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Immunohistochemical Distribution of Laminin and Fibronectin in Oral Squamous Cell Carcinoma : Relationship to Lymph Node Metastasis

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ABSTRACT. This study examined the immunohistochemical distribution of extracellular matrix components, laminin for basement membrane and fibronectin for stroma, at the invasive border of 56 oral squamous cell carcinomas and their respective nodal metastases and the relationship between those distribution and regional lymph node metastasis. Laminin expression significantly correlated with low metastatic potential and well-differentiated tumors, but not with the mode of cancer invasion. Stromal fibronectin expressin with a different intensity at the invasive front was observed in the majority (90%) of the 52 cases. The tumors with diffuse strong expression of stromal fibronectin were significantly related to high metastatic potential, moderate or poor differentiation of the tumor and loss or disruption of laminin. Lymph node metastases showed more frequently laminin expression than the corresponding primary tumor and showed fibronectin in all cases examined. Laminin and fibronectin were not expressed at the interface between metastases and parenchymal lymphoid tissue, but were expressed at interface between metastases and their stroma. These results suggest that alterations in extracellular matrix components reflect the interaction between tumor cells and the extracellular matrix and are related to the clinical behavior of the tumor.

Key words : laminin — fibronectin — extracellular matrix — lymph node metastasis — oral squamous cell carcinoma

The process of cancer invasion and metastasis consists of a complex series of sequential steps, involving specific tumor cells and host properties. It is believed that the interaction between tumor cells and the basement membrane (BM) is a critical step in the initiation of tumor invasion and subsequent metastasis.[1] It has been clarified that in human carcinoma the alterations of the extracellular matrix components are associated with tumor invasion and metastasis. Several studies in human carcinomas have shown that the tumor BM is lost in invasive and/or metastizing tumors and that it acts as a barrier to be breached against tumor invasion.[2,3] These findings are explained by the three-step hypothesis, cell attachment, degradation of the matrix and tumor cell locomotion, of cancer invasion of the extracellular matrix.[4] There is also increasing evidence that the loss or disruption of the BM results from localized degradation of the exracellular matrix by proteolytic enzymes such as the plasminogen activator/plasmin system and matrix metalloproteinases which are produced by stromal cells or tumor cells.[5] However, it has also been proposed

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that many invading carcinomas retain their BM in both primary and secondary sites and that the tumor BM does not always serve as a barrier.[6–8]

Moreover, the ability of tumor cells to migrate through a stromal matrix that is rich in fibronectin (FN) and related glycoproteins may also be a critical parameter in tumor invasion.

The BM is an extracellular matrix that separates epithelial cells from the underlying connective tissue stroma and consists of three major intrinsic components, type IV collagen, laminin, and heparan sulfate proteoglycan. Laminin is the major glycoprotein confined to the BM, and it mediates the attachment of endothelial and epithelial cells to type IV collagen of the BM by binding to cell surface integrins.[9] Tissue FN is found in the connective tissue in close apposition to the BMs and is considered as a high molecular matrix glycoprotein. It is also regarded as an important mediator of tissue modeling and organization by influencing growth as well as adhesion, differentiation, and motility of cells.[10,11]

Metastasis to neck lymph nodes is an important prognostic factor in squamous cell carcinoma of head and neck.[12] Many clinicopathological investigations have focused on prediction of the metastatic aggressiveness of the individual tumor, and on the prevention and treatment of occult micrometastasis to the lymph nodes,[13,14] and have revealed risk factors related to lymph node metastasis and survival.[15,16] However, little is known of the effect of extracellular matrix on lymph node metastasis of oral squamouse cell carcinoma. The purpose of this study was to clarify the correlation of distribution of laminin for BM and FN for stroma in primary tumor tissues of oral squamouse cell carcinoma with regional lymph node metastasis by means of immunohistochemical staining.

MATERIALS AND METHODS

Patients

Fifty-six patients with squamous cell carcinoma of the oral cavity who were biopsied from previously untreated lesions in the Department of Oral Surgery, Kawasaki Medical School Hospital, were included in this study. Of these 56 patients, 30 had neck lymph node metastasis that could be confirmed histologically by neck dissection. The remaining 26 patients had no evidence of lymph node metastasis histologically. Twenty-one metastatic cervical lymph nodes obtained from neck dessection were also examined. In nearly all cases, preoperative radiotherapy and/or chemotherapy were applied and thereafter neck dissection was performed. The cases without histological cervical lymph node metastasis (pN0) were further followed for at least two years after treatment to confirm that no distant metastasis had occurred.

Tissue preparation

The specimens were fixed in 10% buffered formalin and embedded in paraffin. The serial sections were cut at 4 μm, deparaffinized, and stained with hematoxylin and eosin for histological diagnosis. Immunodetection of laminin and FN was performed on paraffin sections using the avidin-biotin immunoperoxidase technique. The paraffin sections were dewaxed and dehydrated in grades of ethanol and then washed with phosphate buffered

saline(PBS). Proteolytic treatments with 0.4% pepsin at 37°C for 30 minutes or 0.1% trypsin at 37°C for 10 minutes were needed prior to the immunohistochemical procedures. Slides were then immersed in 10% H_2O_2 in absolute methanol for 30 minutes at room temperature to block endogenous peroxidase activity. The tissue samples were exposed for 30 minutes to 2% normal horse serum. Then the serum was drained and the samples were immediately incubated with 1 : 100 laminin polyclonal antibody (Bio-Science Products AS, Switzerland) in Tris-buffered saline, pH 7.6. The samples for FN staining were incubated with 1 : 150 FN polyclonal antibody (Lipshaw, Michigan) in Tris-buffered saline. After incubation for 45 minutes in a humidity chamber at room temperature, the slides were washed three times with PBS, then incubated for 30 minutes with biotinylated goat anti-rabbit IgG (diluted 1 : 200 in Tris-buffered saline), washed three times in PBS, and finally incubated for 30 minutes with the avidin-biotin-peroxidase complex (Vectastain kit, Vector Laboratories, Burlingame, CA). The peroxidase activity was revealed by incubating the slides in 0.5 mg per ml 3-3′ diaminobenzidine tetra-hydrochloride (Sigma Chemical Co., St. Louis, MO) in 50 millimol per liter Tris-buffer and by the addition of 2 μl of 2% H_2O_2 per milliliter. The sections were then counterstained with Myer's hematoxylin, cleared, and mounted. As negative controls, normal serum of the same species as the primary antibodies was used in place of the primary antibodies.

Evaluation of staining results

Evaluation of the staining pattern for laminin and FN was performed without any knowledge of the clinical data for each patient. The staining pattern for laminin was evaluated in areas of the invasive border of the cancer, which were represented in at least one section from each tumor, and the intensity of laminin staining around the cancer nest was compared with that for the BM of normal squamous epithelium or the subendothelium of blood vessels. The type of laminin expression of the tumors was classified according to the proportion of laminin positive cancer nests as follows : positive, cancer nests are surrounded by a continuous laminin positive line which is present in $\geq$90% of the section ; negative, cancer nests showing a disrupted linear pattern (+/−) and negative staining for laminin.

Evaluation of stromal FN staining along the invasive border was compared with that for the subendothelium of the normal blood vessels and defined[17] as : positive, if the invasive fronts of cancer nests were surrounded by a constant FN-positive, diffuse or basement membrane-like (BM-like) staining pattern, and negative, if this pattern was interrupted or lacking. The diffuse staining pattern was further classified according to the staining intensity with the amount of FN-positive strands : ++, many (strong pattern) ; +, few (fine pattern). The BM-like staining pattern was defined as continuous, thin FN-positive lines surrounding the tumor nests without diffuse staining of the stroma.

Clinical parameters

The clinicopathological stage of the tumors was classified according to the TNM classification system of the International Union against Cancer,[18] and their histological type was evaluated based on the World Health Organization classification.[19] Their histological degree of malignancy was also graded

according to the criteria of Jakobsson.[20]

The data were analyzed for statistical significance by the Chi-square test and Fischer's exact test.

RESULTS

Normal squamous epithelium exhibited a prominent and continuous linear patten corresponding to the BM that was readily identified in all the sections with antibodies for laminin. The blood vessels showed a consistent laminin positive staining reaction, which was located subendothelially corresponding to the BM area. The same positive pattern for laminin was also observed around skeletal muscle bundles and nerve fibers. In contrast to these normal tissues, various staining patterns were observed in cancer tissues (Fig 1a-d). Of the 56 primary tumors examined, 25 tumors (45%) were classified as laminin-positive and 31 tumors (55%) as laminin-negative. The relationship between laminin

TABLE 1. Relationship between expression of laminin and clinicopathological parameters of oral squamouse cell carcinoma

	No.	Laminin positive No. (%)	Laminin negative No.			P
			(+/−)	(−)	Subtotal(%)	
Total	56	25(45)	14	17	31(55)	
T stage						
1	14	7(50)	3	4	7(50)	
2	29	13(45)	7	9	16(55)	
3	9	3(33)	3	3	6(67)	
4	4	2(50)	1	1	2(50)	
pN stage						
0	26	18(69)	2	6	8(31)	<0.01
1~3	30	7(23)	12	11	23(77)	
Tumor grade						
G1	28	15(54)	3	10	13(46)	
G2	20	9(45)	8	3	11(55)	<0.05
G3	8	1(12)	3	4	7(88)	
Mode of invasion						
M1	6	4(67)	1	1	2(33)	
M2	11	4(36)	4	3	7(64)	
M3	27	12(44)	5	10	15(56)	
M4	12	5(42)	4	3	7(58)	
Cellular response						
Marked	20	12(60)	6	2	8(40)	
Moderate	19	5(26)	5	9	14(74)	
Slight or None	17	8(47)	3	6	9(53)	

T, pN stage: UICC classification (1987), Tumor grade: WHO grading
Mode of invasion: M1; well defined border, M2; cords, less marked borderline, M3; groups of cells, no distinct borderline, M4; diffuse growth

expression and the clinicopathological parameters is shown in Table 1. No relationship between tumor size (T stage) and laminin expression was observed. Concerning the regional lymph node metastasis (pN stage), 18 (69%) of 26 primary tumors without lymph node metastasis were evaluated as laminin-positive, whereas 23 (77%) of 30 primary tumors with lymph node metastasis were evaluated as laminin-negative. There was a strong relationship between regional lymph node metastasis and laminin expression in primary tumor tissues ($P<0.01$). The frequency of laminin positive staining in well differentiated tumors (15 of 28 tumors, 54%) was significantly higher than that in poorly differentiated tumors (one of eight tumors, 12%) ($P<0.05$). There was no significant relationship between the laminin staining pattern and the mode of cancer invasion, or the mononuclear cell infiltration in the tumor stroma.

Although the incidence of lymph node metastasis tended to increase with a high grade of the T stage, tumor differentiation and the mode of cancer invasion, there were no statistically significant differences between the lymph node metastasis and these clinicopathological parameters. The relationship between laminin expression in the primary tumor and the metastatic potential

TABLE 2. Incidence of lymph node metastasis related to laminin immunostaining, clinical stage and pathological grades in patients with oral squamous cell carcinoma

		Laminin staining		
	Meta/Total(%)	Positive(%)	Negative(%)	P
Total	30/56(54)	7/25(28)	23/31(74)	<0.01
T stage				
1	6/14(43)	0/7 (0)	6/7 (86)	<0.01
2	14/29(48)	3/13(23)	11/16(69)	<0.05
3	7/9 (78)	2/3 (67)	5/6 (83)	
4	3/4 (75)	2/2(100)	1/2 (50)	
Tumor grade				
G1	15/28(54)	7/15(47)	8/13(62)	
G2	9/20(45)	0/9 (0)	9/11(82)	<0.01
G3	6/8 (75)	0/1 (0)	6/7 (86)	
Mode of invasion				
M1	1/6 (17)	0/4 (0)	1/2 (50)	
M2	6/11(55)	0/4 (0)	6/7 (86)	<0.05
M3	15/27(56)	5/12(42)	10/15(67)	
M4	8/12(67)	2/5 (40)	6/7 (86)	
Cellular response				
Marked	11/20(55)	4/12(33)	7/8 (88)	<0.05
Moderate	12/19(63)	2/5 (40)	10/14(71)	
Slight or None	7/17(41)	1/8 (13)	6/9 (67)	<0.05

T: UICC classification (1987), Tumor grade: WHO grading
Mode of invasion: M1; well defined border, M2; cords, less marked borderline, M3; groups of cells, no destinct borderline, M4; diffuse grouth

to regional lymph nodes was examined according to the respective grade of the clinicopathological parameters (Table 2). In T1 and T2 stage tumors, the frequency of lymph node metastatsis of laminin negative tumors was significantly higher than that of laminin positive tumors ($P<0.01$ and $P<0.05$ respectively). Also, in the grade 2 (moderately differentiated) tumors, there was no lymph node metastasis in the tumor with laminin positive staining, whereas 9 of 11 tumors (82%) with laminin negative staining developed lymph node metastasis. There was a significant correlation ($P<0.01$) between the laminin staning pattern and lymph node metastasis. A similar significant relationship between the laminin staning pattern and lymph node metastasis was found in tumors showing a mode of invasion such as cords or a less marked borderline (M2), tumors with marked mononuclear cell infiltration and tumors with a slight or scanty cellular response. These variables showed that the laminin expression of primary tumors was strongly related to reduced lymph node metastasis.

Laminin expression in the metastatic lymph nodes of 21 patients was investigated and compared with that of corresponding primary tumors

TABLE 3. Relationship between laminin expression in metastatic lymph nodes and that in the primary tumor

Primary tumor		No.	Metastatic lymph node: Laminin Positive	Metastatic lymph node: Laminin Negative
Laminin	Positive	6	5	1
	Negative	15	7	8

(Table 3). Laminin was expressed at the border between the metastatic tumor and its stroma, but was not expressed at the interface between the metastatic tumor nest and the lymphoid parenchyma (Fig 2a-d). In 13 of the 21 metastatic lesions, laminin was expressed in the same pattern as the corresponding primary lesions. However, in 7 patients whose primary lesions were laminin negative, the metastatic lymph nodes were evaluated as laminin-positive. The tumor growth pattern in metastatic lymph nodes (Table 4), 12 of 15 metastatic tumors with well-defined growth (80%) were evaluated as laminin-positive, whereas the 6 remaining tumors with cord-like or diffuse growths were evaluated as laminin-negative (Fig 2c, d). There was a strong relationship between the tumor growth pattern in metastatic lymph nodes and the laminin

TABLE 4. Correlation of metastatic tumor growth pattern and laminin expression in metastatic lymph nodes

Laminin expression	No.	Growth pattern of metastatic tumor: Well-defined	Cord like	Diffuse
Positive	12	12(100)	0 (0)	0 (0)
Negative	9	3 (33)	4(44)	2(22)

($P<0.01$)

expression ($P<0.01$).

FN positive staining was observed in the subendothelial area of blood vessels and smooth muscle fibers in non-neoplastic connective tissue. The stromal FN staining reaction along the invasive border of the primary tumor was evaluated in 52 oral squamous cell carcinomas. Forty-seven out of 52 tumors (90%) expressed FN positive staining that was observed as a diffuse pattern in 40 tumors (diffuse++, 20 tumors and diffuse+, 20 tumors) (Fig 3a, b) and as a BM-like pattern (Fig 3c) in 7 tumors. The other five tumors were defined as FN negative (Fig 3d). The relationship between the FN staining pattern and the clinicopathological parameters was evaluated (Table 5). The T stage of the tumor was not significantly related to the FN staining pattern.

TABLE 5. Stromal fibronectin staining pattern at invasive border of 52 primary oral squamous cell carcinoma related to clinicopathological parameters

	No.	FN positive			P	FN negative
		Diffuse ++	Diffuse +	BM-like		
Total	52	20	20	7		5
T stage						
1	13	5	6	1		1
2	27	9	9	6		3
3	8	4	3	0		1
4	4	2	2	0		0
pN stage						
0	25	5	15	5	<0.01	0
1~3	27	15	5	2		5
Tumor grade						
G1	24	5	14	3	<0.05	2
G2	20	11	6	2		1
G3	8	4	0	2		2
Mode of invasion						
M1	6	0	2	3	<0.05	1
M2	11	5	5	1		0
M3	23	9	10	1		3
M4	12	6	3	2		1
Cellular response						
Marked or Moderate	35	13	11	6		5
Slight or None	17	7	9	1		0
Laminin staining						
Positive	23	4	14	5	<0.01	0
Negative	29	16	6	2		5

T, pN stage: UICC classification (1987), Tumor grade: WHO grading
Mode of invasion: M1; well defined border, M2; cords, less marked borderline, M3; groups of cells, no destinct borderline, M4; diffuse growth
The Dotted box shows a significant relationship between FN positive intensity and the pathological parameters.

Twenty-two out of 47 FN positive tumors (47%) developed cervical lymph node metastases (pN1～3) and all five of the FN negative tumors had lymph node involvement (pN1～3). This difference was not statistically significant. However, in the 47 FN positive tumors, there were significant relationships between the intensity of FN positive staining and the pN stage, tumor grade and mode of invasion. The tumors with FN-diffuse+ staining and a BM-like staining pattern showed a lower frequency of lymph node metastsis and higher differentiation than those of the tumors with FN-diffuse++ staining. The tumors with a well-defined mode of invasion (M1) more frequently showed a BM-like FN staining pattern than tumors with other modes of invasion. The 52 tumors stained for FN were also available for evaluation of their laminin staining pattern on serial sections from the same primary tumor tissue. Twenty-three out of 47 FN positive tumors (49%) were also laminin positive,

TABLE 6. Frequency of lymph node metastasis related to fibronectin staining pattern and clinicopathological parameters

Parameter	Meta/Total(%)	FN Staining (%)			P
		Diffuse++	Deffuse+ BM-like	Negative	
Total	27/52(52)	*15/20(75)	7/27(26)	*5/5(100)	<0.01
T stage					
1	5/13(38)	3/5 (60)	1/7 (14)	1/1(100)	N.S
2	13/27(48)	*7/9 (78)	3/15(20)	*3/3(100)	<0.05
3	6/8 (75)	3/4 (75)	2/3 (67)	1/1(100)	N.S
4	3/4 (75)	2/2(100)	1/2 (50)	0	N.S
Tumor grade					
G1	12/24(50)	*5/5(100)	5/17(29)	2/2(100)	<0.01
G2	9/20(45)	7/11(64)	1/8 (13)	1/1(100)	N.S
G3	6/8 (75)	3/4 (75)	1/2 (50)	2/2(100)	N.S
Mode of invasion					
M1	1/6 (17)	0	0/5 (0)	1/1(100)	N.S
M2	6/11(55)	*5/5(100)	1/6 (17)	0	<0.05
M3	12/23(52)	5/9 (56)	4/11(36)	3/3(100)	N.S
M4	8/12(67)	5/6 (83)	2/5 (40)	1/1(100)	N.S
Cellular response					
Marked or Moderate	20/35(57)	*10/13(77)	5/17(29)	**5/5(100)	**<0.01 *<0.05
Slight or None	7/17(41)	5/7 (71)	2/10(20)	0	N.S
Laminin staining					
Positive	6/23(26)	2/4 (50)	4/19(21)	0	N.S
Negative	21/29(72)	13/16(81)	3/8 (38)	5/5(100)	N.S

T, pN stage: UICC classification (1987), Tumor grade: WHO grading
Mode of invasion: M1; Well defined border, M2; cords, less marked borderline, M3; groups of cells, no distinct borderline, M4; diffuse growth
**, *: Significant difference of metastatic frequency between the FN+ or BM-like staining pattern and the other FN staining pattern, N.S: no significance

and all five tumors with FN-negative staining were laminin negative. This difference was not significant ($P=0.052$). However, the tumors with a FN-diffuse++ staining pattern were more frequently laminin negative (Fig 4a, b) than tumors with FN-diffuse+ staining or ones with a BM-like pattern (16/20, 8/27 respectively). This difference was statistically significant ($p<0.01$).

The frequency of lymph node metastasis of the tumors with FN diffuse+ or BM-like staining was significantly less than that of tumors with FN diffuse++ or FN negative staining ($p<0.01$ respectively) (Table 6). These significant differences were indicated in the tumors with the T2 stage, G1, M2 and a marked or moderate cellular response. The clinicopathological parameters including T stage, tumor grade, mode of invasion and cellular response, except for laminin reactivety, were not significantly related to lymph node metastasis. Though the frequency of lymph node metastasis of the tumors with laminin positive staining was significantly less than that of the tumors with laminin negative staining, no significant differences were found between the FN staining pattern and lymph node metastasis in laminin positive or negative tumors.

All of the metastatic lymph nodes from 19 patients were FN positive, and there was no correlation in FN expression between the primary tumor and metastatic lymph nodes. FN in the metastatic lymph nodes was expressed at the interface between the tumor cells and the stroma, but was absent at the interface with lymphoid parenchyma (Fig 5a, b). In addition, in the metastatic lymph nodes, FN was consistently observed in the stroma irrespective of concomitant deposits of laminin (Fig 6a, b)

DISCUSSION

In the present study, the relationship between the distribution of laminin along the invasive border of primary squamous cell carcinoma of the oral cavity and the frequency of regional lymph node metastasis was clarified by an immunohistochemical study with statistical analysis. The laminin negative (loss or disruption) staining pattern at the invasive front of the primary tumor was a risk factor for regional lymph node metastasis, whereas the primary tumors with continuous linear staining for laminin were less metastatic to regional lymph nodes. A similar relationship between the expression of BM components and cervical lymph node metastasis has been reported in a few investigations of oral squamous cell carcinoma.[21-23] The experimental model of lymphatic metastasis has demonstrated that tumor cells can directly invade into the lymphatic vessels around the tumor nest because lymphatic capillaries possess no BM around endothelial cells.[24,25] It would seem plausible that foci of invasive squamous cell carcinoma without BM are more likely to attain access to lymphatics and penetrate the lymphatics. Consequently, the results of this study suggest that continuous BMs around the squamous cell carcinoma may act as a barrier against lymphatic metastasis, and that interaction between tumor cells and the BM is a critical step in the initiation of lymph node metastasis. On the other hand, it has been proposed in gastric cancers with liver metastasis that, rather than serving as barriers to metastasis, BMs can mediate the interaction of tumor cells with vessels and play an active role in hematogenous metastasis.[8,26,27] It would appear that the role of the tumor BM differs with the mode of metastasis.

Although the laminin negative staining at the invasive front correlated with the lymph node metastasis, there was no significant relationship with tumor invasiveness, which was assessed by the mode of invasion. Furthermore, this study found that the laminin expression of lymph node metastases tends to be more frequent than that in corresponding primary tumors though preoperative radiotherapy and/or chemotherapy may affect lymph node metastases. These results indicate that invasive carcinomas do not always show loss or disruption of BM. Whether loss of BM components indicates tumor invasiveness is controversial. It has been proposed that loss of BM components is associated with invasive activity. Evidence of this association comes from many studies in which BM-staining patterns of various benign and malignant tumors have been compared.[2,28,29] In addition, tumors with widespread loss of the BM have been found to be significantly associated with a mode of diffuse invasion for oral squamous cell carcinoma.[21,22] However, the BM breaks do not invariably indicate active invasion, since they are present in dysplasia and in situ carcinoma of the head and neck,[30] skin[31] and cervix.[32]

Although the present study could not differentiate whether the loss or disruption of BM components is due to a lack of production by tumor cells or to increased degradation of BM components by specific enzymes, there is increasing evidence that the BM breaks are due to increased breakdown by tumor cell derived proteases, such as plasminogen activator/plasmin system and matrix metalloproteinases.[5,33] Also, the possibility that adjacent inflammatory cells release proteolytic enzymes degrading BM components must be considered.[34] In this study, there was no evidence that inflammatory infiltrates affected the distribution of the staining pattern of BM laminin. It has also been proposed that BM discontinuities in xenografted human carcinomas occur independently of invasion processes and are caused by qualitative imbalances of the composition of the synthesized BM material.[35] In addition, there is evidence that epithelial BM production depends on cooperative interaction between neoplastic epithelial cells and stromal cells[36] and is also influenced by some matrix proteins such as type-I collagen.[8] If stromal cells are required for BM production, this may explain the marked BM laminin deficiency observed at the interface between metastases and parenchymal lymphoid tissue in this study because parenchymal cells do not have the same functions as stromal fibroblasts. Similar findings have been reported for lymph node metastases from a variety of carcinomas.[37] These findings are thought to suggest that host tissues may influence invasive activity through their effects on epithelial BM continuity.[37]

On the other hand, it has also been proposed that laminin expression in invasive carcinoma indicates the preservation of the normal function of differentiation in tumor cell[6,7] and that tumors demonstrating laminin around the nest are no longer actively invading through the adjacent stroma.[30] In this study, in agreement with other reports,[30,38,39] laminin expression was related to the degree of tumor defferentiation. Further studies are required to clarify the mechanism inducing the alteration of BM components and its significance in tumor invasion and metastasis.

The present study revealed that most oral squamous cell carcinomas display a positive stromal FN staining pattern in their peripheral invasive border. In addition, the FN positive staining patterns at this location could be

divided into three patterns accoding to the intensity of staining. The FN-diffuse++ (strongly positive) tumors and FN negative tumors significantly correlated with high metastatic potential to regional lymph nodes. These findings contradict, in part, those indicating that FN-positive staining reaction at the infiltrating border of invasive breast carcinoma is significantly associated with a low metastatic potential.[17]

Although FN is known to suppress the invasive and metastatic potentials of melanoma cells in vitro by its attachment function,[40] the majority of human solid tumors express stromal FN, and there is no significant relationship between the expression of FN and low metastatic potential.[8,38,41] However, Harada *et al*[21] reported that in oral squamous cell carcinomas, the expression of FN significantly increased in the primary tumors of metastatic cases. The results of the present study indicated that the difference in metastatic potential between tumors with FN-diffuse++ staining and ones with FN-diffuse+ staining or a BM-like staining pattern (75%, 25%, 29% respectively) may depend on a mutual interrelationship between FN and laminin reactivities. The 19 out of 27 tumors (70%) showing stromal FN+ staining or BM-like staining coexpressed laminin positive staining and displayed low metastatic potential, whereas the 16 out of 20 tumors (80%) showing stromal FN-diffuse++ staining did not stain for laminin, and had a high metastatic rate. These results suggest that the metastatic potential of a tumor with stromal FN positive staining is associated with the laminin reactivity of the tumor rather than stromal FN expression.

Regarding the mechanism of FN expression of the tumor stroma, there have been some proposals. The findings of FN+ or BM-like staining in laminin positive tumor may reflect preexisting FN in the host connective tissue,[8] whereas the strong stromal FN staining pattern in laminin negative tumors, as with similar observations reported in various types of carcinoma,[29,38] may reflect the FN produced and secreted by the interaction between the tumor cell and its stroma or stromal cells such as fibroblasts or myofibroblasts. Terranova *et al*[42] indicate that fibroblasts grow best in the presence of antibody to laminin in cocultures of fibroblasts and epithelial cells. Kao *et al*[43] showed in vitro that the matrix of human breast carcinoma cells was mitogenic for fibroblasts, and they considered the fibrotic reaction of the stroma as a host response to the invasive tumor. On the other hand, some authors[44,45] have indicated that in vitro squamous cell lines synthesize and secrete larger amounts of FN into culture medium than colon carcinoma cell lines. Christensen *et al*[46] reported that cytoplasmic FN in human breast carcinoma is found if specimens were fixed in ethanol-glacial-acetic acid (1%). In the present immunohistochemical study using formalin-fixed material, cytoplasmic FN was not observed in any tumor cells.

These results suggest that the distribution of stromal FN reflects the interaction between tumor cells and the stromal matrix.

The primary conclusions of this study may be summarized as follows:

1. The expression of the BM antigen, laminin, of the invasive border is related to a low metastatic potential to the cervical lymph nodes and differentiation of the tumor, rather than to the mode of tumor invasion;

2. FN expression is observed in the stroma of the invasive front of the majority of carcinomas and the intensity of staining is related to the laminin reactivity of the tumor and to its metastatic potential;

3. Cervical lymph node metastases more frequently show BM laminin expression than corresponding primary tumors and all cases in this study showed FN-strong expression at the interface between the metastatic tumor and its stroma ;

4. Alterations in the extracellular matrix components, laminin for BM and FN for stroma, in the primary tumor and the lymph node metastasis indicate the interaction between tumor cells and the extracellular matrix and are related to the clinical behavior of the tumor.

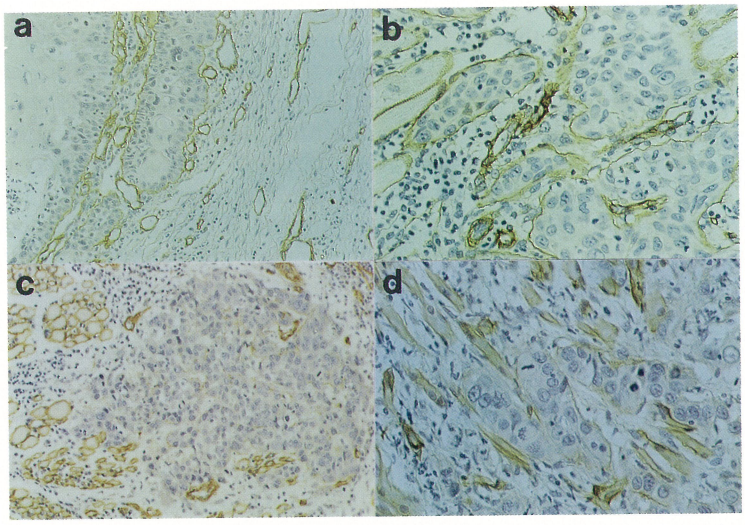

Fig 1. The laminn immunostaining pattern of primary oral squamous cell carcinoma at the invasive border (Immunoperoxidase-avidin-biotin-complex staining for laminin, counterstained with hematoxylin)
a : Continuous and linear staining for laminin is shown corresponding to the basement membrane of well-defferentiated squamous cell carcinoma and subendothelia of blood vessels. (×100) b : The invasive cancer nests are surrounded by continuous line for basement membrane laminin. (×200) c : Laminin positive reactivities are lost around the invasive tumor nest, but blood vessels and muscle bundles are clearly stained with laminin antibody. (×100) d : The diffusely invasive squamous cell carcinomas to musle layer lack laminin positive reactivities. (×200)

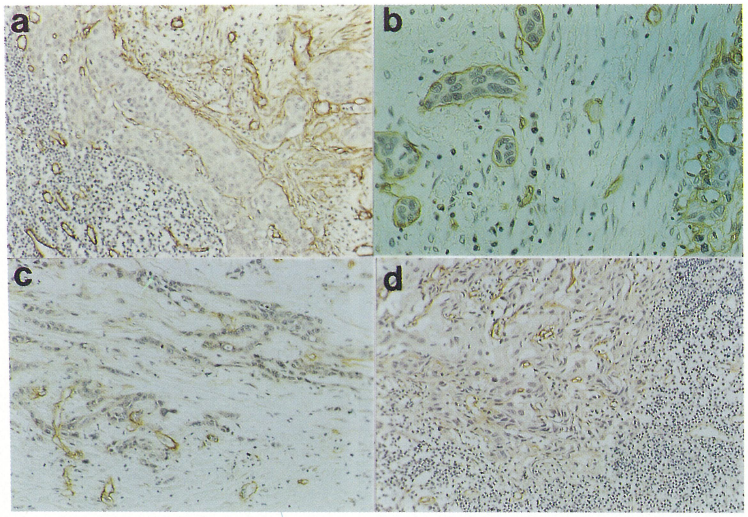

Fig 2. The laminin staining pattern of metastatic lymph node (Immunoperoxidase-avidin-biotin-complex staining for laminin, counterstained with hematoxylin)
a : Laminin positive staining is seen at the interface between metastatic tumor cells and stroma, but is absent at the interface with lymphoid parenchyma (×100). b : The metastatic tumor cells invading to the capsule of the lymph node are surrounded by contimuous line for laminin antibody (×200). c : The metastatic tumors showing cord-like growth pattern lack laminin reactivities at the interface between tumor sells and stroma (×100). d : The tumor with diffuse growth pattern shows poor formation of tumor stroma and a loss of laminin reactivity (×100).

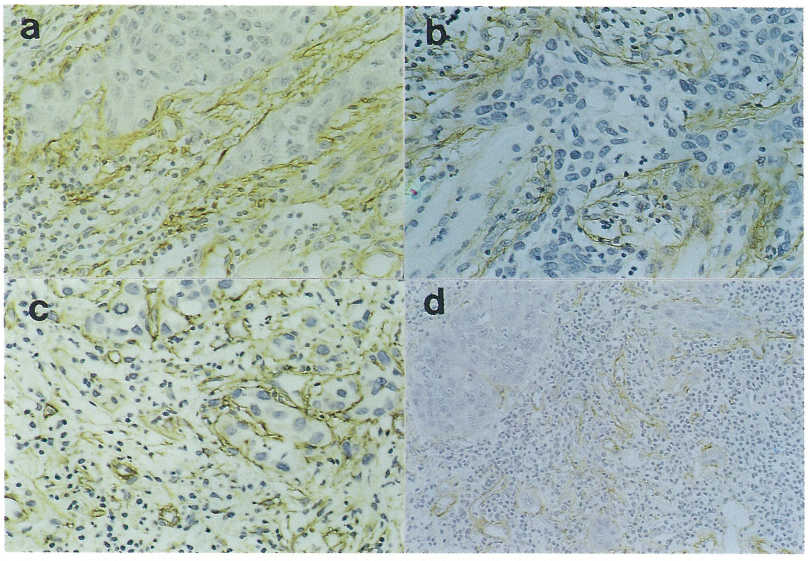

Fig 3. The stromal fibronectin (FN) staining pattern at the invasive border of the primary tumor (Immunoperoxidase-avidin-biotin-complex staining for FN, counterstained with hematoxylin)
a : FN diffuse++ (strong) staining, which shows many FN positive strands (×200). b : FN diffuse+ staining, which shows few FN positive strands (×200). c : FN basement membrane-like (BM-like) or a pericellular staining around tumor islands (×400). d : The tumor is absent stromal FN staining at the invasive border. The small vessels show distinct, subendothelial staining reaction for FN (×200).

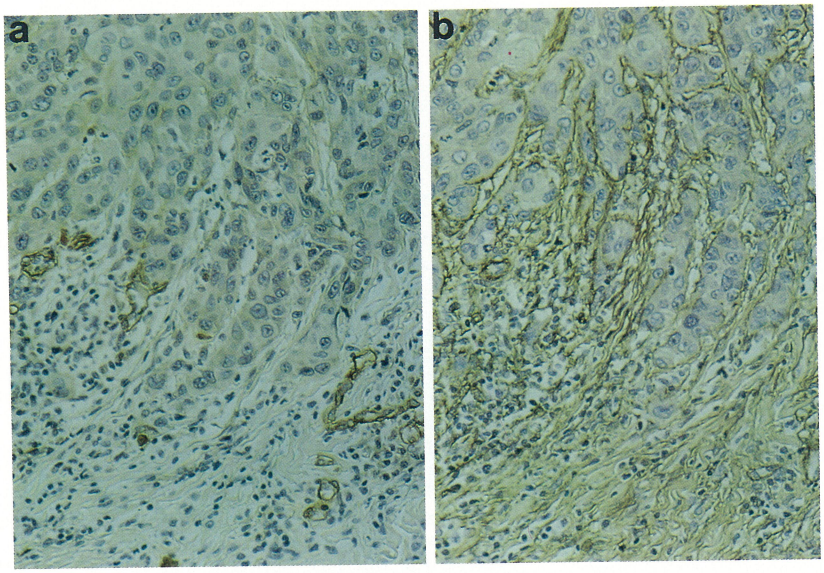

Fig 4. The relationship between laminin staining and FN staining pattern in the same primary tumor tissue is shown. (Immunoperoxidase-avidin-biotin-complex staining for laminin and FN, counterstained with hematoxylin)
a : Laminin reactivity is absent at invasive front (×100). b : FN diffuse++ staining is seen at invasive front of the same tumor tissue as (a) (×100).

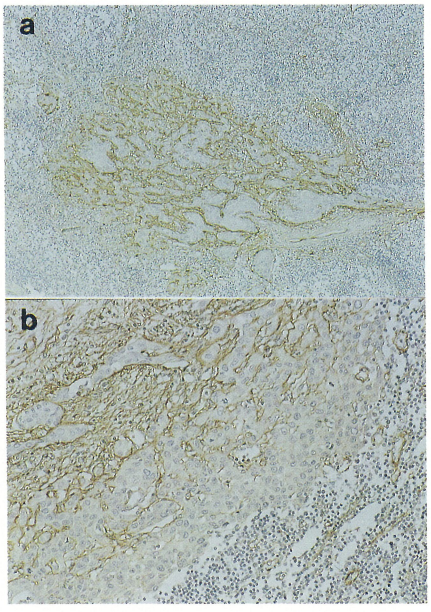

Fig 5. The FN staining pattern of the metastatic cervical lymph nodes. (Immunoperoxidase-avidin-biotin-complex staining for FN, counterstained with hematoxylin)
a : The FN staining locarizes in the metastaic foci (×40). b : The FN diffuse++ staining pattern is found in the stroma of metastatic tumor, but is absent at interface between the metastases and the lymphoid parenchyma (×100)

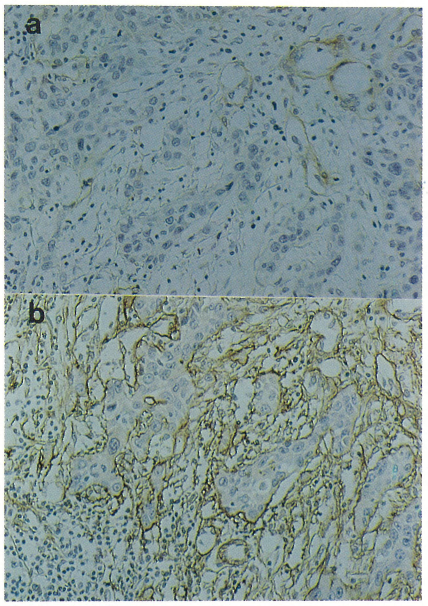

Fig 6. The relationship between laminin staining and FN staining pattern in the metastases of the lymph nodes is shown. (Immunoperoxidase-avidin-biotin-complex staining for laminin and FN, counterstained with hematoxylin)
a : Laminin reactivity is absent at invasive front (×100). b : FN diffuse++ staining is seen at invasive front of the same tumor tissue as (a) (×100).

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