Expression of nm23H1 and MMP2 in laryngeal carcinoma and its role in aggressiveness of the tumour and node metastasis

Wael Shehata[a] and Manal Salman[b]

[a]Department of Otorhinolaryngology, October 6 University and [b]Department of Pathology, Ain Shams University, Cairo, Egypt

Correspondence to Wael Shehata, 14 Dannish Street, Beside Air Force Military Hospital, Abbassia, Cairo, Egypt
Tel: +01005684945;
e-mail: waelent@yahoo.com

Received 26 November 2012
Accepted 3 January 2013

The Egyptian Journal of Otolaryngology
2013, 29:86–92

Introduction
Squamous cell carcinomas of the head and neck are known for their aggressive growth and propensity to metastasize. The invasion is facilitated by matrix metalloproteinases (MMPs). The overexpression of many MMPs is positively associated with tumour metastasis. The nm23H1 gene has been implicated as a suppressor gene and reduced expression of its gene product has been observed in patients with positive lymph node metastasis.

Objective
The aim of this study was to examine the degree of expression of both MMP2 and nm23H1 proteins in 24 patients with primary laryngeal carcinoma using immunohistochemical technique and also to correlate the results with the clinical, radiological and histopathological data, in order to evaluate their role in predicting the local spread and lymph node metastasis.

Patients and methods
This study included 24 patients with primary laryngeal carcinoma involving various regions of the larynx and was carried out at the Department of Otolaryngology, Ain Shams University Hospitals.
The laryngeal and nodal specimens were examined histopathologically and immunohistochemical analysis was carried out for the nm23H1 and MMP2 proteins.

Results
There was a significant correlation between MMP2 expression and the site of the tumour, as a drastic reduction in MMP2 expression was mainly associated with glottic carcinoma ($P<0.05$). The tumour stages were associated with an increase in MMP2 expression, but this was not statistically significant ($P=0.07$). However, the presence of lymph node metastasis was significantly related to the overexpression of MMP2 ($P<0.05$). As regards nm23H1, a statistically significant correlation was found between nm23H1 expression and the tumour stage ($P<0.05$). Moreover, the presence of lymph node metastasis was significantly correlated to the loss of nm23H1 expression and vice versa ($P<0.05$). However, the correlation between the expression levels of MMP2 and nm23H1 was found to be statistically insignificant ($P>0.05$).

Conclusion
The increased expression of MMP2 was related to the occurrence of nodal metastasis of the tumour and also to the tumour sites characterized by frequent metastasis (supraglottic and transglottic). In contrast, nm23H1 expression was inversely related to the advancement of the tumour stage and the nodal metastatic state.

Keywords:
cancer larynx, matrix metalloproteinase-2, nm23H1

Egypt J Otolaryngol 29:86–92

1012-5574

Introduction

Laryngeal squamous cell carcinoma (LSCC) is one of the most common head and neck malignancies. It is generally accepted that LSCC is strongly associated with tobacco smoking, alcohol consumption and viral infection [1]. Early-stage well-differentiated LSCC has a good prognosis but the survival rate is significantly lower in patients with regional and distant metastasis; therefore, invasion and metastasis are important factors that greatly impact the prognosis in LSCC.

The procedure of invasion and metastasis during malignancy involves a very complicated process [2]. Degradation of the extracellular matrix (ECM) is an essential step that is required for the invasion [3].

Matrix metalloproteinase-2 (MMP2), which is one of many matrix-degrading enzymes, has been identified as a basement membrane-degrading enzyme and is thought to play a role in the malignant behaviour of cancer cells [4]. Presently, there are at least 24 members of the MMP2 family that can be classified into a number of

subgroups [5]. Several studies have proven that MMP2 may play an important role in the development of acute lymphoblastic leukaemia [6], breast cancer, renal cancer and head and neck carcinoma [7]. The development of nodal metastasis is a major adverse event in patients with squamous cell carcinoma of the upper aerodigestive tract, reducing the survival by about 50% [8]. The metastatic cells undergo alternation in the genes and their products, including overexpression of the metastasis-promoting factors or loss of expression of suppressing factors [9].

Steeg *et al.* [10] reported a family of closely related tumour suppressor genes (nm23) that is associated with the inhibition of the metastatic process through an unknown molecular mechanism. Metastasis suppressor proteins regulate multiple steps in the metastatic cascade, including cancer cell invasion, survival in the vascular and lymphatic circulation and colonization of the distant organ sites. Tumour suppressor genes may positively or negatively regulate the subsequent development of primary tumours [11].

Two closely related genes namely nm23H1 and nm23H2 have identical protein products and similar activities and shown to be identical to the human nucleotide diphosphate kinase (NDP3). Therefore, the nm23H1 gene has been demonstrated to correlate inversely with the metastatic potential in several tumours, as the data suggests that the normal expression of nm23H1 contributes to normal cell growth, whereas altered or lowered levels of nm23H1 expression aid in cancer metastasis [12].

Aim of the study

The aim of the study was to examine the degree of expression of both MMP2 and nm23H1 in primary laryngeal carcinoma using immunohistochemical techniques and correlating it with the findings of the clinical, radiological and conventional histopathological examination.

Patients and methods

Patients

A total of 24 patients with LSCC involving various regions of the larynx (and at various stages) were included in this study, which was carried out at the Departments of Otolaryngology and Pathology of Ain Shams University Hospitals, between March 2007 and April 2009.

Methods

Preoperative clinical evaluation of these patients included a full history taking, especially for hoarseness of the voice, stridor and neck masses. A thorough ENT and neck examination was performed for each patient.

Direct laryngoscopy was performed to evaluate the extent of the tumour for provisional staging and obtaining samples for biopsy.

Radiological assessment through a computed tomography (CT) scan of the neck comprised thin axial cuts at a soft tissue window. The CT scan was performed to study the site of the lesion and the extent of tumour spread; the staging was confirmed depending on the findings of the CT scan.

The specimens obtained from partial or total laryngectomy and the removed lymph nodes underwent fixation to prepare paraffin-embedded tissue blocks, from which sections of 5 μm thickness were cut and stained with haematoxylin and eosin.

A routine histopathological examination of the specimens was performed and the tumour type, grade, freedom or involvement of surgical margins, depth of invasion and lymph nodal status were determined.

For immunohistochemical staining, 5 μm sections were cut and placed on coated slides and then deparaffinized. The endogenous peroxidase activity was blocked using 0.3% H_2O_2. The sections were then incubated for 10 min after which the non-specific-binding sites were blocked by treatment with 3% normal goat serum for 30 min. The slides were incubated with the primary antibodies for nm23H1 (mouse monoclonal anti-nm23H1 antibody diluted to 1 : 100) and MMP2 proteins (rabbit monoclonal anti-MMP2 antibody diluted to 1 : 50).

Each primary antibody was incubated for 1 h at 37°C. After washing with PBS, the biotinylated secondary antibodies, which were anti-mouse immunoglobulin G for nm23H1 and anti-rabbit immunoglobulin G for MMP2, were added, followed by an incubation of 45 min. After rinsing with PBS, peroxidise-conjugated streptavidin was added for 20 min, followed by another rinse with PBS. The immunohistochemical reactions were developed in 20 mg of freshly prepared 3,3′ diaminobenzidine tetrahydrochloride for 10 min and were then rinsed with distilled water. After counterstaining with haematoxylin for 1 min, the specimens were observed under a light microscope. The sections were scanned at low power (× 40) to assess the heterogeneity of the stain distribution, and multiple fields with diffused staining were examined at high power (× 200) for scoring. As regards the scoring of the nm23H1 staining, the tissues were scored as follows:

0 = very light or no staining.

1 = for widespread faint staining or localized strong staining.

2 = for widespread strong staining.

As regards the MMP2 protein expression, a semiquantitative analysis based on a 4-point scale was used depending on the percentage of positively stained cells:

0 = no staining.

1 = low expression (< 10% positive cells).

2 = moderate expression (10–50% positive cells).

3 = strong expression (> 50% positive cells).

After scoring both MMP2 and nm23H1 staining, correlations were drawn on the basis of the clinical, radiological and histopathological data of the patients. Statistical analysis of the results was performed using the χ^2 test, one-way analysis of variance test and Student *t*-test by using the SPSS software version 10.0 for windows.

Results

A total of 24 patients with LSCCs were involved in this study that was carried at Ain Shams University Hospitals between March 2007 and April 2009. The age of the patients ranged from 42 to 89 years (with an average of 58 ± 5 years). There was a strong male predominance with 21 men and three women.

The results were classified into two groups (a) clinical and radiological results and (b) pathological and immunohistochemical results.

Clinical and radiological results

In all patients, the clinical evaluation and CT scan staging revealed the exact site of the tumour, its extent and presence or absence of lymph node metastasis.

Eight patients (33.3%) had supraglottic carcinoma of various T stages (one patient with T1, three with T2, two with T3 and two with T4), 11 patients (45.9%) had transglottic carcinoma involving the ventricle and the glottis areas, with subglottic extension in two patients only (three patients with T2, seven with T3 and only one with T4), five patients (20.8%) had glottic carcinoma of T1 or T2 stages only (three patients with T1 and two with T2).

The tumour was localized within the larynx in 21 patients (87.5%) but extended beyond this limit, through the thyroid cartilage and into the prelaryngeal muscles in two patients (8.3%) and into the pharyngeal mucosa in one patient (4.1%).

As regards the T stage, nine patients (37.5%) were classified under the T3 stage, eight (33.3%) under T2, four (16.7%) under T1 and three (12.5%) under T4.

As regards the N stage, nine patients (37.5%) had positively enlarged lymph nodes and 15 (62.5%) showed no lymph node enlargement.

Based on the American Joint Committee on Cancer staging system, we staged the patients as follow: three patients (12.5%) with stage I, seven (29.1%) with stage II, six (25%) with stage III and eight (33.3%) with stage IV.

Histopathological and immunohistochemical results

The histopathological examination revealed that squamous cell carcinomas were present in all patients and were graded (according to Broder's classification) into: well-differentiated in 10 patients (41.1%), moderately differentiated in 10 (41.6%) and poorly differentiated in four (16.7%).

Metastatic deposits in the cervical lymph nodes were seen in nine patients (37.5%) with radiologically enlarged lymph nodes, whereas the remaining 15 patients showed no malignant deposits within their lymph node specimens (Table 1).

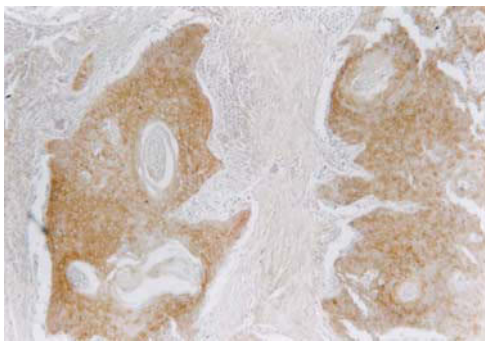
The immunohistochemical results were as follows: MMP2 staining was localized to the cytoplasm; it was detected in 12 patients (50%), of whom seven (29.1%) showed moderate expression and the remaining five (20.8%) showed strong expression. More intensive staining was seen at the advanced edge of the tumour. Figure 1 shows high MMP2 expression in a

Table 1 Clinicopathologic features and immunohistochemical results

No.	Age (years)	Sex	Main site	TNM status	Stage	Grade	Lymph node	MMP2	nm23H1
1	59	M	Supraglottic	T1N3M0	IV	II	Positive	+	0
2	42	M	Supraglottic	T4N1M0	IV	II	Positive	+	0
3	71	M	Supraglottic	T2N0M0	II	I	Negative	+	1
4	50	M	Supraglottic	T4N3M0	IV	III	Positive	–	0
5	62	M	Transglottic	T3N1M0	III	II	Positive	+	1
6	70	M	Transglottic	T3N3M0	IV	III	Positive	+	0
7	89	M	Transglottic	T3N0M0	III	I	Negative	+	II
8	59	M	Transglottic	T2N1M0	III	II	Positive	+	II
9	60	M	Glottic	T1aN0M0	I	I	Negative	–	I
10	48	F	Glottis	T2N0M0	II	II	Negative	–	I
11	72	M	Transglottic	T3N0M0	III	I	Negative	–	II
12	65	M	Transglottic	T3N2M0	IV	II	Positive	+	I
13	55	M	Glottic	T1aN0M0	I	II	Negative	–	II
14	44	M	Glottic	T1aN0M0	I	I	Negative	–	II
15	50	M	Supraglottic	T2N0M0	II	I	Negative	–	I
16	52	F	Transglottic	T2N0M0	II	I	Negative	–	I
17	48	M	Transglottic	T2N0M0	II	I	Negative	–	0
18	72	M	Supraglottic	T3N2M0	IV	III	Positive	+	0
19	49	M	Supraglottic	T2N0M0	II	II	Negative	+	II
20	60	F	Glottic	T2N0M0	II	I	Negative	–	I
21	42	M	Transglottic	T3N0M0	III	II	Negative	–	II
22	50	M	Transglottic	T3N0M0	III	I	Negative	+	0
23	71	M	Supraglottic	T3N2M0	IV	II	Positive	–	I
24	45	M	Transglottic	T4N0M0	IV	III	Negative	+	II

F, female; Grade differentiation: I, well; II, moderate; III, poor; M, male; MMP2, matrix metalloproteinase-2.

Figure 1



High matrix metalloproteinase-2 (MMP2) expression in a tumour with lymph node metastasis (MMP2 ×40).

Figure 2

Low matrix metalloproteinase-2 (MMP2) expression in a tumour without lymph node metastasis (MMP2 ×40).

tumour with lymph node metastasis and Fig. 2 shows low MMP2 expression in a tumour without lymph node metastasis.

For statistical analysis, the patients were divided into two groups according to the expression of MMP2: those with absent or low expression and those with moderate or strong expression. The correlation of MMP2 expression with the clinicopathological data is shown in Table 2.

There was no statistically significant correlation between the level of MMP2 expression and the mean age and sex of the patients ($P>0.05$). Meanwhile, a significant correlation was found between MMP2 expression and the site of the tumour, as drastically low expression of MMP2 was mainly associated with glottic carcinoma ($P>0.05$).

As regards the tumour stage, advanced tumour stages were associated with an increase in MMP2 expression; however, this was not statistically significant ($P=0.07$).

However, the presence of lymph node metastasis was significantly related to overexpression of MMP2 ($P<0.05$).

The expression of the nm23H1 protein was localized to the cytoplasm, with staining of the lymphocytes serving as an internal positive control. Seven patients showed

Table 2 Correlation of the level of matrix metalloproteinase-2 expression with the clinicopathologic features of the patients

	n (%)			
Variable	MMP2 – (or low)	MMP2 +	*P* value	Significance
Age	Mean=54.3 years	Mean=61 years	0.08[a]	NS
Sex				
Male	9 (37.5)	12 (50)	0.06	NS
Female	3 (12.5)	0 (0)		
Site				
Supraglottic	3 (12.5)	5 (20.8)	0.04	S
Transglottic	4 (16.67)	7 (29.7)		
Glottic	5 (20.8)	0 (0)		
Grade				
I	7 (29.7)	3 (12.5)	0.2	NS
II	4 (16.67)	6 (25)		
III	1 (4.17)	3 (12.5)		
Stage				
I	3 (12.5)	0 (0)	0.07	NS
II	5 (20.8)	2 (8.3)		
III	2 (8.3)	4 (16.67)		
IV	2 (8.3)	6 (25)		
Lymph node metastasis				
Absent	10 (41.67)	5 (20.8)	0.03	S
Present	2 (8.3)	7 (29.3)		
nm23				
0	2 (8.3)	5 (20.8)	0.3	NS
I	6 (25)	3 (12.5)		
II	4 (16.67)	4 (16.67)		
Total	12	12		

MMP2, matrix metalloproteinase-2; S, significant.
[a]Student's *t*-test, the rest by χ^2 test.

negative staining (0), nine showed reduced staining (1) and eight showed increased level of expression (2).

Correlation of the level of expression of the nm23H1 protein with the clinicopathological data is shown in Table 3. There was no statistically significant correlation between nm23H1expression and the mean age, sex and tumour site ($P>0.05$).

Moreover, as regards the tumour grade, no statistically significant correlation was found between nm23H1 expression and the tumour grade ($P>0.05$), whereas with respect to the tumour stage, a statistically significant correlation was found ($P<0.05$), such that in more advanced tumour stages, the expression of nm23H1 was lost.

The presence of lymph node metastasis was significantly correlated to the loss of nm23H1 expression and vice versa ($P<0.05$).

Figure 3 shows a tumour with lymph node metastasis accompanied by marked reduction of nm23H1 expression, whereas Fig. 4 shows a tumour without lymph node metastasis associated with increased nm23H1 expression. The correlation between the level of expression of MMP2 and nm23H1 was statistically insignificant ($P>0.05$).

Discussion

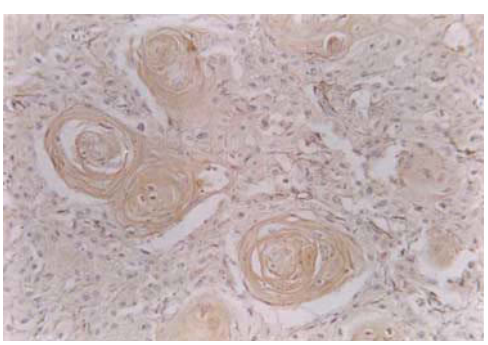
Lymph node metastasis is an important factor in the treatment and prognosis of patients with head and neck

Table 3 Correlation of the level of nm23 expression with the clinicopathologic features of the patients

	n (%)				
Variable	nm23 0	nm23 I	nm23 II	*P* value	Significance
Age	55.85 years	59.88 years	56.87 years	0.7[a]	NS
Sex					
Male	7 (29.7)	6 (25)	8 (33.3)	0.05	NS
Female	0 (0)	3 (12.5)	0 (0)		
Site					
Supraglottic	4 (16.67)	3 (12.5)	1 (4.17)	0.2	NS
Transglottic	3 (12.5)	3 (12.5)	5 (20.8)		
Glottic	0 (0)	3 (12.5)	2 (8.3)		
Grade					
I	2 (8.3)	5 (20.8)	3 (12.5)	0.2	NS
II	2 (8.3)	4 (16.67)	4 (16.67)		
III	3 (12.5)	0 (0)	1 (4.17)		
Stage					
I	0 (0)	1 (4.17)	2 (8.3)	0.04	S
II	1 (4.17)	5 (20.8)	1 (4.17)		
III	1 (4.17)	1 (4.17)	4 (16.67)		
IV	5 (20.8)	2 (8.3)	1 (4.17)		
Lymph node metastasis					
Absent	2 (8.3)	6 (25)	7 (29.7)	0.04	S
Present	5 (20.8)	3 (12.5)	1 (4.17)		
MMP2					
–	2 (8.3)	6 (25)	4 (16.67)	0.3	NS
+	5 (20.8)	3 (12.5)	4 (16.67)		
Total	7 (29.7)	9 (37.5)	8 (37.5)		

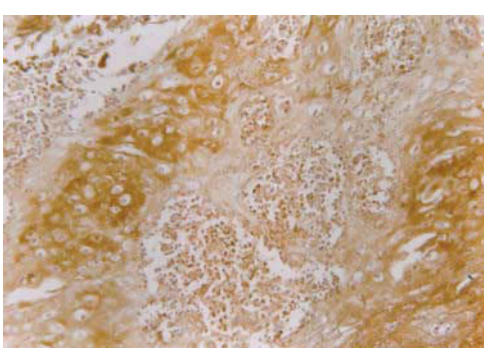
MMP2, matrix metalloproteinase-2; S, significant.
[a]One-way analysis of variance test, the rest by χ^2 test.

Figure 3



A tumour with lymph node metastasis with a marked reduction in nm23H1 expression (nm23H1 × 200).

Figure 4



A tumour without lymph node metastasis with increased nm23H1 expression and staining of the inflammatory cells (nm23H1 × 200).

squamous cell carcinoma [13]. Successful metastasis is dependent on the promoters and suppressors involved in each step [14]. The proteolysis of the ECM macromolecules is related to tumour progression, including invasion, growth and metastasis [15]. The MMPs are a family of proteolytic zinc-containing enzymes that are responsible for the breakdown of the ECM components in pathological conditions; they are involved in the disruption of the basement membrane, penetration of the stroma and blood vessels and metastasis [16]. The production of large amounts of these enzymes is an important factor in predicting the aggressive behaviour and hence, the prognosis of patients with malignant tumours. Moreover, such enzymes have been demonstrated to correlate well with the metastatic potential of tumour cells [17].

Répássy *et al.* [18] found that laryngeal cancers lacking MMP2 expression belonged to low TNM stages, whereas those expressing MMP2 belonged to the advanced stages. Moreover, Sariolu *et al.* [19] concluded that an altered expression of MMP2 was seen during early neoplastic transformation, with a consecutive increase during progression of the laryngeal carcinomas. On studying the expression of MMP2 in squamous cell carcinoma, Magary *et al.* [20] found that the expression of MMP2 was strongly correlated with more aggressive tumours and a poor treatment outcome. The present study shows that the increase in MMP2 expression was associated with advanced stages, but this was not statistically significant ($P = 0.07$); this is probably because of the small number of our patients, almost all of whom presented with advanced tumour stages.

Magary *et al.* [20] found that MMP2 was strongly expressed in 77% of patients with lymph node metastasis

and it was detected in only 25% of patients without nodal metastasis. There was a positive correlation between a high level expression of MMP2 and the clinical stage and status of lymph node metastasis [21]. Wang *et al.* [22] found significant differences in the expression of MMP2 among the different pathological degrees of cancer (I, II, III), but the age of the patient and the clinical stage of the cancer had no effect on the expression of MMP2.

Our results support the previously recorded results that present a strong correlation between the level of MMP2 expression and the presence of lymph node metastasis. We reported MMP2 overexpression in 83% of patients with pathologically metastatic lymph nodes and in only 41% of those without lymph node metastasis, suggesting the increased metastatic tendency of tumour cells to express high values of MMP2. Nevertheless, the percentage of patients without lymph node metastasis showing overexpression of MMP2 (41%) is not as low as expected; this could be attributed to the probability that the tumours in some of these patients without nodal metastasis are already passing through the nodal metastatic process but have not yet reached the stage of pathological detection. Similarly, Bogusiewicz *et al.* [23] concluded that MMP2 might play a role in the lymphatic spread of laryngeal carcinoma.

As previously reported, the incidence of nodal metastasis varies with the primary site and size of the laryngeal carcinoma, as in the case of supraglottic carcinoma that shows a higher incidence of nodal metastasis [8]. The results of the present study agree with such oncological findings by demonstrating a strong association between MMP2 expression and supraglottic carcinoma, suggesting that MMP2 expression might be one of the factors facilitating the nodal metastatic spread of tumours arising in a particular region of the larynx. In contrast, a drastic reduction in the expression of MMP2 was associated with increased occurrence of glottic carcinoma, which is classically known to have a lower rate of nodal metastasis.

One of the putative metastatic suppressor genes is nm23H1 that encodes nucleotide diphosphatase kinase [12]. The nm gene has been implicated as a suppressor gene involved in the control of the metastatic process of malignant cells. Reduced levels of the nm23 gene product have been found in tumour cells with high metastatic potential, such as rodent ulcers and human breast, colorectal and lung carcinoma. Reduced expression of the nm23 gene product was observed in patients with positive lymph nodes metastasis [24]. Takes *et al.* [9] demonstrated a profound reduction in nm23H1 levels in the metastatic tissues compared with their primary laryngeal tumours, and hence suggested that the nm23H1 gene was related inversely to the process of metastasis.

Other studies confirm the nature of nm23H1 as a suppressor gene, whose levels are reduced in tumour cells of high metastatic potential [25].

The results of our study agreed with those of all the aforementioned studies and showed a statistically significant reduction in the expression of nm23H1 ($P = 0.04$) in patients with enlarged lymph node metastasis. Moreover, the presence of lymph node metastasis was significantly correlated with the loss of nm23H1 expression and vice versa ($P < 0.05$).

As regards our results correlating nm23H1 expression and staging of the tumour, a statistically significant correlation was found between nm23H1 expression and the tumour stage ($P < 0.05$), to the extent that in the most advanced stages, the tumour lost the expression of nm23H1. In other words, downregulation of nm23H1 expression was associated with increased staging of the T stage and N stage, both of which were statistically significant ($P = 0.04$); this suggests that the loss of the tumour metastatic suppressor effect of the nm23H1 gene might be partially responsible for the aggressive biological behaviour of the primary laryngeal carcinoma and hence increasing its metastatic potential.

Although the expression of both MMP2 and nm23H1 was correlated to the aggressiveness of the malignancy, the statistical correlation of expression of MMP2 with that of nm23H1 was insignificant ($P > 0.05$). Collectively, increased expression of MMP2 was related to the occurrence of nodal metastasis of the tumour and also to the tumour site (within the larynx) that was characterized by frequent metastasis (supraglottic and transglottic). In contrast, nm23H1 expression was inversely related to the advancement of the tumour stage and the nodal metastatic state.

Conclusion

Many factors are responsible for the aggressive biological behaviour of laryngeal carcinoma. Increased production of ECM-degrading enzymes (MMP2) and reduction in the expression of the tumour suppressor gene nm23H1 may be among the factors that increase the metastatic tendency in laryngeal carcinoma.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

1 Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010; 11: 781–789.

2 Nelson AR, Fingleton B, Rothenberg ML, Matrisian LM. Matrix metalloproteinases: biologic activity and clinical implications. J Clin Oncol 2000; 18:1135–1149.

3 Fidler IJ, Poste G. The 'seed and soil' hypothesis revisited. Lancet Oncol 2008; 9:808.

4 Kusukawa J, Sasaguri Y, Shima I, Kameyama T, Morimatsu M. Expression of matrix metalloproteinase-2 related to lymph node metastasis of oral squamous cell carcinoma: a clinicopathologic study. Am J Clin Pathol 1993; 99:18–23.

5 Nabeshima K, Inoue T, Shimao Y, Sameshima T. Matrix metalloproteinases in tumor invasion: role for cell migration. Pathol Int 2002; 52: 255–264.

6 Klein G, Vellenga E, Fraaije MW, Kamps WA, De Bont ESJM. The possible role of matrix metalloproteinase (MMP)-2 and MMP-9 in cancer, e.g. acute leukemia. Crit Rev Oncol/Hematol 2004; 50:87–100.

7 Kondratiev S, Gnepp DR, Yakirevich E, Sabo E, Annino DJ, Rebeiz E, Laver NV. Expression and prognostic role of MMP2, MMP9, MMP13, and MMP14 matrix metalloproteinases in sinonasal and oral malignant melanomas. Hum Pathol 2008; 39:337–343.

8 Helliwell TR. Molecular markers of metastasis in squamous carcinomas. J Pathol 2001; 194:289–293.

9 Takes RP, Baatenburg De Jong RJ, Wijffels K, Schuuring E, Litvinov SV, Hermans J, Van Krieken JHJM. Expression of genetic markers in lymph node metastases compared with their primary tumours in head and neck cancer. J Pathol 2001; 194:298–302.

10 Steeg PS, Cohn KH, Leone A. Tumor metastasis and nm23: current concepts. Cancer Cells 1991; 3:257–262.

11 Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100:57–70.

12 Gunduz M, Ayhan A, Gullu I, Onerci M, Hosal AS, Gursel B, *et al*. nm23 Protein expression in larynx cancer and the relationship with metastasis. Eur J Cancer 1997; 33:2338–2341.

13 Takes RP, Baatenburg De Jong RJ, Schuuring E, Hermans J, Vis AA, Litvinov SV, Van Krieken JHJM. Markers for assessment of nodal metastasis in laryngeal carcinoma. Arch Otolaryngol Head Neck Surg 1997; 123:412–419.

14 Iizumi M, Liu W, Pai SK, Furuta E, Watabe K. Drug development against metastasis-related genes and their pathways: a rationale for cancer therapy. Biochim Biophys Acta 2008; 1786:87–104.

15 Groblewska M, Siewko M, Mroczko B, Szmitkowski M. The role of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in the development of esophageal cancer. Folia Histochem Cytobiol 2012; 50:12–19.

16 Peschos D, Damala C, Stefanou D, Tsanou E, Assimakopoulos D, Vougiouklakis T, *et al*. Expression of matrix metalloproteinase-9 (gelatinase B) in benign, premalignant and malignant laryngeal lesions. Histol Histopathol 2006; 21 (4–6): 603–608.

17 Yoshizaki T, Sato H, Maruyama Y, Murono S, Furukawa M, Park C-S, Seiki M. Increased expression of membrane type 1-matrix metalloproteinase in head and neck carcinoma. Cancer 1997; 79:139–144.

18 Répássy G, Forster-Horváth C, Juhász A, Ádány R, Tamássy A, Timár J. Expression of invasion markers CD44v6/v3, NM23 and MMP2 in laryngeal and hypopharyngeal carcinoma. Pathol Oncol Res 1998; 4:14–21.

19 Sariolu S, Özer E, Kirimca F, Sis B, Pabucuolu U. Matrix metalloproteinase-2 expression in laryngeal preneoplastic and neoplastic lesions. Pathol Res Pract 2001; 197:483–486.

20 Magary SP, Ryan MW, Tarnuzzer RW, Kornberg L. Expression of matrix metalloproteinases and tissue inhibitor of metalloproteinases in laryngeal and pharyngeal squamous cell carcinoma: a quantitative analysis. Otolaryngol Head Neck Surg 2000; 122:712–716.

21 Gou XX, Jin F, Chen HX, Wu WL, Chen L, Zeng Y. Expressions of CD147, MMP-2 and MMP-9 in laryngeal carcinoma and clinical significance. Zhonghua Yi Xue Za Zhi 2010; 90:1264–1267.

22 Wang X, Shan Y, Wang J, Luo S. Expression and clinical significance of matrix metalloproteinase-2 in laryngeal squamous cell carcinoma. J Clin Otorhinolaryngol 2006; 20:1109–1110.

23 Bogusiewicz M, Stryjecka-Zimmer M, Szymanski M, Rechberger T, Golabek W. Activity of matrix metalloproteinases-2 and -9 in advanced laryngeal cancer. Otolaryngol Head Neck Surg 2003; 128:132–136.

24 Song AU, Mais DD, Groo S, Wright JR, Yoshida GY. Expression of nm23 antimetastatic gene product in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg 2000; 122:96–99.

25 Steeg PS, Horak CE, Miller KD. Clinical-translational approaches to the Nm23-H1 metastasis suppressor. Clin Cancer Res 2008; 14:5006–5012.