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Main Article

Significance of fascin expression in laryngeal squamous cell carcinoma

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Abstract

Objective: Fascin is an actin-binding protein which is expressed in the basal areas of healthy squamous epithelium. Although overexpression of fascin has been shown in many tumours, the relationship between fascin and laryngeal squamous cell carcinoma has not previously been investigated, to the best of our knowledge. This study aimed to investigate the relationship between fascin expression and tumour behaviour in 30 cases of laryngeal squamous cell carcinoma.

Materials and methods: For all lesions, a section of paraffin-embedded tissue was immunohistochemically stained for fascin. The percentage of positive, stained cells was scored from one to five (one = 0–5 per cent, two = 6–25 per cent, three = 26–50 per cent, four = 51–75 per cent and five = 76–100 per cent), and the staining intensity from one to three (one = mild, two = moderate and three = strong). A total immunohistochemical fascin expression score was obtained by multiplying the staining percentage and intensity. The relationship between the total fascin score and each case's age, sex, tumour localisation, tumour–node–metastasis stage and differentiation was evaluated statistically.

Results: Various amounts of fascin expression were observed in all cases. There was a statistically significant relationship between high levels of fascin expression (i.e. a total fascin score of 10 or more) and the cases' tumour stage ($p = 0.022$), node stage ($p = 0.024$) and clinical stage ($p = 0.014$). In addition, worsening tumour differentiation was associated with an increasing fascin score, but this finding was statistically insignificant.

Conclusion: These results suggest that laryngeal squamous cell carcinomas with high levels of fascin expression may be more aggressive than those with low expression levels. Further studies with larger series are needed to support these results and to clarify rationales.

Key words: Larynx Neoplasms; Squamous Cell Carcinoma; Fascin

Introduction

Laryngeal squamous cell carcinoma (SCC) is the 11th most common form of cancer with a male predominance, and the second most common malignancy of the head and neck.[1] The major prognostic factors for laryngeal SCC are tumour–node–metastasis (TNM) stage, clinical stage, cartilage invasion, differentiation, perineural invasion and lymphatic invasion.[2] Studies of laryngeal SCC have investigated the effect of genetic and epigenetic modification, alteration in tumour environment, and immune evasion, in order to better understand the tumour's development and course.[1,3] Protein-based alterations in tumour cells may also contribute to carcinogenesis.[3]

Fascin is a 55-kDa, actin-binding protein. Actin bundle proteins are concentrated in cell membrane protrusions, and these protrusions provide motility for the cell. These actin bundle proteins are rearranged by fascin proteins.[4,5] During the neoplastic transformation of cells, the development of motility and migratory capacity is important for the tumour's invasive and metastatic potential. A relationship between fascin overexpression and an unfavourable prognosis has been demonstrated for many malignant tumours.[6–9] However, the relationship between fascin expression and laryngeal SCC aggressiveness has not previously been reported, to the best of our knowledge.

In this study, we aimed to investigate the relationship between fascin expression and age, sex, tumour localisation, TNM stage and tumour differentiation, in laryngeal SCC patients.

Materials and methods

The surgical specimens of 30 patients who had undergone surgical treatment for laryngeal SCC at the

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Gulhane Military Medical Academy between 1995 and 2007 were randomly retrieved for immunohistochemical study.

We chose a representative block from each tumour for immunohistochemical analysis. A 5-mm section from each formalin-fixed, paraffin-embedded tumour tissue block (which consisted almost entirely of tumour tissue) was automatically immunostained (Autostainer-480-LV1; Labvision, Fremont, California, USA) using a primary antibody against fascin (FCN01, 1:80, mouse monoclonal antibody; Neomarkers, Fremont, California, USA), employing the avidin-biotin peroxidase technique. The sections were incubated with the primary antibody at room temperature for 60 minutes.

All immunohistochemically stained slides for each case were reviewed by two pathologists, using a previously described fascin staining and scoring method, as follows.[10] Cytoplasmic staining was accepted as positive. The percentage of positively stained cells was assessed semiquantitatively and scored from one to five, as follows: one = 0–5 per cent, two = 6–25 per cent, three = 26–50 per cent, four = 51–75 per cent and five = 76–100 per cent. The intensity of immunohistochemical staining was also scored, as either one (=weak; Figure 1a), two (=moderate; Figure 1b) or three (=strong; Figure 1c). Overall immunohistochemical scores for fascin expression were calculated for each case by multiplying the scores for staining percentage and staining intensity; possible overall fascin scores ranged from one to 15. Finally, we categorised the overall fascin scores into two groups: eight and below, and more than eight.[10]

The overall immunohistochemical fascin score was compared with the age, sex, localisation, TNM stage and differentiation status of the cases. Statistical significance was assessed by the chi-square test. All analyses were performed using the Statistical Package for the Social Sciences version 11.0 software program (SPSS Inc, Chicago, Illinois, USA).

Results

Patients' ages ranged from 29 to 79 years. There were 28 men and two women.

Tumours were supraglottic in 11 cases, transglottic in 10, glottic in four, supraglottic with glottic extension in three and glottic with subglottic extension in two. Four of the cases were well differentiated, 21 moderately differentiated and five poorly differentiated. Nine cases had one to four metastatic lymph nodes. No lymph node metastasis was found in 21 cases.

Fascin expression of varying degrees was found in all tissue samples. The overall fascin score was 12 in nine cases, 10 in 10 cases, eight in six cases and six or less in five cases (Table I).

There was no significant relationship between the cases' age or sex and the overall fascin score (p = 0.571 and 0.126, respectively).

An overall fascin score of more than eight was found in five of the 11 supraglottic cases, nine of the 10 transglottic cases, two of the four glottic cases, two of the three supraglottic with glottic extension cases, and one of the two glottic with subglottic extension cases. Although most transglottic laryngeal SCC cases had high overall fascin scores, we found no statistically significant relation between cases' overall scores and their tumour localisation ($p = 0.282$).

The overall fascin score was more than eight in one of the four well differentiated cases, 14 of the 21 moderately differentiated cases, and four of the five poorly differentiated cases. There was no statistically significant relationship between tumour

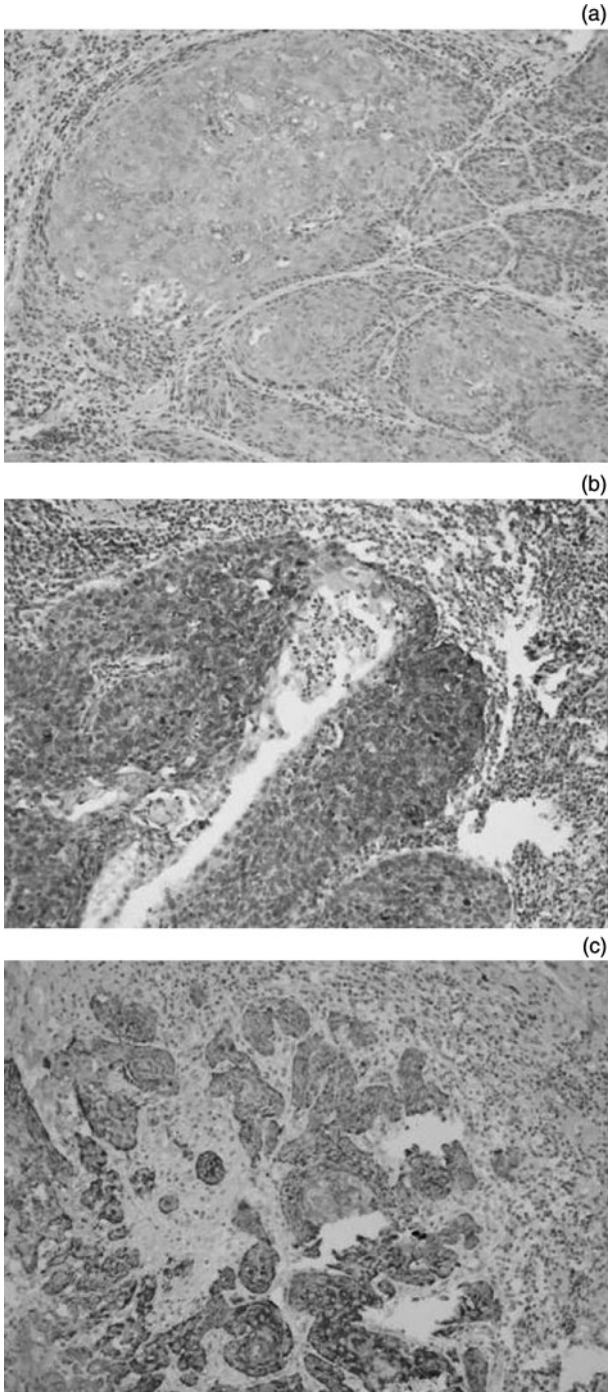

FIG. 1

Photomicrographs showing tissue sections with (a) weak, (b) moderate and (c) strong fascin immunohistochemical staining intencity (×200).

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TABLE I

CASES' CLINICOPATHOLOGICAL VARIABLES AND FASCIN EXPRESSION SCORES

Age (y)	Sex	T site	Stage				T differentiation	Score
			T	N	M	Clinical		
74	Ma	Transglottic	T_3	N_1	M_0	III	Moderate	10
73	Ma	Supraglottic	T_3	N_0	M_0	III	Moderate	2
62	Ma	Supraglottic-glottic	T_2	N_0	M_0	II	Moderate	6
29	Ma	Transglottic	T_3	N_0	M_0	III	Moderate	8
78	Ma	Supraglottic	T_1	N_0	M_0	I	Moderate	3
56	Ma	Transglottic	T_{4a}	N_0	M_0	IVA	Poor	10
61	Ma	Supraglottic	T_3	N_1	M_0	III	Moderate	12
77	Ma	Transglottic	T_{4a}	N_0	M_0	IVA	Moderate	12
65	Fe	Supraglottic	T_1	N_0	M_0	I	High	8
45	Ma	Transglottic	T_{4a}	N_0	M_0	IVA	Poor	12
50	Ma	Glottic	T_{1b}	N_0	M_0	I	Moderate	2
61	Ma	Supraglottic	T_3	N_{2c}	M_0	IVA	Moderate	12
62	Fe	Supraglottic	T_1	N_0	M_0	I	High	8
70	Ma	Transglottic	T_4	N_0	M_0	IVA	Moderate	10
79	Ma	Glottic	T_{1b}	N_0	M_0	I	Moderate	10
70	Ma	Transglottic	T_4	N_1	M_0	IVA	Moderate	12
59	Ma	Glottic-subglottic	T_3	N_0	M_0	III	Moderate	5
55	Ma	Supraglottic-glottic	T_{4a}	N_1	M_0	IVA	Moderate	12
68	Ma	Glottic-subglottic	T_3	N_0	M_0	III	Moderate	10
66	Ma	Supraglottic	T_2	N_0	M_0	II	Moderate	12
57	Ma	Glottic	T_1	N_0	M_0	I	Moderate	10
61	Ma	Transglottic	T_{4a}	N_1	M_0	IVA	Poor	10
54	Ma	Supraglottic	T_1	N_0	M_0	I	High	12
63	Ma	Supraglottic-glottic	T_{4a}	N_{2b}	M_0	IVA	Moderate	12
75	Ma	Transglottic	T_4	N_{2c}	M_0	IVA	Moderate	10
64	Ma	Glottic	T_1	N_0	M_0	I	High	8
65	Ma	Supraglottic	T_3	N_0	M_0	III	Moderate	8
74	Ma	Supraglottic	T_1	N_0	M_0	I	Poor	8
57	Ma	Transglottic	T_4	N_0	M_0	IVA	Moderate	10
74	Ma	Supraglottic	T_3	N_1	M_0	III	Poor	10

Y = years; T = tumour; N = node; M = metastasis; score = overall immunohistochemical fascin expression score; Ma = male; Fe = female

differentiation and overall fascin score ($p = 0.199$) (Table II).

Since none of the cases in our series had distant metastasis, we were not able to evaluate the significance of fascin expression for this parameter.

All T_4, N_1 to N_2, and stage IV cases had high overall fascin scores. Half of stage II and III cases had high overall fascin scores, whereas the remainder had low scores. Only 33 per cent of stage I cases had high overall fascin scores. Similar results were observed for T staging. Ten of the 21 N_0 cases had low overall fascin scores. There was a statistically significant relationship between cases' T stage ($p = 0.022$), N stage ($p = 0.024$) and clinical stages ($p = 0.014$) and their overall fascin score (Table II).

Discussion

Despite the availability of new surgical techniques and organ preservation protocols, laryngeal SCC is still a serious health problem, with low survival rates especially for patients with advanced cancers. Prevention and early diagnosis of laryngeal carcinoma is the most effective way of maximising cure rates and preserving function. In addition, a better understanding of the steps involved in malignant transformation may assist the development of novel diagnostic, prognostic and therapeutic strategies.

In this study, we investigated the relationship between fascin expression and clinicopathological prognostic parameters in laryngeal SCC. We demonstrated that fascin expression was greater in more aggressive tumours.

Fascin expression was first reported in dendritic cells, and then in Hodgkin lymphoma, glial, neuron and muscle cells.[4,5] It is only expressed in the basal layer of the epidermis within normal epithelium. The fascin protein may be related to tumour invasion and distant metastasis, due to its association with motility. It has been studied in cancers of the breast, oesophagus, stomach, lung, colon, pancreatic and biliary tract, ovary, and bladder, and an association with an unfavourable prognosis has been demonstrated.[11–18]

Tumoural protein expression is known to play a role in carcinogenesis, in addition to genetic and epigenetic factors. Various studies have addressed the relationship between laryngeal SCC prognosis and epidermal growth factor receptor, dysadherin, cyclooxygenase-2 (COX-2) and vascular endothelial growth factor C.[19–21]

Epidermal growth factor receptor is a transmembrane tyrosine kinase receptor. It plays an important role in cell survival and proliferation. Overexpression has been demonstrated in more than 90 per cent of head and neck SCC cases. Overexpression in early glottic carcinomas has been reported to be associated with an unfavourable prognosis.[19]

Dysadherin is another cancer-associated cell membrane glycoprotein which has recently been defined and characterised. Kyzas *et al.* have demonstrated a

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TABLE II

CASES' CLINICOPATHOLOGICAL VARIABLES BY OVERALL FASCIN EXPRESSION SCORE

Variable	Score (*n* (%))		Total (*n*)	*p*
	>8	≤8		
Age (y)				
≥60	13 (61.8)	8 (38.1)	21	0.571
<60	6 (66.7)	3 (33.3)	9	
Sex				
Ma	19 (67.9)	9 (32.1)	28	0.126
Fe	0	2 (100)	2	
T site				
Supraglottic	5 (45.5)	6 (54.5)	11	0.282
Transglottic	9 (90)	1 (10)	10	
Glottic	2 (50)	2 (50)	4	
Supraglottic-glottic	2 (66.7)	1 (33.3)	3	
Glottic-subglottic	1 (50)	1 (50)	2	
T stage				
T_1	3 (33.3)	6 (66.7)	9	0.022
T_2	1 (50)	1 (50)	2	
T_3	5 (55.6)	4 (44.4)	9	
T_4	10 (100)	0	10	
N stage				
N_0	10 (47.6)	11 (52.4)	21	0.024
N_1	6 (100)	0	6	
N_2	3 (100)	0	3	
M stage				
M_0	19 (63.3)	11 (36.7)	30	–
M_1	0	0	0	
Clinical stage				
I	3 (33.3)	6 (66.7)	9	0.014
II	1 (50)	1 (50)	2	
III	4 (50)	4 (50)	8	
IV	11 (100)	0	11	
T differentiation				
High	1 (25)	3 (75)	4	0.199
Moderate	14 (66.7)	7 (33.3)	21	
Poor	4 (80)	1 (20)	5	

Score = overall immunohistochemical fascin expression score; y = years; Ma = male; Fe = female; T = tumour; N = node; M = metastasis

relationship between high expression of dysadherin in head and neck region SCC and higher clinical stage and presence of lymph node metastasis at the time of diagnosis.[20]

Similarly, it has been shown that high expression levels of COX-2 and vascular endothelial growth factor C are correlated with the presence of lymph node metastasis at the time of diagnosis.[21]

There are no previous studies investigating the relationship between fascin expression and laryngeal SCC aggressiveness. However, fascin has been studied in oral cancers and SCC of the oesophagus.

In 2006, Zhang *et al.* studied a total of 102 sections, comprising tissue from normal regions of cancerous oesophageal tissue, dysplastic cases and SCC cases.[12] They also evaluated 49 fresh cases of oesophageal SCC. They found that high fascin expression levels were associated with high levels of cellular proliferation and lymph node metastasis.

In 2007, Chen *et al.* studied 129 oral SCC cases, and found more fascin expression in tumour tissue than in normal tissue.[22] They also found an association between fascin overexpression and tumour diameter, lymph node metastasis, distant metastasis and clinical stage.

Similarly, we found a high degree of fascin expression to be associated with tumour stage and lymph node metastasis, in laryngeal SCC cases. The overall immunohistochemical fascin score was usually high in cases of advanced stage tumour; however, it is not clear which causes which. Despite this, in both of these situations, knowing a tumour's fascin expression level may assist tumour management, for the following reasons. The fascin protein has been shown to play a role in the carcinogenesis of many cancer types. It does this by forming actin-based structures, changing cellular motility and causing migratory changes.[23] According to recent reports, tumours which overexpress fascin generally have markedly more lymph node metastases than tumours with low fascin expression. Lymph node metastasis is an important negative prognostic indicator for laryngeal SCC.[2] We have found a statistically significant relationship between high fascin expression levels and lymph node metastasis.

- **Fascin is an actin-binding protein which is expressed in the basal areas of healthy squamous epithelium**
- **This study aimed to investigate the relationship between fascin expression and tumour behaviour in 30 laryngeal squamous cell carcinoma (SCC) cases**
- **Results suggest that laryngeal SCC tumours with high levels of fascin expression may be more aggressive than those with low levels**

We did not find a statistically significant relationship between tumour differentiation and overall fascin score. However, the percentage of cases with a high score tended to increase as cases become less differentiated (Table II). The reason for the statistical insignificance of this association may be the small number of patients in our series.

Conclusion

We found an association between a high fascin expression score and the T stage, N stage and clinical stage of our laryngeal SCC cases. This may indicate that tumours with high levels of fascin expression behave more aggressively. In light of recent studies, fascin could be considered as a candidate molecular target for cancer therapy. However, studies employing larger series and evaluating survival versus fascin expression are needed, in order to fully understand the significance of fascin expression for patients with laryngeal SCC.

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