membranous immunoreactivity was noted in the clear and mucous-secreting cells (Fig.3a). The high-grade MEC showed intense metallothionein immunopositivity, particularly in the anaplastic epidermoid cells (Fig.3b).

The difference in the mean percentage area of metallothionein expression between low- and high-grade MEC was calculated. The MA % was higher in the high-grade type than in the low-grade type, with a significant difference ($p < 0.05$). Besides, MOD was higher in high-grade compared to low-grade, but with no significant difference.

Metallothionein expression was evident in all the examined cases of AdCC (6 cases). In the cribriform pattern, positive immunosignals were detected in almost all the malignant cells (Fig. 3c). Meanwhile, in the tubular pattern, focal weak immunoreactivity was detected in some ductal epithelial and myoepithelial cells (Fig.3d). In the solid pattern, intense positive metallothionein expression in the highly malignant basaloid tumor cells was detected. The MOD and MA% were highest in the solid type, followed by the cribriform type, and the lowest in the tubular form.

All examined cases of carcinoma ex pleomorphic adenoma showed positive metallothionein immunoexpression (Fig. 3e).

Using Student's t-test, the MOD of metallothionein immunoexpression was higher in carcinoma ex pleomorphic adenoma compared to pleomorphic adenoma, but the difference in MA% between these two groups was not significant.

The only case examined of salivary duct carcinoma showed intense metallothionein immunosignals within the highly anaplastic ductal epithelial cells (Fig. 3f). Myoepithelial carcinoma cases exhibited nuclear and cytoplasmic metallothionein immunosignals in the malignant epithelioid and plasmacytoid myoepithelial cells. In

the only case of oncocytic carcinoma examined, uneven cytoplasmic distribution of metallothionein immunoreactivity was noted in malignant oncocytes with granular cytoplasm. The nuclei were free from any reaction. In both cases of acinic cell carcinoma (clear type), membranous immunoreactivity was noted in the malignant, clear, vacuolated cells.

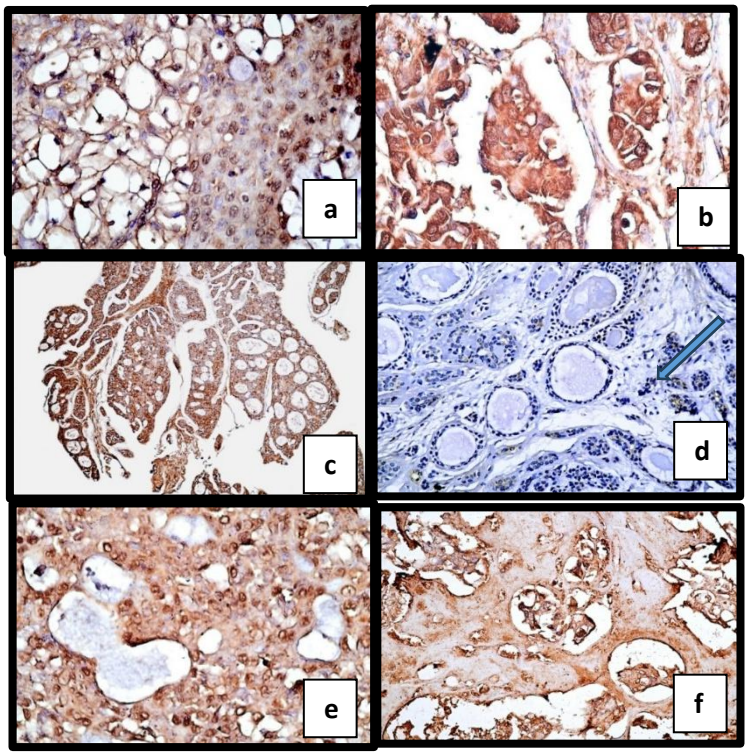

Figure 3: (a) Low- grade mucoepidermoid carcinoma showing metallothionein immunoreactivity in the epidermoid, intermediate and clear cells (x400). (b) Higher magnification of the previous case showing intense positive reaction in both the cytoplasm and nucleus of the anaplastic epidermoid cells "Total Cell Reactivity" (x400). (c) Cribriform pattern of adenoid cystic carcinoma showing positive reaction in the cytoplasm of basaloid myoepithelial cells (x 100). (d)Trabecular pattern of adenoid cystic carcinoma exhibiting focal metallothionein immunopositivity in the malignant tumor cells (Arrow) (x200). (e) Carcinoma ex-pleomorphic adenoma showing positive immunosignals of metallothionein in the malignant glandular epithelial cells (x 400). (f) Salivary duct carcinoma on top of pleomorphic adenoma, revealing intense immunoreactions in the anaplastic glandular cells (x 200).

In polymorphous low-grade adenocarcinoma, metallothionein was expressed in almost all malignant glandular cells. A targetoid pattern with a single-file, concentric arrangement of cells around a nerve bundle was evident.
The intensity of immunostaining of metallothionein between benign and malignant salivary gland tumors was calculated. There was a statistically significant difference ($p < 0.001$) in the MOD, which was 76.86±9.89 and 41.65±11.62 for malignant and benign salivary gland tumors, respectively (Table 2). The difference in MA% between these two groups was also significantly higher in malignant than benign tumors (Table 3).
No significant differences were found between metallothionein immunoreactivity and age, sex or site. On the other hand, there were no cases presented with lymph node metastasis.

Table (2): Difference in Mean Optical Density of Metallothionein Immunoexpression Between Benign and Malignant Tumors.

	Benign tumors	**Malignant tumors**	**T**	**P**
Mean ± SD	41.65 ±11.62	76.86±9.89	5.907	<0.001*

t: Student's t-test
*: Statistically significant at $p \leq 0.05$

Table (3): Difference in Area percent of Metallothionein Immuno-expression Between Benign and Malignant Tumors.

	Benign tumors	Malignant tumors	T	P
Mean ± SD	16.48 ±6.21	28.87±10.65	2.499	0.030*

t: Student's t-test
*: Statistically significant at $p \leq 0.05$

Discussion

The salivary glands are the site of origin of a wide variety of neoplasms. The histopathology of these tumors is said to be the most complex and diverse of any organ in the body [(20)].

In this work, the frequency of malignant tumors (80%) was higher than that of the benign tumors (20%). Lopes et al., [(21)] reported a higher prevalence of malignant tumors in minor salivary gland tumors. In contrast with other authors, it was found that benign tumors are predominant in salivary gland tumors worldwide [(22,23)].

The World Health Organization reported that the incidence of salivary gland tumors is slightly higher in females than in males [(22)]. This goes with the results of the present work.

The mean age in this study of patients with benign and malignant tumors was 34 and 47 years, respectively, which is similar to the findings of Pinkston and Cole [(2)], who found that tumor incidence increased with age, with peaks in the fifth and sixth decades of life.

Most salivary gland tumors encountered in the present research occurred in the minor salivary glands (52.5%), especially in the palate while 47.5 % of tumors were found in the major salivary glands, with predominance in the parotid gland. This agrees with Eveson and Cawson [(24)], who stated that adenocarcinoma of the minor salivary glands is more common than that of the major salivary glands. In contrast, many authors found that most salivary gland tumors occurred in the major salivary glands rather than the minor ones [(24,25)].

The clinical importance of metallothionein in cancer has been investigated since its discovery in 1957 as a cadmium (CD)-binding protein in the horse kidney [(9-11)].

In the current study, the normal control section of salivary gland tissues showed positive metallothionein immunoreactivity in ductal epithelial cells and in myoepithelial cells lining the acini periphery. This is consistent with the findings reported by many investigators [(26, 27)]. They showed that MT was expressed in normal salivary glands as well as in benign and malignant tumors mainly in cells resembling the phenotype of myoepithelial cells. MTs are important in acinar cell morphological differentiation and are the most frequently detected gene induced during laminin-1-dependent differentiation of human salivary gland cell line [(28, 29)].

Although MT expression has been largely studied in breast cancer, few studies have evaluated MT in salivary gland tumors and its role in these lesions remains to be elucidated [(26-31)].

In this work, the pleomorphic adenoma cases showed intense nuclear and cytoplasmic metallothionein immunosignals. These findings agree with Miranda et al., [(32)] and Sunardhi-Widyaputra et al., [(27)]. They noted that positivity for MT was striking in cells exhibiting myoepithelial differentiation, with variation in staining intensity and in nuclear and cytoplasmic distribution. In addition, they reported that MT may be necessary for the growth and differentiation of myoepithelial cells due to its role in intracellular zinc storage, an important cofactor for protein synthesis and metabolism during increased cellular metabolism.

Moreover, Adriance et al., [(32)] evidenced that myoepithelial cells play a key role in the organizational development of the glandular tissues and that the changes of myoepithelial cell function are a key step in the development of the tumors.

In the present study, one case of myoepithelioma was evaluated. It exhibited total cell (nuclear and cytoplasmic) immunopositivity of myoepithelial cells. This is in accordance with Sunardhi-Widyaputra et al., [(27)] and Gao et al., [(28)]. They reported that almost all myoepithelial tumor cells showed an intense reactivity of MT.

In this work, one case of papillary cystadenoma lymphomatosum exhibited intense MT immunoreactivity in the oncocytic epithelial lining. However, the lymphoid stroma was free from any reaction. Similarly, Sunardhi-Widyaputra et al. (27) in their work on salivary gland tumors found strong immunoreactivity for MT in the basal cells of the epithelial lining of the Warthin tumor, while only a few luminal cells were stained. Lymphoid stroma was devoid of staining.

In this study, 28.1% of the examined malignant salivary gland tumors were mucoepidermoid carcinoma, with low- and high-grade types. Low-grade MEC expressed intense nuclear and cytoplasmic immunoreactivity in the intermediate cells and epidermoid cells, whereas membranous immunoreactions were noted in the clear and mucous-secreting cells, while the high-grade MEC showed MT intense immunopositivity, particularly in the anaplastic epidermoid cells. These findings agree with Sunardhi-Widyaputra et al., [(27)] who reported that tumor cells in the periphery of tumor clusters were stained strongly for MT.

All the AdCC cases encountered in the present research exhibited positive MT immunosignals. This is in agreement with the results of a study done by Prasad et al., [29]. They stated that diffuse MT expressions in combination with smooth muscle actin, calponin and smooth muscle myosin heavy chain were strongly predictive for adenoid cystic carcinoma. Moreover, Alves et al., [26] reported that MT expression in adenoid cystic carcinomas varied according to the histological subtype. MT showed intense staining in solid and cribriform as compared to tubular subtypes of this tumor. These findings align with the results of the present work.

In the current investigation, myoepithelial carcinoma cases exhibited nuclear and cytoplasmic immunosignals for MT in the malignant epithelioid and plasmacytoid myoepithelial cells. This is in accordance with Sunardhi-Widyaputra et al., [27] and Gao et al., [28], who found that MT expression may vary regarding tumor degree of differentiation with the most heterogeneous expression in the most differentiated and immature tumors.

In the present work, increased expression of MT was detected in high-grade tumors such as carcinoma ex pleomorphic adenoma, oncocytic carcinoma as well as salivary duct carcinoma. These findings confirm the aggressive behavior of these tumors. This is supported by Sunardhi-Widyaputra et al., [27], who found the expression of MT in salivary gland tumors parallels the morphological heterogeneity and may correlate with the degree of differentiation and maturation of the tumor cells, as also seen in other tumors.

In the current study, low-grade salivary adenocarcinoma, such as acinic cell carcinoma and polymorphous low-grade adenocarcinoma, showed positive expression of MT in almost all the malignant glandular epithelial cells. Similarly, Prasad et al., [29] found that MT is expressed in glandular epithelial cells of polymorphous low-grade adenocarcinomas as a potential marker of differentiation of this tumor.

Some studies have shown that the subcellular distribution of MT may be related to its function in apoptosis [26, 27]. They detected the nuclear expression of MT, which is required for zinc-donor activity toward transcription factors, including p53 and others. In the present work, a significant number of cases revealed intense immune nuclear reaction. Moreover, Nielsen et al., [10] reported that the anti-apoptotic effects of MT-I+II during chemo- and radiation therapy have two major consequences. On the one hand, MT-I+II can result in malignant cells becoming resistant to treatment. On the other hand, MT-I+II protects surrounding healthy cells from the treatment's toxicity. Thus, selectively inhibiting MT-I+II expression in malignant cells to enhance the therapeutic response, while keeping normal MT-I+II expression in healthy tissue cells, prevents the side effects of treatment, hereby strengthening the patients' chances for survival.

Conclusion

- There is a variable pattern of metallothionein expression among different types of oral tumors.
- The existence of nuclear metallothionein may play a critical role in tumour progression.
- In the future, there is a possibility that it will be used as a prognostic marker in salivary gland adenocarcinomas.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

References

1) Brandwein MS, Ferlito A, Bradley PJ, Hille JJ, Rinaldo A. Diagnosis and classification of salivary neoplasms: pathologic challenges and relevance to clinical outcomes. Acta Otolaryngol 2002; 122: 758-64.
2) Pinkston JA, Cole P. Incidence rates of salivary gland tumors: results from a population-based study. Otolaryngol Head Neck Surg 1999; 120: 834-40.
3) Iezzoni JC, Gaffey MJ, Weiss LM. The role of Epstein-Barr virus in lymphoepithelioma-like carcinomas. Am J Clin Pathol 1995; 103: 308-15.
4) Tsai CC, Chen CL, Hsu HC. Expression of Epstein-Barr virus in carcinomas of major salivary glands: a strong association with lymphoepithelioma-like carcinoma. Hum Pathol 1996; 27: 258-62.
5) Maruoka Y, Sanzhe CUI, Tomohiro ANDO, Yoichiro KATO, Shibata N, Kobayashi M, et al. Detection of Epstein-Barr virus in Warthin's tumor. J Tokyo Wom Med Univ 2001; 71: 99-106.
6) Peter KJ, Wittekindt C, Florian PS, Al AA, Schroeder U, Guntinas-Lichius O. High risk for bilateral Warthin tumor in heavy smokers-review of 185 cases. Acta Otolaryngol 2006; 126: 1213-7.
7) Saku T, Hayashi Y, Takahara O, Matsuura H, Tokunaga M, Tokunaga M, et al. Salivary gland tumors among atomic bomb survivors, 1950-1987. Cancer 1997; 79: 1465-75.
8) Horn-Ross PL, Ljung BM, Morrow M. Environmental factors and the risk of salivary gland cancer. Epidemiology 1997; 8: 414-9.
9) Thirumoorthy N, Manisenthil Kumar KT, Shyam Sundar A, Panayappan L, Chatterjee M. Metallothionein: an overview. World J Gastroenterol 2007; 13: 993-6.
10) Nielsen AE, Bohr A, Penkowa M. The balance between life and death of cells: roles of metallothioneins. Biomarker Insights 2007; 2: 99-111.

11) Trnkova L, Krizkova S, Adam V, Hubalek J, Kizek R. Immobilization of methallothionein to carbon paste electrode surface via anti-MT antibodies and its use for biosensing of silver. Biasens Bioelectron 2011; 26: 2201-7.
12) Adam V, Petrlova J, Wang J, Eckschlager T, Trnkova L, Kizek R. Zeptomole electrochemical detection of metallothioneins. PLoSone 2010;5(7):8.
13) Cherian MG, Kang YJ. Metallothionein and liver cell regeneration. Exp Biol Med (Maywood) 2006; 231: 138-44.
14) Klaassen CD, Liu J, Choudhuri S. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu Rev Pharmacol Toxicol 1999; 39: 267-94.
15) Ferrario C, Lavagni P, Gariboldi M, Miranda C, Losa M, Cleris L, et al. Metallothionein 1G acts as an oncosupressor in papillary thyroid carcinoma. Lab Invest 2008; 88: 474-81.
16) Tao X, Zheng JM, Xu AM, Chen XF, Zhang SH. Down regulated expression of metallothionein and its clinicopathological significance in hepatocellular carcinoma. Hepatol Res 2007; 37: 820-7.
17) Vasak M, Hasler DW. Metallothioneins: new functional and structural insights. Curr opin chem. Biol 2000; 4: 177-83.
18) Ogawa Y. Immunocytochemistry of myoepithelial cells in the salivary glands. Prog Histochem Cytochem 2003; 38: 343-426.
19) Irie Y, Mori F, Keung WM, mizushima Y, Wakabayashi K. Expression of neuronal growth inhibitory factor (metallothionein-III) in the salivary gland. Physiol Res 2004; 53: 719-23.
20) Subhashraj K. Salivary gland tumors: a single institution experience in India. Br J Oral Maxillofac Surg 2008; 46: 635-8.
21) Lopes MA, Kowalski LP, da Cunha SG, Paes de AO. A clinicopathologic study of 196 intraoral minor salivary gland tumors. J Oral Pathol Med 1999; 28: 264-7.
22) Barnes L, Eveson JW, Reichart P, Sidransky D. World Health Organization classification of tumors: pathology and genetics of head and neck tumors. Lyon: IARC Press, 2005.
23) Shishegar M, Ashraf MJ, Azarpira N, Khademi B, Hashemi B, Ashrafi A. Salivary gland tumors in maxillofacial region: a retrospective study of 130 cases in a southern Iranian population. Patholog Res Int Patholog Res Int 2011; 2011: 934350.
24) Eveson JW, Cawson RA. Salivary gland tumors. A review of 2410 cases with particular reference to histological types, site, age and sex distribution. J Pathol 1985; 146: 51-8.
25) Li LJ, Li Y, Wen YM, Liu H, Zhao HW. Clinical analysis of salivary gland tumor cases in West China in past 50 years. Oral Oncol 2008; 44: 187-92.
26) Alves SM, Cardoso SV, de Fatima Bernardes V, Machado VC, Mesquita RA, Vieira Do Carmo MA. Metallothionein immunostaining in adenoid cystic carcinomas of the salivary glands. Oral Oncology 2007; 43: 252-6.
27) Sunardhi-Widyaputra S, van den Oord J, van Houdt K, Deley M, van Damme B. Identification of metallothionein and parathy-roid hormone-related peptide (PTHrp)-positive cells in salivary gland tumor. Pathol Res Pract 1995; 191: 1092-8.
28) Gao Y, Han Z, Liu X. Metallothionein expression and its significance in salivary gland tumors. Zhonghua Kou Qiang Yi Xue Za Zhi 1997; 32: 282-4.
29) Prasad ML, Barbacioru CC, Rawal YB, Husein O, Wen P. Hierarchical cluster analysis of myoepithelial/basal cell markers in adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma. Mod Pathol 2008; 21: 105-14.
30) Hecht D, Jung D, Prabhu VV, Munson PJ, Hoffman MP, Kleinman HK. Metallothionein promotes laminin-1 induced acinar differentiation in vitro and reduces tumor growth in vivo. Cancer Res 2002; 62: 5370-4.
31) Irie Y, Mori F, Keung WM, Mizushima Y, Wakabayashi K. Expression of neuronal growth inhibitory factor (metallothionein-III) in the salivary gland. Physiol Res 2004; 53: 719-23.
32) Miranda Viana Ade C, Ribeiro DC, Florêncio TN, Santos VT, Sousa AA, Aguiar MC. Immunohistochemical expression of metallothionein in pleomorphic adenoma of minor salivary glands: A role in the control of apoptosis? Acta Histochemica 2013; 115: 564-8.
33) Adriance MC, Inman JL, Petersen OW, Bissel MJ. Myoepithelial cells: good fences make good neighbors. Breast Cancer Res 2005; 7: 190-7.

