American Journal of Life Sciences
2014; 2(3): 182-189
Published online June 30, 2014 (http://www.sciencepublishinggroup.com/j/ajls)
doi: 10.11648/j.ajls.20140203.18

Mapsin expression in mucoepidermoid carcinoma and adenoid cystic carcinoma

Sahar Mohamed El-Sheikh[1], Amani Noureldin Abd El-Latif[2, *]

[1]Professor of oral pathology, Faculty of dentistry, Alexandria University, Egypt
[2]Associatte professor of oral pathology, Faculty of dentistry, Pharos University, Alexandria, Egypt

Email address:
Sahar.elsheikh@gmail.com (S. M. El-Sheikh), Amany.noureldin@pua.edu.eg (A. N.Abd El-Latif)

To cite this article:
Sahar Mohamed El-Sheikh, Amani Noureldin Abd El-Latif. Mapsin Expression in Mucoepidermoid Carcinoma and Adenoid Cystic Carcinoma. *American Journal of Life Sciences.*Vol. 2, No. 3, 2014, pp. 182-189. doi: 10.11648/j.ajls.20140203.18

Abstract: Salivary gland tumors comprise a significant proportion of oral tumors. Maspin is a non-inhibitory serine protease inhibitor (serpin). It is supposed to suppress tumor-induced angiogenesis, tumor cell motility, invasion and metastasis. Objectives: To evaluate salivary gland tumors clinically, investigate the immunoexpression of maspin in mucoepidermoid and adenocystic carcinoma of the salivary gland tumors and to detect the correlation of maspin expression with biological behavior of these tumors.Materials and methods: Fourteen cases of malignant salivary gland tumors were retrieved from the Oral Pathology files of Faculty of Dentistry, Alexandria University, Egypt. The study included eight cases of mucoepidermoid carcinoma, six cases of adenoid cystic carcinoma. Two specimens of normal salivary tissue were taken as control.Results: The difference in mean optical density of maspin expression between low-grade and high-grade MEC was calculated. Optical density was higher in low-grade type compared to the high grade one with a significant difference ($P< 0.05$).Moreover, the results of this study showed difference between the MOD of adenoid cystic carcinoma subtypes, with the solid subtype expressing the lowest percentage. Conclusion: Maspin protein is overexpressed in low grade malignant salivary gland tumors, and decrease as the histological malignancy rises so it can be used as prognostic marker in salivary gland carcinoma. In addition, maspin could serve as a therapeutic target in salivary gland tumors including recombinant protein or gene therapy.

Keywords: Mucoepidermoid Carcinoma, Adenoid Cystic Carcinoma, Maspin

1. Introduction

Salivary gland tumors comprise a significant proportion of oral tumors and are the next most common tumors of the mouth after squamous cell carcinoma (1). They represent less than 1% of all tumors, and 3-5% of all head and neck tumors (2-4), with an overall global incidence of 0.4-13.5 cases per 100.000 populations. The World Health Organization (WHO) lists twenty four malignant tumors, with a wide spectrum of biological behavior, and variable responsiveness to systemic therapies (5, 6).

Knowledge about the biological changes occurring during tumor initiation and progression is the basis for prevention, prediction of prognosis of the adequate therapy of malignant tumors. (7)

Progression of neoplastic diseases requires the acquisition of certain properties that promote tumor aggressiveness. Proteases and protease inhibitors are known to play important roles in tumor invasion and metastasis. Two classes of proteases have been extensively studied in breast and other cancers: serine proteases (such as plasminogen activator) and their inhibitors (PAI 1 & PAI 2) and metalloproteases (such as collagenases) and their inhibitors (TIMP 1 and TIMP 2). (8, 9)

Maspin (mammary serine protease inhibitor) is a member of the serine protease inhibitor superfamily. Maspin gene (located in the serpin cluster on chromosome 18q21.3 q23) was first identified by subtractive hybridization as candidate tumor suppressor gene which may play a role in human breast cancer development. (10, 11) Several reports show that maspin expression is down regulated in breast, prostate and gastric cancers, (12, 13) but over expressed in pancreatic, lung, thyroid and endometrial cancers,(14,15) suggesting that maspin may play different functions in different cell types. Further studies suggest that the tumor suppressor activity of maspin

depends on its involvement in the stimulation of the adhesion to the extracellular matrix or triggering the extracellular matrix mediated pathways that negatively regulate tumor migration and invasion.(16,17) Some investigators suggest that the induction of apoptosis and the inhibition of angiogenesis may represent other important mechanisms by which maspin inhibits tumor progression.(18,19) As the mechanism of regulation of maspin expression is not well differentiated, it was shown that p53 may regulate maspin expression by direct binding to maspin promotor.(20) Promotor hypermethylation of the maspin gene leading to gene silencing, was identified in several human cancers, including breast, thyroid, skin and colon.(21) It was found that overexpression of maspin in some cancers (ovarian and pancreatic) may result from promoter demethylation, suggesting that methylation and demethylation may regulate maspin expression.(22, 23)

Because maspin is expressed in the epithelium of other glandular tumors, it is possible that it participates in salivary gland biology as well. Only few reports of maspin expression in malignant salivary gland tumors were published (24), so, the aim of this research was to evaluate salivary gland tumors clinically, to investigate the immunoexpression of maspin inmucoepidermoid and adenocystic carcinoma salivary gland tumors and to detect the correlation of maspin expression with biological behavior of these tumors.

2. Material and Methods

2.1. Tissue Samples

Fourteen casesof malignant salivary gland tumors were retrieved from the Oral Pathology files of Faculty of Dentistry, Alexandria University, Egypt during the period from 2007- 2013. The study included eight cases of mucoepidermoid carcinoma, six cases of adenoid cystic carcinoma. Two specimens of normal salivary tissue are taken as control.

2.2. Methods

Clinical data were collected from the files of the departments of Maxillofacial and Plastic surgery and Oral Pathology at the faculty of dentistry, Alexandria University. Data included:Age and sex of the patient,Location of the tumor, its recurrence, lymph node involvement and its distance metastases.

Histopathological examination using haematoxylin and eosin stain was used to confirm diagnosis.

For immunohistochemical analysis: all lesions were sectioned at 5 µm for immunohistochemical staining. Maspin immunohistochemical staining was performed using the universal ABC, peroxidase kit (ultra vision detection system, Anti polyvalent, ready to use, LAB VISION, USA, cat.1767). All the slides were deparaffinized using xylene and then rehydrated in decreasing concentrations of ethanol. Antigen retrieval using microwave heating (three times of 10 min; 10mM citrate buffer, pH 6.0) after inhibition of endogenous peroxidase activity (0.3 hydrogen peroxidase for 15 min). The slides were incubated overnight with the primary antibody at room temperature, and then washed using phosphate buffered solution (PBS) and then incubated with secondary antibody for 15 min followed by PBS. Finally the detection of bound antibody was accomplished using the ABC reagent for 20 min then washed with PBS. A 0.1% solution of diaminobenzidine (DAB) was used for 5 min as a chromagen. The slides were counterstained with Mayer's hematoxylin for 5 10 min.

2.3. Immunohistochemical Interpretation

Immunohistological sections were examined by the image analyzer computer system using the software Leica Qwin 500.

The intensity of immunostaining of maspin was calculated in terms of mean area percent (MA %) and mean optical density (MOD)(Fig.1, 2)

The clinical data was statistically estimated using the student's t test and Chi-square test.

A p value less than 0.01 was considered highly significant. The experimental values are given as a mean value ± SD (standard deviation).

3. Results

3.1. Clinical Results

Fourteen patients with malignantsalivary gland tumors were included in this study. Among those 14 cases, 9 (64.29%) were femalesand 5 (35.7%) were males. Patient’s age ranged between 26 and 82 years with a mean age 42.5 years.

The anatomical distribution of the recorded cases showed that the most common site of occurrence was the parotid salivary gland; 8 cases (57.14%) followed by the palatal minor salivary gland(42.8%).

3.2. Histopathological Results

In the current research, the two grades of mucoepidermoid carcinoma (MEC) were included, low grade mucoepidermoid carcinoma (4 cases) and the high grade one. (4cases).

Six histological patterns of AdCC were included in this study, the cribriform,tubular-trabecularand thesolid form (2cases each).

3.3. Immunohistochemical Results

Positive expression of the maspin protein was observed in the control group. (Fig.3)

Of all tumors analyzed (n=14), 85.7% showed positive maspin expression (n=12), while 14.3% (n=2) were negative.

Out of the total 8 cases of mucoepidermoid carcinoma,

6case (75%) were positive for maspin. Four cases were of the low-grade type and two case of the high-grade type. Low-grade MEC expressed intense nuclear and cytoplasmic immunoreactivity in the intermediate cells and epidermoid cells, whereas membranous immunoreactions were noted in the mucous-secreting cells (Figs. 4-5). While the high-grade MEC showed maspin immunopositivity particularly in the anaplastic epidermoid cells, some of these cells showed only cytoplasmic reaction, and others showed both nuclear and cytoplasmic immunosignals(Figs. 6,7).

The MOD was higher in low-grade type compared to the high grade one. There was significant difference ($P < 0.05$) was found (Table 1,). While MA% was higher in high-grade compared to low-grade. (Table 2)

Table (1). *Different in optical Density of Maspin Immunoexpression between Low grade and High grades Mucoepidermoid Carcinoma.*

	Low grade MEC)	High grade MEC	T	P
Mean ± SD	38.28 ± 0.12	33.14 ± 0.11	45.431*	<0.001

t: Student's t-test
*; statistically significant

Table (2). *Different in Area percent of Maspin Immunoexpression between Low grade and High grade Mucoepidermoid Carcinoma.*

	Low grade MEC)	High grade MEC	t	p
Mean ± SD	8.276 ±1.1	26.501 ±9.52	4.2485**	<0.001

t: Student's t-test **: highly statistically significant

Maspin expression was evident in all the examined cases of AdCC. In the cribriform pattern, positive immunosignals was detected in almost all the malignant cells. Only few cells were negative (Fig. 8). Meanwhile in tubular - trabecular patterns, intense nuclear and cytoplasmic staining was detected in the ductal epithelial and myoepithelial cells (Figs.9, 10). In the solid pattern of AdCC the maspin expression was focally positive in basaloid tumor cells of some nests (Fig.11), and the other nests were completely negative

The MOD and MA% were highest in tubular-trabecular type, followed by cribriform type; the lowest MOD and MA% were recorded in the solid AdCCs.

The mean optical density of maspin immune expression was higher in low grade malignant tumors compared to high grade tumors. Using student's t- test, but the difference in MOD between these two groups did not reach a significant value, (Table 3).

Table (3). *Different in Mean Optical Density of MaspinImmuno-expression Between Low and High Grade Malignant Salivary Tumors.*

	High grade tumors	Low grade tumors	t	p
Mean ± SD	26.55 ±16.02	32.83 ± 6.64	0.897	0.387

t: Student's t-test

No significant differences were found between maspin immunoreactivity and age, sex as well as site. On the other hand, there were no cases presented with lymph node metastasis.

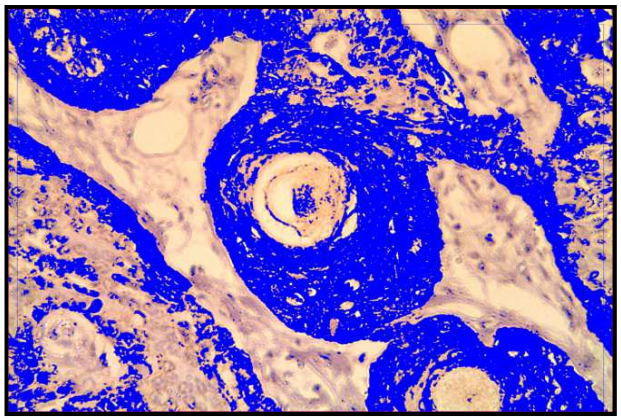

Fig. (1). *Area percent (blue binary color).*

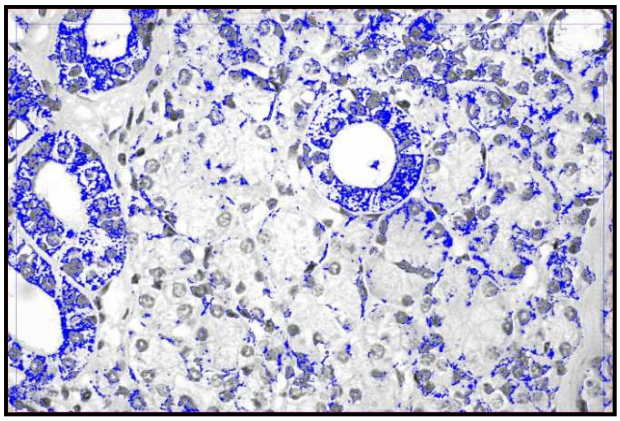

Fig. (2). *Optical density (OD) (blue binary color).*

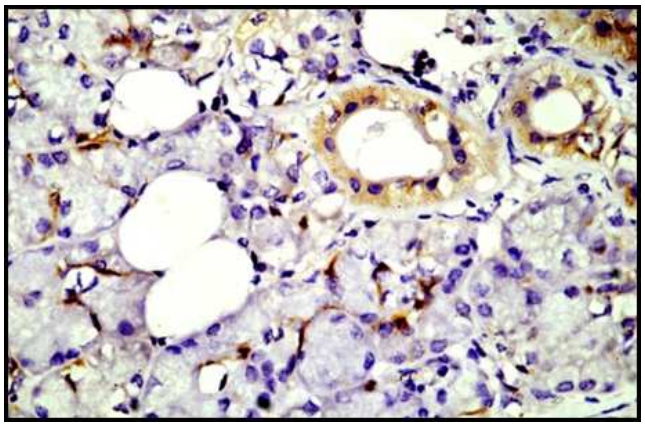

Fig. (3). *Normal salivary gland tissue showing positive expression of maspin in the ductal epithelium and myoepithelial cells lining periphery. (x 400)*

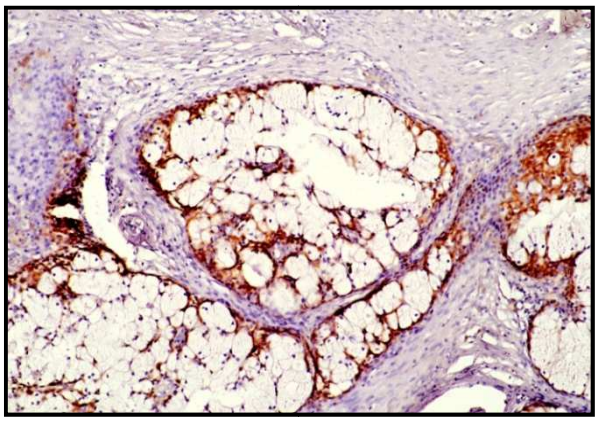

Fig. (4). *Low-grade mucoepidermoid carcinoma showing positive maspin immunoreactivity in the intermediate cells. The mucous secreting cells exhibited membranous reaction. (x 100)*

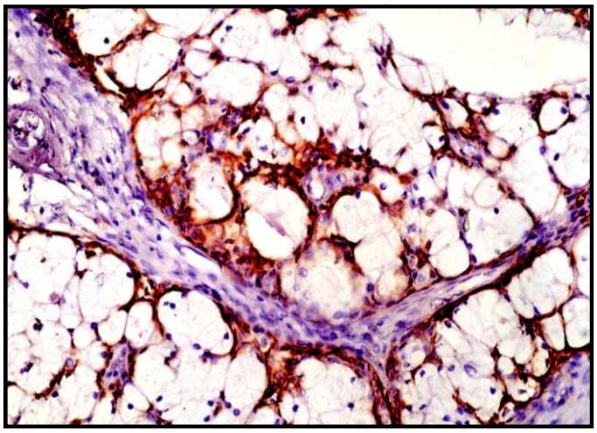

Fig. (5). *Higher magnification of the previous case revealing intense maspin immunoreaction. (x 200)*

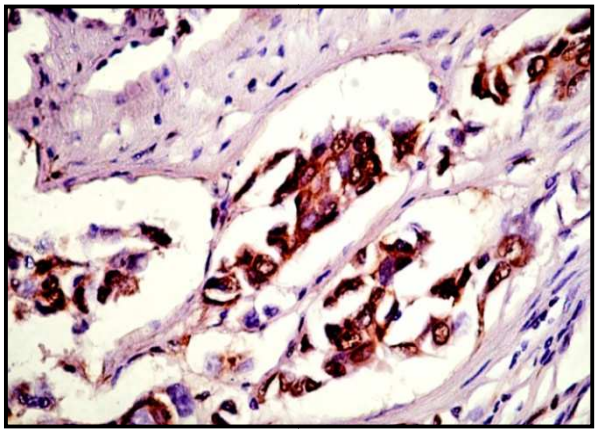

Fig. (6). *High-grade of mucoepidermoid carcinoma showing nuclear and cytoplasmic maspin immunoexpression in the anaplastic epidermoid cells within central spaces. Negative immunosinglas to maspin are also noted in some cells. (x 400)*

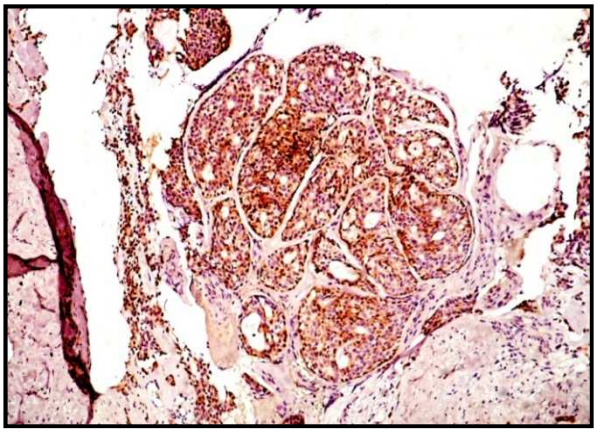

Fig. (7). *Cribriform pattern of adenoid cystic carcinoma exhibiting maspin immunopostitivity in almost all the malignant basaloid tumors cells. (x 100)*

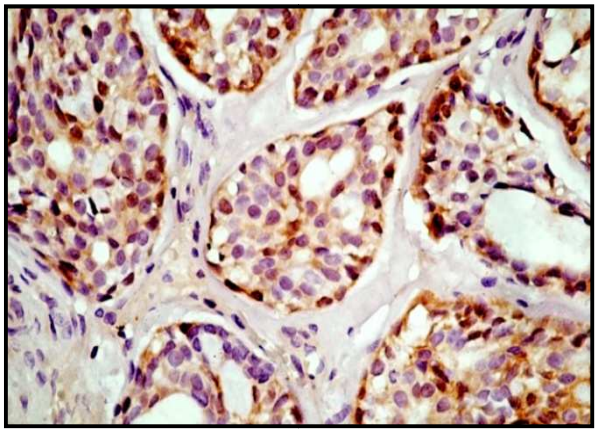

Fig. (8). *Higher magnification of previous case notice only few cells are negative. (x 400)*

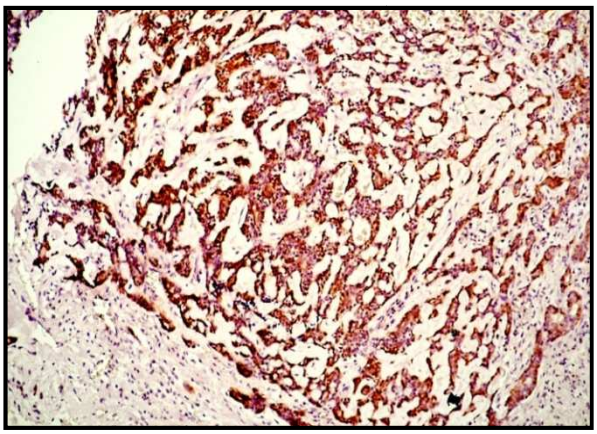

Fig. (9). *Trabecular pattern of adenoid cystic carcinoma revealing an intense maspin immunosignals in the malignant basaloid cells. (x 100)*

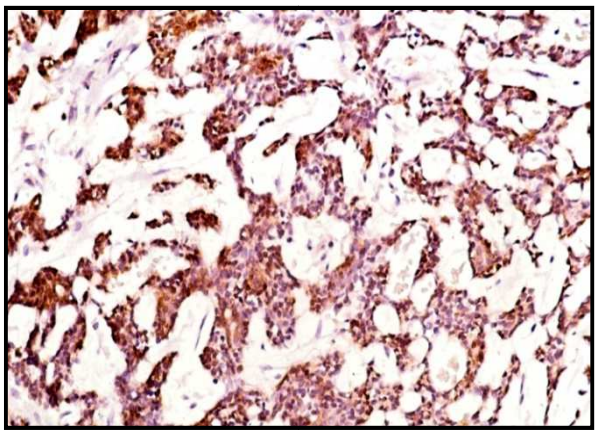

Fig. (10). *Higher magnification of the previous case showing nuclear and cytoplasmic maspin immunopositivity. (x 400)*

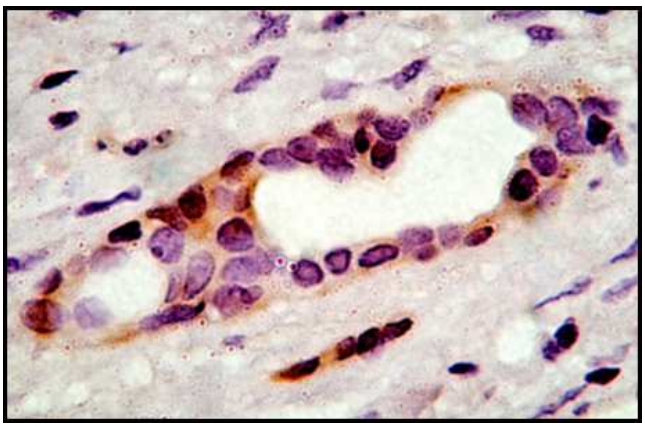

Fig. (11). *Tumbular pattern of adenoid cystic carcinoma exhibiting strong maspin immunosignals in the glandular ductal cells. (x 400)*

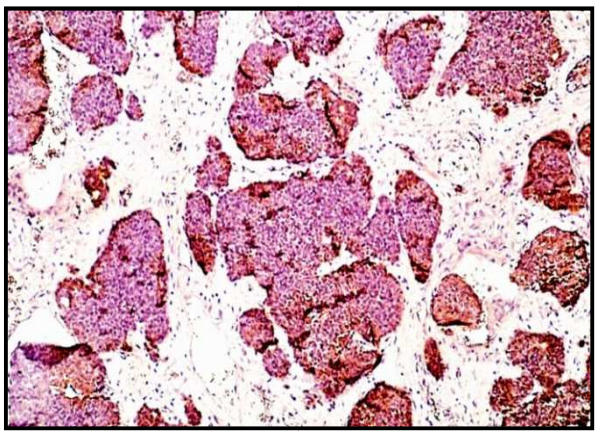

Fig. (12). *Solid pattern of adenoid cystic carcinoma exhibiting focal maspin positivity in the malignant tumor cells. (x 100)*

4. Discussion

Salivary gland tumors comprise a significant proportion of oral tumors and are the next most common tumors of the mouth after squamous cell carcinoma (25).The World Health Organization (WHO) listed twenty four malignant tumors, with a wide spectrum of biological behavior, and variable responsiveness to systemic therapies (5, 6).In their report they mentioned that the incidences of salivary gland tumors are slightly higher in females thanmales (5). This goes with the results of the present work as well as other studies (26-28). However, other Asians researchers found that the incidence of male out-numbered the females (29, 30). They stated that, this may be due to that most females being confined to their homes and do not come for treatment. In the present study, the mean age of patients with malignant tumors was 42.5 years. This goes with the results of several reports(26,29,31).The vast majority of salivary gland tumors encountered in the present research occurred in the major salivary glands(57.14 %) especially in the parotid gland. while, 42.8 % of tumors found in the minor salivary glands with predominance in the palate. This is in agreement with Shishhegar et al (29), Oliveira et al (26), and Li LJ et al (32).In most reports (5, 31, 33), mucoepidermoid carcinoma was the most common malignant tumor diagnosed among salivary gland tumors followed by adenoid cystic carcinoma.However, other studies reported that adenoid cystic carcinoma are more common (29, 34), these findings might suggest a geographic variation in the frequencies of both malignant tumors.(30).

The clinical importance of maspin in human cancers has been investigated since its discovery in 1994. (10) Experimental studies revealed that maspin suppressed tumor growth, angiogenesis, invasion and metastasis. (16,17,36,38) Maspin is also involved in the process of cell apoptosis.(18,22) The exact function of maspin, as a tumor suppressor is not known. Moreover, its localization in different cell compartments (cytoplasm, nucleus, extracellular matrix) suggests that it may be involved in different biological processes. (37)

Several studies were performed to detect the expression

of maspin protein in different malignant tumors such as ovarian carcinoma (38), pulmonary adenocarcinoma (39), prostate carcinoma (40), oral squamous cell carcinoma (41), and breast cancer (23) to clarify its role in malignancy. Most researchers found that the increased maspin expression does correlate with better prognosis of these tumors.

However few researches studying maspin expression in salivary gland tumors have been published (24).

In the current research, the expression of maspin protein in normal salivary gland tissues and salivary gland tumors were undergoexamination. The normal control section of salivary gland tissues revealed positive immunoreactivity to maspin in the ductal epithelial cells as well as in myoepithelial cells lining acini periphery. This is in consistent with the findings of De Lima Navarro et al(24) and Nakashima et al(42).

The tumor suppressor maspin was expressed in most of the examined malignant salivary gland tumors where 14.3% of them were negative to the maspin protein, and 85.7% of theses tumors showed positive immunosignals to maspin with different intensities.

All cases of AdCC in this research revealed a positive maspin expression with different degrees of intensity and location. This was in agreement with the findings of other studies (24, 42). Controversially, in a study done by Ghazy et al (43) he found that all cases of solid pattern of AdCC demonstrated negative immunoreaction. However, in this research the solid pattern showed focal positivity in the tumor cells of some nests while others were completely negative. Moreover, it was demonstrated that the pattern as well as the intensity of immunostaining (as shown by image analysis) varied between the histologic subtypes of AdCC. The solid pattern showed a focal immunoreactivity, lower MOD and area% than the cribriform or tubular pattern. De Lima Navarro et al (24) reported that maspin expression in these tumors coincides with their biological behavior. Much evidence showed that the histologic subtypes of AdCC are directly related to prognosis, where the tubular pattern having the best prognosis and the solid pattern the worst prognosis (44, 45).

In this study, 57.145% of the examined malignant salivary gland tumors were mucoepidermoid carcinoma, with equal distribution between low and high grade types. Intermediate type of mucoepidermoid carcinoma was not included in the present work. 75% of mucoepidermoid carcinoma cases exhibited positive maspin immunoreactivity. This is in accordance with Schwarz et al (46) and Ghazy et al (43). They reported that 86.7% and 100% of their examined cases of MEC showed positivity to maspin respectively. However, low grade MEC showed significantly higher expression of the maspin protein more than the high grade carcinoma. High grade MEC was characterized by decreased maspin expression. Loss or decreased expression of maspin indicates loss of it is role in inhibition of tumor invasion, metastasis, and angiogenesis (46).

Some authors (47-48) found that maspin nuclear staining was associated with good prognostic factors, whereas, cytoplasmic staining was associated with poor outcome, these findings might suggest the critical role played by mapsin in the biological function of the tumors.

In the present research, the majority of the malignant tumors expressed cytoplasmic maspin reaction. This finding denotes that maspin cellular localization has an influence on its role as tumor suppressor gene (49). Sood et al (50) demonstrated that mixed nuclear end cytoplasmic maspin localization in ovarian cancer is indicative of a more benign lesion than neoplasms with cytoplasmic expression only.

Regarding patient age, and sex, no significant correlation was found in the present work or reported in previous studies (46). On the other hand, the correlation between maspin expression and the lymph node metastasis cannot be evaluated due to lack of cases presented in the present work. Other previous studies had evaluated this correlation, and their results were conflicting (43, 46).Most hypothesis suggested that maspin, which inhibits tissue plasminogen activator, may be a tumor-suppressor factor. Moreover, Seftor et al(51) reported that maspin protein has the ability to induce upregulation of some specific integrin levels, resulting in suppression of an invasive phenotype of the breast cancer cell line. These results suggested that up regulation of integrin expression in addition to plasminogen activator inhibition might be another suppressive mechanisms against tumor invasion and metastasis stimulated by maspin (52). Again, similar results documenting a correlation between the absence of maspin and poor prognosis have been reported for breast and prostate cancer (53, 54). Boltze et al (55) reported that colorectal carcinoma with loss of maspin expression was associated with shorter survival. However, in contrast to these observations, studies on ovarian and pancreatic cancer revealed a significant overexpression of maspin in the analyzed samples (46, 56). Maspin seems to behave as an oncogene rather than as a tumor suppressor. Conversely, an investigation on breast cancer suggests that the treatment of tumor cells with recombinant maspin will lead to decrease activation of Ras signaling which is associated with invasion and metastasis and maspin in turn will lead to decreased invasion and metastasis (57).

5. Conclusions

Based on the immunohistochemical results of this study, the following conclusions were obtained:

1- Highly expression of maspin in low grade malignancy, whereas progressive loss of maspin is noted as histological malignancy raise.
2- Nuclear maspin may play a critical role in tumor suppression, whereas cytoplasmic maspin localization is associated with aggressive behavior in salivary gland carcinomas.

Recommendations

1- Further studies with large sample size are required to clarify the correlation between maspin expression and salivary gland tumors.
2- Further studies involving samples with lymph node metastasis are required to clarify the correlation between maspin expression and lymph node metastasis.
3- Maspin could serve as a therapeutic target in salivary gland tumors including recombinant protein or gene therapy.

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(7):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(6):834-40.

[3] Speight PM, Barrett AW. Salivary glandtumors. Oral Dis 2002;8(5):229-40.

[4] Califano J, EiseleDW. Benign salivary gland neoplasms. OtolaryngolClin North Am 1999; 32(5):861-73.

[5] 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.

[6] Speight PM, Barrett AW. Prognosticfactors in malignant tumors of the salivary glands. Br J Oral MaxillofacSurg 2009;47(8):587-93

[7] Abraham J, Allegra CJ, Gulley JL. Bethesda Handbook of Clinical Oncology. Philadelphia: Lippincott Williams and Wilkins; 2001.

[8] Gettins P, Patston PA, Schapira M. Structure and mechanism of action of serpins. HematolOncolClin North Am 1992 Dec;6(6):1393-408.

[9] Chen WT. Membrane proteases: roles in tissue remodeling and tumor invasion. CurrOpin Cell Biol 1992 Oct;4(5):802-9.

[10] Zou Z, Anisowicz A, Hendrix MJ, Thor A, Neveu M, Sheng S, et al. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. Science 1994 Jan 28; 263(5146):526-9.

[11] Schneider SS, Schick C, Fish KE, Miller E, Pena JC, Treter SD, et al. A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. ProcNatlAcadSci U S A 1995 Apr 11;92(8):3147-51.

[12] Maass N, Hojo T, Zhang M, Sager R, Jonat W, Nagasaki K. Maspin--a novel protease inhibitor with tumor-suppressing activity in breast cancer. ActaOncol 2000; 39(8):931-4.

[13] Ito R, Nakayama H, Yoshida K, Oda N, Yasui W. Loss of maspin expression is associated with development and progression of gastric carcinoma with p53 abnormality. Oncol Rep 2004 Nov; 12(5):985-90.

[14] Maass N, Hojo T, Ueding M, Luttges J, Kloppel G, Jonat W, et al. Expression of the tumor suppressor gene Maspin in human pancreatic cancers. Clin Cancer Res 2001 Apr; 7(4):812-7.

[15] Li HW, Leung SW, Chan CS, Yu MM, Wong YF. Expression of maspin in endometrioid adenocarcinoma of endometrium. Oncol Rep 2007 Feb; 17(2):393-8.

[16] Abraham S, Zhang W, Greenberg N, Zhang M. Maspin functions as tumor suppressor by increasing cell adhesion to extracellular matrix in prostate tumor cells. J Urol 2003 Mar; 169(3):1157-61.

[17] Cella N, Contreras A, Latha K, Rosen JM, Zhang M. Maspin is physically associated with [beta]1 integrin regulating cell adhesion in mammary epithelial cells. FASEB J 2006 Jul;20(9):1510-2.

[18] Zhang W, Shi HY, Zhang M. Maspin overexpression modulates tumor cell apoptosis through the regulation of Bcl-2 family proteins. BMC Cancer 2005 May;20;5:50.:50

[19] Solomon LA, Munkarah AR, Schimp VL, Arabi MH, Morris RT, Nassar H, et al. Maspin expression and localization impact on angiogenesis and prognosis in ovarian cancer. GynecolOncol 2006 Jun;101(3):385-9.

[20] Zou Z, Gao C, Nagaich AK, Connell T, Saito S, Moul JW, et al. p53 regulates the expression of the tumor suppressor gene maspin. J BiolChem 2000 Mar 3; 275(9):6051-4.

[21] Khalkhali-Ellis Z. Maspin: the new frontier. Clin Cancer Res 2006 Dec 15; 12(24):7279-83.

[22] Rose SL, Fitzgerald MP, White NO, Hitchler MJ, Futscher BW, De GK, et al. Epigenetic regulation of maspin expression in human ovarian carcinoma cells. GynecolOncol 2006 Aug; 102(2):319-24.

[23] Magdalena Machowska1, Katarzyna Wachowicz13, Mirosław Sopel2 and Ryszard Rzepeck Nuclear location of tumor suppressor protein maspin inhibits proliferation of breast cancer cells without affecting proliferation of normal epithelial cells Biomed central(BMC)Cancer 2014, 14:142

[24] Navarro RL, Martins MT, de AraujoVC. Maspin expression in normal and neoplastic salivary gland. J Oral Pathol Med 2004;33(7):435-40.

[25] Brandwein MS, Ferlito A, Bradley PJ, Hille JJ, Rinaldo A. Diagnosis and classification of salivary neoplasms: pathologic challenges and relevance to clinical outcomes. ActaOtolaryngol 2002; 122(7):758-64.

[26] de Oliveira FA, Duarte EC, Taveira CT, Maximo AA, de Aquino EC, Alencar RC, et al. Salivary gland tumor: a review of 599 cases in a Brazilian population. Head Neck Pathol 2009; 3(4):271-5.

[27] Jones AV, Craig GT, Speight PM, Franklin CD. The range and demographics of salivary gland tumours diagnosed in a UK population. Oral oncology 2008; 44(4):407-17.

[28] Ito FA, Ito K, Vargas PA, de Almeida OP, Lopes MA. Salivary gland tumors in a Brazilian population: a retrospective study of 496 cases. Int J Oral MaxillofacSurg 2005; 34(5):533-6.

[29] 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 Int2011. 934350.

[30] Tian Z, Li L, Wang L, Hu Y, Li J. Salivary gland neoplasms in oral and maxillofacial regions: a 23-year retrospective study of 6982 cases in an eastern Chinese population. Int J Oral MaxillofacSurg 2010; 39(3):235-42.

[31] Gill MS, Muzaffar S, Soomro IN, Kayani N, Hussainy AS, Pervez S, et al. Morphological pattern of salivary gland tumours. J Pak Med Assoc 2001; 51(10):343-6.

[32] 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(2):187-92.

[33] Vargas PA, Gerhard R, AraujoFilho VJ, de Castro IV. Salivary gland tumors in a Brazilian population: a retrospective study of 124 cases. Rev HospClinFac Med Sao Paulo 2002; 57(6):271-6.

[34] Kayembe MK, Kalengayi MM. Salivary gland tumours in Congo (Zaire). Odontostomatol Trop 2002; 25(99):19-22.

[35] Zhang M, Volpert O, Shi YH, Bouck N. Maspin is an angiogenesis inhibitor. Nat Med 2000 Feb; 6(2):196-9.

[36] Cher ML, Biliran HR, Jr., Bhagat S, Meng Y, Che M, Lockett J, et al. Maspin expression inhibits osteolysis, tumor growth, and angiogenesis in a model of prostate cancer bone metastasis. ProcNatlAcadSci U S A 2003 Jun 24; 100(13):7847-52.

[37] Klasa-Mazurkiewicz D, Narkiewicz J, Milczek T, Lipinska B, Emerich J. Maspin overexpression correlates with positive response to primary chemotherapy in ovarian cancer patients. GynecolOncol 2009 Apr; 113(1):91-8.

[38] Solomon LA, Munkarah AR, Schimp VL, Arabi MH, Morris RT, Nassar H, et al. Maspin expression and localization impact on angiogenesis and prognosis in ovarian cancer. GynecolOncol 2006; 101(3):385-9.

[39] Lonardo F, Li X, Siddiq F, Singh R, Al-Abbadi M, Pass HI, et al. Maspin nuclear localization is linked to favorable morphological features in pulmonary adenocarcinoma. Lung Cancer 2006; 51(1):31-9.

[40] Colmenero RC, Patron RM, Martin PM. Salivary duct carcinoma: a report of nine cases. J Oral MaxillofacSurg 1993; 51(6):641-6.

[41] Yasumatsu R, Nakashima T, Hirakawa N, Kumamoto Y, Kuratomi Y, Tomita K, et al. Maspin expression in stage I and II oral tongue squamous cell carcinoma. Head Neck 2001; 23(11):962-6.

[42] Nakashima D, Uzawa K, Kasamatsu A, Koike H, Endo Y, Saito K, et al. Protein expression profiling identifies maspin and stathmin as potential biomarkers of adenoid cystic carcinoma of the salivary glands. Int J Cancer 2006; 118(3):704-13.

[43] Ghazy SE, Helmy IM, Baghdadi HM. Maspin and MCM2 immunoprofiling in salivary gland carcinomas. DiagnPathol 2011; 6:89.

[44] Szanto PA, Luna MA, Tortoledo ME, White RA. Histologic grading of adenoid cystic carcinoma of the salivary glands. Cancer 1984; 54(6):1062-9.

[45] Santucci M, BondiR. New prognostic criterion in adenoid cystic carcinoma of salivary gland origin. Am J ClinPathol 1989; 91(2):132-6.

[46] Schwarz S, Ettl T, Kleinsasser N, Hartmann A, Reichert TE, Driemel O. Loss of Maspin expression is a negative prognostic factor in common salivary gland tumors. Oral Oncol 2008; 44(6):563-70.

[47] Al-Ayyoubi M, Gettins PG, Volz K. Crystal structure of human maspin, a serpin with antitumor properties: reactive center loop of maspin is exposed but constrained. J BiolChem 2004; 279(53):55540-4.

[48] Al-Ayyoubi M, Schwartz BS, Gettins PG. Maspin binds to urokinase-type and tissue-type plasminogen activator through exosite-exosite interactions. J BiolChem 2007; 282(27):19502-9.

[49] Bailey CM, Khalkhali-Ellis Z, Seftor EA, Hendrix MJ. Biological functions of maspin. J Cell Physiol 2006; 209(3):617-24.

[50] Sood AK, Fletcher MS, Gruman LM, Coffin JE, Jabbari S, Khalkhali-Ellis Z, et al. The paradoxical expression of maspin in ovarian carcinoma. Clin Cancer Res 2002; 8(9):2924-32

[51] Seftor RE, Seftor EA, Sheng S, Pemberton PA, Sager R, Hendrix MJ. maspin suppresses the invasive phenotype of human breast carcinoma. Cancer Res 1998; 58(24):5681-5.

[52] Hojo T, Akiyama Y, Nagasaki K, Maruyama K, Kikuchi K, Ikeda T, et al. Association of maspin expression with the malignancy grade and tumor vascularization in breast cancer tissues. Cancer Lett 2001; 171(1):103-10.

[53] Sheng S, Carey J, Seftor EA, Dias L, Hendrix MJ, Sager R. Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells. ProcNatlAcadSci U S A 1996; 93(21):11669-74.

[54] Prasad CP, Rath G, Mathur S, Bhatnagar D, Ralhan R. Expression analysis of maspin in invasive ductal carcinoma of breast and modulation of its expression by curcumin in breast cancer cell lines. ChemBiol Interact 2010; 183(3):455-61.

[55] Boltze C. Loss of maspin is a helpful prognosticator in colorectal cancer: a tissue microarray analysis. Pathol Res Pract 2005; 200(11-12):783-90.

[56] Latha K, Zhang W, Cella N, Shi HY, Zhang M. Maspin mediates increased tumor cell apoptosis upon induction of the mitochondrial permeability transition. Mol Cell Biol 2005; 25(5):1737-48.

[57] Odero-Marah VA, Khalkhali-Ellis Z, Chunthapong J, Amir S, Seftor RE, Seftor EA, et al. Maspin regulates different signaling pathways for motility and adhesion in aggressive breast cancer cells. Cancer Biol Ther 2003;2(4):398-403.