The expression and significance of laminin receptor in squamous cell carcinoma of the tongue

Running title: LR and squamous cell carcinoma

Katsuhisa Matsuo[1,2], Jun Akiba[3], Sachiko Ogasawara[1], Reiichiro Kondo[1], Yoshiki Naito[3], Hironori Kusano[1], Sakiko Sanada[1], Tatsuyuki Kakuma[4], Jingo Kusukawa[2], Hirohisa Yano[1]

1 Department of Pathology, Kurume University School of Medicine, Kurume, Japan
2 Dental and Oral Medical Center, Kurume University School of Medicine, Kurume, Japan
3 Department of Diagnostic Pathology, Kurume University Hospital, Kurume, Japan
4 Department of Biostatistics Center, Kurume University, Kurume, Japan

Acknowledgements: We thank Ms. Kumiko Tsubone and Dr. Kazuya Murata for their excellent assistance in our experiments.

Abstract

Background: Laminin receptor is a non-integrin cell-surface receptor that binds laminin present on the basement membrane. It has been reported to be associated with infiltration and metastasis of various malignant tumors. However, no studies regarding tongue cancer have been reported. This study aimed to clarify the role of laminin receptor in squamous cell carcinoma of the tongue. **Methods:** We performed immunohistochemical staining of specimens from 66 patients with squamous cell carcinoma of the tongue and assessed laminin receptor expression and clinicopathological factors. As epithelial-mesenchymal transition has been shown to be associated with infiltration and metastasis of malignant tumors, staining for E-cadherin, vimentin, and N-cadherin was also performed. **Results:** Of 20 patients with postoperative recurrence, 14 exhibited high laminin receptor expression (P = 0.0025). Kaplan-Meier analysis revealed a significantly shorter time to postoperative recurrence for the high laminin receptor expression group than that for the low laminin receptor expression group (P = 0.0008). Based on multivariate analyses for postoperative recurrence, high laminin receptor expression was associated with poor prognosis (high expression vs. low expression; HR = 3.19, 95% CI = 0.92–11.08; P = 0.0682). There was a correlation between laminin receptor and N-cadherin (P = 0.0089), but not between laminin receptor and E-cadherin (P = 0.369) or vimentin (P = 0.4221). **Conclusion:** These results suggest that high laminin receptor expression is a useful prognostic factor for postoperative recurrence and may be a target for molecular therapy to treat squamous cell carcinoma of the tongue.

Keywords: laminin receptor; squamous cell carcinoma; tongue cancer; prognostic factor

Introduction

Head and neck cancers are the sixth most common malignant tumors worldwide, accounting for approximately 1%–2% of all cancer deaths. One-third of head and neck cancers are oral squamous cell carcinomas, which account for 90% or more of all oral malignant tumors. Among the oral squamous cell carcinomas, the most common is squamous cell carcinoma of the tongue. Risk factors include alcohol consumption, smoking, chronic irritation, HPV infection, and oral hygiene conditions.

Factors associated with prognosis of oral squamous cell carcinomas include depth of invasion (DOI), degree of differentiation, vascular invasion, lymphatic vessel invasion, and recurrence or metastasis.[1] With recurrence or metastasis being an important factor of poor prognosis, it is important to identify biomarkers that allow the identification of patients who may develop these secondary tumors.

The laminin receptor (LR) is a non-integrin cell-surface receptor that binds with laminin present on the basement membrane. The level of LR increases in breast,[2] cervical,[3] colon,[4] stomach,[5] liver,[6] and lung[7] cancer cells when compared to that in normal cells and is known to be associated with cancer infiltration and metastasis. With regard to the head and neck region, LR has been studied in laryngeal cancer and shown to be expressed higher in patients with cervical lymph node metastasis than in those without cervical lymph node metastasis.[8]

The association between factors involved in infiltration and epithelial-mesenchymal transition (EMT) have been reported in many malignant tumors. EMT is associated with tumor development, progression, and tumor cell activities such as migration, infiltration, metastasis, and development of refractoriness to therapy. In highly migratory and invasive cells, there is a decrease in cell adhesiveness with

decreases in the expression of epidermal cell markers such as E-cadherin and increases in expression of mesenchymal cell markers such as vimentin and N-cadherin.[9] A study of lung adenocarcinoma showed that LR promotes EMT induction and is associated with tumor infiltration and metastasis.[10]

LR expression has been studied in malignant tumors of various organs. However, its expression in tongue cancer has not yet been reported. To clarify the role of LR in squamous cell carcinoma of the tongue, we assessed LR expression and clinicopathological factors, as well as the relationship between LR and EMT.

Materials and Methods

Patients and tissue specimens

The study included 66 patients with squamous cell carcinoma of the tongue, excluding intraepithelial carcinoma, that had not yet metastasized to any lymph nodes. All enrolled participants underwent resection at the Dental and Oral Medical Center, Kurume University School of Medicine (Kurume, Japan) between 2010 and 2017 without receiving treatment prior to surgery, such as chemotherapy or radiation therapy. The removed tissue specimens were fixed in 10% buffered formalin, embedded in paraffin, and 4 mm-thick slices were prepared for staining with hematoxylin and eosin (H&E). Pathological tumor-node-metastasis (TNM) staging was assessed according to the Union for International Cancer Control (UICC) TNM Classification of Malignant Tumors, 8th edition (James D Brierley, Mary K Gosopdarowicz, Christian Wittekind).[11]

The infiltrated area was defined as the deepest region from the surface-stratified squamous epithelial cells. Tumor DOI was measured from the basement membrane of the adjacent normal mucosa to the deepest point of infiltration

using a 5-mm cut-off value, which was recently added to the pT classification of the UICC. A cut-off value of 20 mm was used for tumor size. Infiltration growth pattern (INF) was assessed and classified as follows: INFa, cases whose basal layer borders were clear or somewhat disturbed; INFb, cases whose borders were unclear and formed large or small tumor nests; and INFc, cases whose borders were unclear and infiltrated as small tumor nests, in trabecular patterns, or diffusely without nest formation.

Postoperative recurrence was defined as lymph node (LN) metastasis and/or locoregional relapse. In this study, seven of 66 cases had locoregional relapse. Of these seven cases, one case each showed dysplasia, squamous cell carcinoma in situ, and invasive squamous cell carcinoma in the surgical margin. We excluded these three cases from the analysis of prognosis. Of the four remaining cases with locoregional relapse, for one case, LN metastasis and locoregional relapse were simultaneously detected. During the observation period, 20 cases, including 16 cases with LN metastasis, three cases with locoregional relapse, and one case with both LN metastasis and locoregional relapse, recurred. In this study, four of 66 patients died during the observation period. As only four cases were not sufficient, patient prognosis based on overall survival could not be evaluated.

In this study, 12 of 66 patients underwent postoperative therapies, such as chemotherapy and/or radiation therapy. Nine of 12 patients underwent cervical lymphadenectomy and/or re-operation due to lymph node metastasis and/or locoregional recurrence, respectively. The other three patients underwent preemptive postoperative chemotherapy because of the large tumor size.

The current study adhered to the ethical guidelines designated by the Declaration of Helsinki and guidelines by the ethics committee of Kurume University

for research involving human subjects. Prior institutional review and approval was obtained for the study (approval number: 444). Clinical specimens were collected from each patient after obtaining informed consent.

Immunohistochemistry

Immunohistochemical staining of the specimens was performed using paraffin-embedded sections. After blocking with skim milk for 10 min, the tissue sections were processed using a BenchMark XT automated slide preparation system (Ventana Medical Systems, Inc., Tucson, AZ, USA). The primary antibodies used were the LR antibody clone H-2 (dilution 1:2,000, cat no. sc-74515; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), anti-E-cadherin antibody clone NCH-38 (dilution 1:100; Dako, Glostrup, Denmark), anti-vimentin antibody clone V9 (dilution 1:100; Dako), anti-N-cadherin antibody clone IAR06 (dilution 1:50; Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, UK), and anti-p16 antibody clone JC8 (dilution 1:200, Santa Cruz Biotechnology, Inc.). We used an automated streptavidin-biotin complex method in which 3,3- diaminobenzidine was the chromogenic substrate (Ventana iVIEW DAB Detection Kit). Invasion of tumor cells into lymphatic vessels was identified using anti-D2-40 antibody clone D2-40 (dilution 1:1; Nichirei Bioscience, Tokyo, Japan). Elastica van Gieson stain was used to identify tumor cell invasion into veins.

LR expression was assessed based on staining intensity in the entire tumor region and the area of staining. For the assessment of the staining, the Allred scoring system[12] of breast cancer was used to calculate a total score (TS) based on the proportion score (PS) and intensity score (IS) of each specimen. The IS was determined by comparing the intensity of staining in the tumor region with that of the normal

endothelial cells within the specimens. The cases with TS ≥ 8 were classified as the high-expression group and those with TS < 8 were classified as the low-expression group. Representative microscopic images of immunostaining for LR and H&E staining are shown in Figure 1. The correlation of EMT with the staining intensities of LR, E-cadherin, vimentin, and N-cadherin were assessed in the leading edge of the tumors. The intensity of LR staining was assessed using a method similar to that described above and cases with a staining intensity of ≥ 3 were classified as the high-expression group and those with an intensity of 1 or 2 were classified as the low-expression group.

The staining intensity of E-cadherin, vimentin, and N-cadherin in the tumor region was compared with that in the background normal stratified squamous epithelium, endothelial cells, and neurons, respectively. The staining intensity was scored as follows: 0, no staining in the tumor region; 1, lower intensity than that of controls; and 2, similar intensity to that of controls. Cases with a staining intensity of 2 for any marker were classified to the high-expression group for that respective marker and those with an intensity of 0 or 1 were classified to the respective low-expression group. Representative images of the low-expression and high-expression groups are shown in Figure 2. Immunohistochemical analysis was independently performed by two pathologists (K.M. and J.A.). For cases in which the results were inconsistent between the pathologists, a discussion was held until a consensus decision was made.

Statistical analysis

The association between the LR expression and the EMT markers as well as various clinicopathologic factors were tested by using a $\chi 2$ test or Fisher's exact test. Correlation analysis between LR and EMT markers was performed by employing polychoric correlation. In order to access effect of LR expression on the postoperative

recurrence of squamous cell carcinoma of the tongue, following two statistical methods were employed. First, the cumulative postoperative recurrence-free rates for the two LR expression groups were estimated by using the Kaplan-Meier method. The log-rank test was then employed to compare two rates. Second, effect of LR expression on the postoperative recurrence rates after controlling the clinicopathologic factors were examined by using Cox proportional hazards regression models. Univariate analyses were carried out to estimate unadjusted hazard rates for each risk factor. Since several factors were highly correlated, clinically important risk factors were then entered into the multivariate analysis, and adjusted hazard rates were estimated. The day of imaging or clinical diagnosis of LN metastasis and/or locoregional relapse was considered the day of postoperative recurrence. The period from the day of surgery to the day of LN metastasis and/or locoregional relapse or from the day of surgery to the day of the last follow-up was considered the postoperative recurrence-free survival time. Statistical significance was set at $p < 0.05$. We used JMP software (version 15.0; SAS Institute Inc., Cary, NC, USA) for all statistical analyses.

Results

Patient characteristics

The clinicopathological characteristics of the 66 patients with squamous cell carcinoma of the tongue included in the current study are shown in Table 1 in detail.

Expression pattern of LR in squamous cell carcinoma of the tongue

LR expression was positive in the cytoplasm of normal stratified squamous epithelial cells of the basal/parabasal layer. In the tumor region, LR expression was positive in the cytoplasm of the tumor cells and surrounding atypical stratified squamous epithelial

cells. LR expression in all cases was higher in tumor cells than in normal stratified squamous epithelial cells.

Clinicopathological factors were compared between the low-expression and high-expression groups (Table 2). Tumor size, DOI, pT classification, postoperative lymph node metastasis and postoperative recurrence were significantly associated with high LR expression. Relationship among LR, E-cadherin, vimentin, and N-cadherin expression was also shown in Table 2. LR and vimentin stained in the cytoplasm of tumor cells in the infiltrated area and E-cadherin and N-cadherin stained in the cell membrane of tumor cells in the infiltrated area were considered positive. There was a significant difference between LR and N-cadherin ($P = 0.022$) but not between LR and E-cadherin ($P = 0.8485$) or LR and vimentin ($P = 0.8041$) based on a $\chi 2$ test. Moreover, we conducted correlation analysis between LR expression and EMT markers (Supplemental Figure 1). A positive correlation was found between LR and N-cadherin ($P = 0.0089$). However, there was no correlation between LR and E-cadherin ($P = 0.369$) or vimentin ($P = 0.4221$).

We then analyzed the association between the expression of LR/EMT markers and drug responses. Of 12 cases that developed postoperative recurrence, eight and four showed high and low LR expression, respectively ($P = 0.0572$). Regarding the association with EMT markers, one, eight, and four cases showed high expression of E-cadherin ($P = 0.1558$), vimentin ($P = 0.3389$), and N-cadherin ($P = 0.0497$), respectively.

A comparison of the cumulative postoperative recurrence-free rates between the low LR expression and high LR expression groups using the Kaplan-Meier curve revealed a significantly shorter time to postoperative recurrence for the high LR

expression group (P = 0.0008; Figure 3). The association between postoperative recurrence-free survival and clinicopathological factors was analyzed using univariate and multivariate analyses (Table 3). Univariate analysis of the time to postoperative recurrence free survival showed that tumor size (P = 0.0004), vascular invasion (P = 0.0039), lymphovascular invasion (P = 0.0013), DOI (P < 0.0001), pattern of invasion (P = 0.0083), muscle layer infiltration (P = 0.0283), differentiation (P = 0.0170), pT classification (P < 0.0001), and LR expression (P = 0.0021) were significant predictive factors of postoperative recurrence.

Multivariate analysis was performed regarding the comparison of vascular invasion, lymphovascular invasion, pattern of invasion, differentiation, pT classification, and LR expression. In the current study, there were significant differences between pT classification and each of these factors (tumor size, P < 0.0001; DOI, P < 0.0001; and muscle layer infiltration, P = 0.0001). pT classification is based on clinicopathological factors such as tumor size, DOI, and muscle layer infiltration. Therefore, these factors were included in pT classification, while the others were excluded. The multivariate analysis showed there were no significant correlations between postoperative recurrence and vascular invasion, lymphovascular invasion, infiltration pattern, or degree of differentiation. The results suggested that high pT classification was an independent poor prognostic factor for postoperative recurrence (P = 0.0435) (pT1 vs. pT2; HR = 1.53, 95% CI = 0.39-5.94, pT1 vs. pT3 ; HR = 5.36, 95% CI = 1.26-22.83). The level of LR expression tended to be associated with poor prognosis, but this did not reach significant statistical difference (high expression vs. low expression; HR = 3.19, 95% CI = 0.92–11.08; P = 0.0682).

Discussion

In the current study, LR expression in the tumor region of tissue specimens was higher than that in non-tumor stratified squamous epithelium. In addition, larger tumor size and greater DOI were significantly more common in the high LR expression group than in the low LR expression group. LR is known to be associated with various stages of malignant tumors involved with growth and progression. Consistent with our current results, LR expression at both the protein and mRNA levels has been reported to be significantly higher in laryngeal and bile duct cancer cells than in non-tumor epithelial cells.[13]

To date, several markers, such as EGFR, cell cycle-associated factors, c-fos, and Akt, have been determined to be associated with patient prognosis in oral squamous cell carcinoma.[14-17] However, studies examining the relationships between LR and these markers are limited. Cell cycle-associated factors, such as cyclins and cyclin-dependent kinases (CDKs), have been shown to regulate LR expression, and LR knockout in a mouse model significantly decreases tumor growth.[18] Moreover, Kumazoe et al reported that the green tea polyphenol (-)-epigallocatechin-3-O-gallate (EGCG) directly binds to cell-surface LR and induces apoptosis through an Akt/endothelial nitric oxide synthase/nitric oxide/cyclic GMP axis.[19] Taken together, LR could be associated with malignant properties in concert with cell cycle-associated factors and/or Akt.

In our current study, postoperative recurrence was observed earlier in the high LR expression group compared to that in the low LR expression group. Peptide G is considered to be a segment of laminin that binds the LR and a synthetic peptide G has been shown to induce a change in the cytoskeleton and increase LR expression on the cell membrane, which leads to enhanced cell adhesiveness and expression of

extracellular matrix (ECM) proteins.[20] Peptide G has also been shown to increase the rate of laminin degradation and expression of the LR, promote the production and secretion of cathepsin B, and reduce the integrity of the basement membrane.[21] We suggest that such a mechanism may have been deeply involved in lymphatic invasion, which is the initial process of lymph node metastasis in this study.

EMT is reported to be highly associated with the growth/progression of many malignant tumors, such as esophageal adenocarcinoma,[22] pancreatic cancer,[23] and breast cancer.[24] Furthermore, LR is reported to be highly associated with tumor infiltration in several carcinomas through the induction of EMT.[25] Therefore, we examined whether there was an association between LR and EMT in squamous cell carcinoma of the tongue. In the current study, we found a correlation between LR and N-cadherin, and thus LR was shown to be associated with EMT. It is possible that LR-positive tumor cells induce EMT, which is associated with tumor invasion and metastasis. However, LR expression did not correlate with E-cadherin or vimentin expression. EMT is generated in a continual spectrum and intermediate EMT states (partial EMTs) exist. In addition, carcinomas possess heterogeneity within tumors and individual tumor cells possess distinct EMT status within the EMT spectrum.[26] For this reason, several EMT markers were considered to have exhibited distinct expression patterns in our study, and thus some cases were presumably in a state of partial EMT. As the EMT state is a key factor for drug resistance, the association between the expression of LR/EMT markers and drug responses was examined. However, it might have been difficult to assess the relationship between drug resistance and EMT markers as all patients underwent chemotherapy and/or radiation therapy after surgical treatment as mentioned.

In general, the expression of LR was enhanced in tumor regions compared to

that in non-tumor regions and strongly correlated with the grade of malignancy. Moreover, high LR expression in tumor cells of breast carcinoma and prostatic carcinoma was reported to be an independent factor of poor prognosis.[27, 28] Thus, LR is considered to be a good therapeutic target. The binding of LR to its ligand generally enhances the malignancy of carcinomas, whereas the adipokine C1q/tumor necrosis factor-related protein-6 binds to LR and suppresses carcinoma growth and progression.[29] In addition, the polyphenol EGCG, a component of green tea, binds LR and increases cancer-specific cGMP to induce selective apoptosis of tumor cells in the presence of a phosphodiesterase 5 inhibitor.[30] As molecules that suppress LR have been identified, LR is considered a potentially good candidate for molecular targeted therapy for carcinomas.

Limitations of the current study include: (1) the sample size was small, (2) it was a retrospective study, (3) the postoperative follow-up period was short, and (4) the expression of each factor at the protein level was only evaluated by immunostaining tissue specimens and functional analysis was not performed.

Conclusion

Findings of the present study suggests that LR may be a biomarker in surgically resected specimens for predicting the prognosis of squamous cell carcinoma of the tongue and may be a good target for molecular therapy to treat squamous cell carcinoma.

Conflict of interest: All authors have declared no conflicts of interest.

Author contributions:

Katsuhisa Matsuo, Jun Akiba, and Hirohisa Yano conceived the study and participated in its design and concordance. Katsuhisa Matsuo and Sachiko Ogasawara conducted immunohistochemical stain. Katsuhisa Matsuo and Jun Akiba assessed immunohistochemical stain. Katsuhisa Matsuo, Jun Akiba, Reiichiro Kondo, Yoshiki Naito, Hironori Kusano, Sakiko Sanada, and Hirohisa Yano participated in pathological diagnosis. Tatsuyuki Kakuma performed statistical analyses and interpretation. Jingo Kusukawa performed surgical treatment and collected clinical information. Katsuhisa Matsuo, Jun Akiba, and Hirohisa Yano drafted the text. All authors have read and approved the final version of the manuscript.

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Tables:

Figure legends:

Figure 1. Hematoxylin and eosin (H&E) staining and immunostaining of laminin receptor (LR) in squamous cell carcinoma of the tongue. (A, C, E) Panels A, C, and E are micrographs of H&E staining corresponding to panels B, D, and E, respectively. (B, D, E) The staining intensity of LR was graded as score of 1–3. (B) Score 1: mild positivity. (D) Score 2: moderate positivity. (F) Score 3: strong positivity. Scale bar indicates 50 μm.

Figure 2. Representative micrographs of hematoxylin and eosin (H&E) staining and immunostaining of E-cadherin, vimentin, N-cadherin, and laminin receptor (LR) in the leading edge of squamous cell carcinoma of the tongue tumors. (A, B) Representative images of H&E staining of cases in which the tumor infiltration depth was ≤ 5 mm (A) and > 5 mm (B). (C–J) Staining intensities of E-cadherin (C, D), vimentin (E, F), N-cadherin (G, H), and LR (I, J) assessed in the low-expression groups (C, E, G, I) and high-expression groups (D, F, H, J). Scale bar indicates 50 μm.

Figure 3. Relationship between laminin receptor (LR) expression and the time to postoperative recurrence based on the Kaplan-Meier method.

Table 1 Clinicopathological characteristics of 66 cases of Squamous cell carcinoma of the tongue

Gender (M/F)	39/27	
Age (Years; mean ± SD)	62.9±16.1	
Location (Rt/Lt)	36/30	
Tumor size, n (%)		
≦2cm		39 (59)
>2cm		27 (41)
Depth of invasion, n (%)		
≦5mm		45 (68)
>5mm		21 (32)
Locoregional relapse, n (%)		7† (11)
Vascular invasion, n (%)		10 (15)
Lymphovascular invasion, n (%)		16 (24)
Pattern of invasion, n (%)		
INF a		12 (18)
INF b		36 (55)
INF c		18 (27)
Muscle layer infiltration, n (%)		44 (67)
p16 positive, n (%)		13 (20)
Differentiation, n (%)		
Well differentiated		47 (71)
Moderately differentiated		16 (24)
Poorly differentiated		3 (5)
pT classification, n (%)		
pT1		31 (47)
pT2		25 (38)
pT3		10 (15)
Lymph node metastasis after surgery, n (%)		17 (26)
Postoperative recurrence, n (%)		20 (30)
Recurrence-free survival (Days; mean± SD)	1062.95±792.72	

†: Of these seven cases, one case each showed dysplasia, squamous cell carcinoma in situ, and invasive squamous cell carcinoma in the surgical margin.

Table 2 The association between laminin-receptor and various clinicopathologic factors and epithelial-mesenchymal transition markers

		Laminin-R High expression	Laminin-R Low expression	P value
Gender	Male	17	22	0.6211
	Female	10	17	
Age	60>	11	12	0.4404
	60≦	16	27	
Location	Rt. tongue	14	22	0.8035
	Lt. tongue	13	17	
Tumor size	2cm≧	11	28	0.0211
	2cm<	16	11	
Depth of invasion	5mm≧	14	31	0.0304
	5mm<	13	8	
pT classification	pT1	9	22	0.0193
	pT2	10	15	
	pT3	8	2	
Differentiation	Well	17	30	0.1188
	Moderately	7	9	
	Poorly	3	0	
Pattern of invasion	INF a	5	7	0.0851
	INF b	11	25	
	INF c	11	7	
Vascular invasion	Absent	22	34	0.7286
	Present	5	5	
Lymphovascular invasion	Absent	17	33	0.0777
	Present	10	6	
Muscle layer infiltration	Absent	7	15	0.4261
	Present	20	24	
P16 expression	Positive	6	7	0.7571
	Negative	21	32	
Post-operative lymph node metastasis	Absent	14	35	0.0012
	Present	13	4	
Postoperative recurrence	Absent	13	33	0.0025
	Present	14	6	
E-cadherin	Low expression	26	21	0.8485
	High expression	11	8	
Vimentin	Low expression	18	15	0.8041
	High expression	19	14	
N-cadherin	Low expression	29	28	0.22
	High expression	8	1	

Table 3 Univariate and multivariate analyses for postoperative recurrence-free survival of individual parameters

		Univariate analysis		Multivariate analysis	
		HR (95%CI)	P-value	HR (95%CI)	P-value
Gender	Male		0.9889		
	Female	1.01 (0.41-2.47)			
Age	60>		0.9156		
	60≦	1.05 (0.42-2.64)			
Location	Rt. tongue		0.2054		
	Lt. tongue	1.78 (0.73-4.36)			
p16 expression	Negative		0.9334		
	Positive	1.05 (0.35-3.14)			
Tumor size	2 cm≦		0.0004		
	>2 cm	6.39 (2.3-17.77)			
Vascular invasion	Absent		0.0039		0.2544
	Present	4.15 (1.58-10.93)		2.62 (0.5-13.68)	
Lymphovascular invasion	Absent		0.0013		0.1534
	Present	4.32 (1.78-10.50)		2.41 (0.72-8.05)	
Depth of invasion	5 mm≦		<.0001		
	>5 mm	7.12 (2.78-18.25)			
Pattern of invasion	INF a		0.0083		0.4892
	INF b	3.77 (0.48-29.79)		2.59 (0.28-23.71)	
	INF c	10.42 (1.33-81.86)		4.59 (0.37-57.40)	
Muscle layer infiltration	Absent		0.0283		
	Present	3.99 (1.16-13.76)			
Differentiation	Well		0.0170		0.4784
	Moderately	1.74 (0.64-4.72)		0.66 (0.12-3.65)	
	Poorly	10.44 (2.68-40.62)		0.24 (0.02-2.41)	
pT classification	pT1		<.0001		0.0435
	pT2	2.86 (0.83-9.85)		1.53 (0.39-5.94)	
	pT3	13.67 (4.04-46.24)		5.36 (1.26-22.83)	
LR	Low expression		0.0021		0.0682
	High expression	4.56 (1.73-11.98)		3.19 (0.92-11.08)	

Figure 1

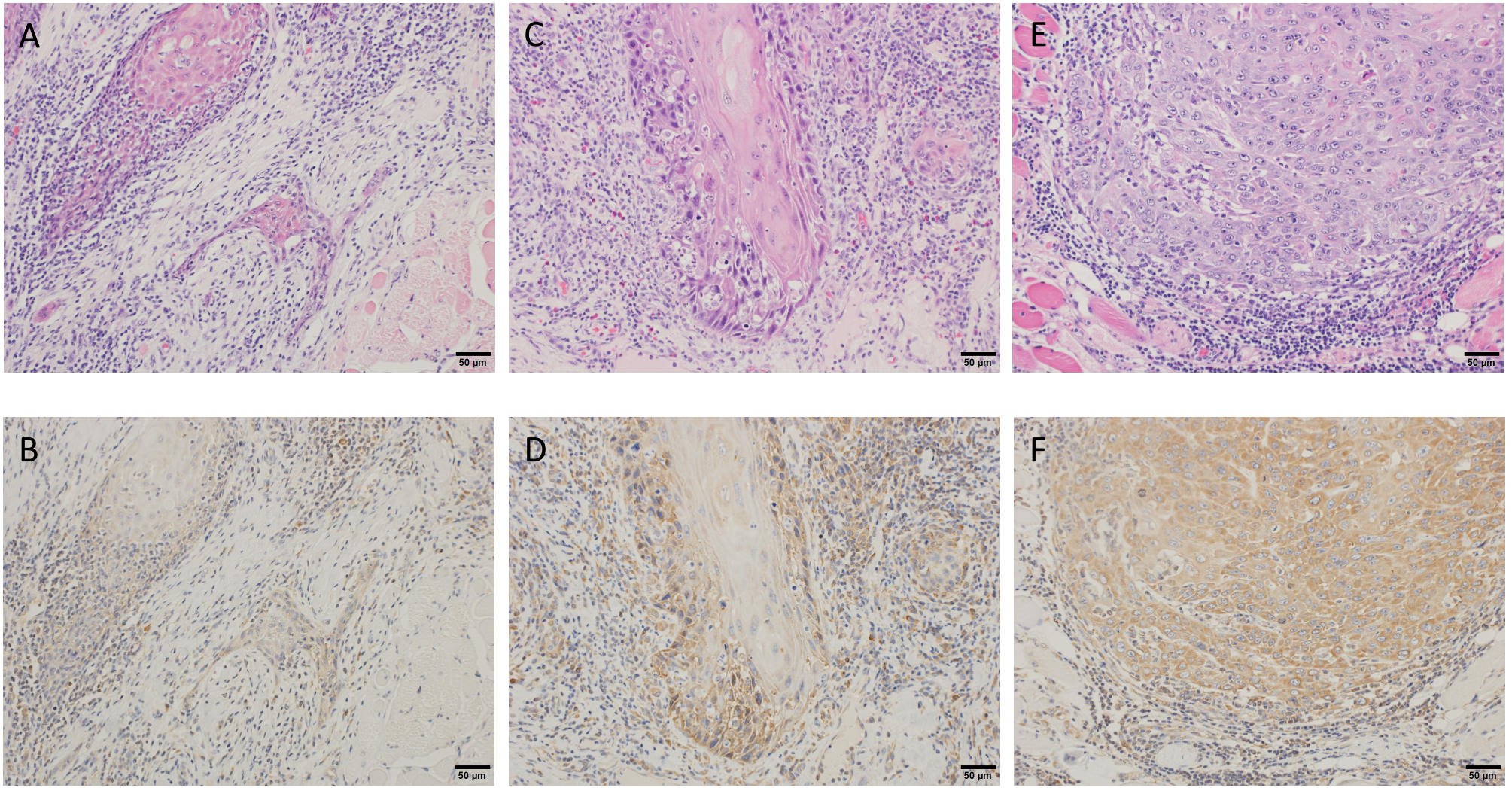


Figure 2

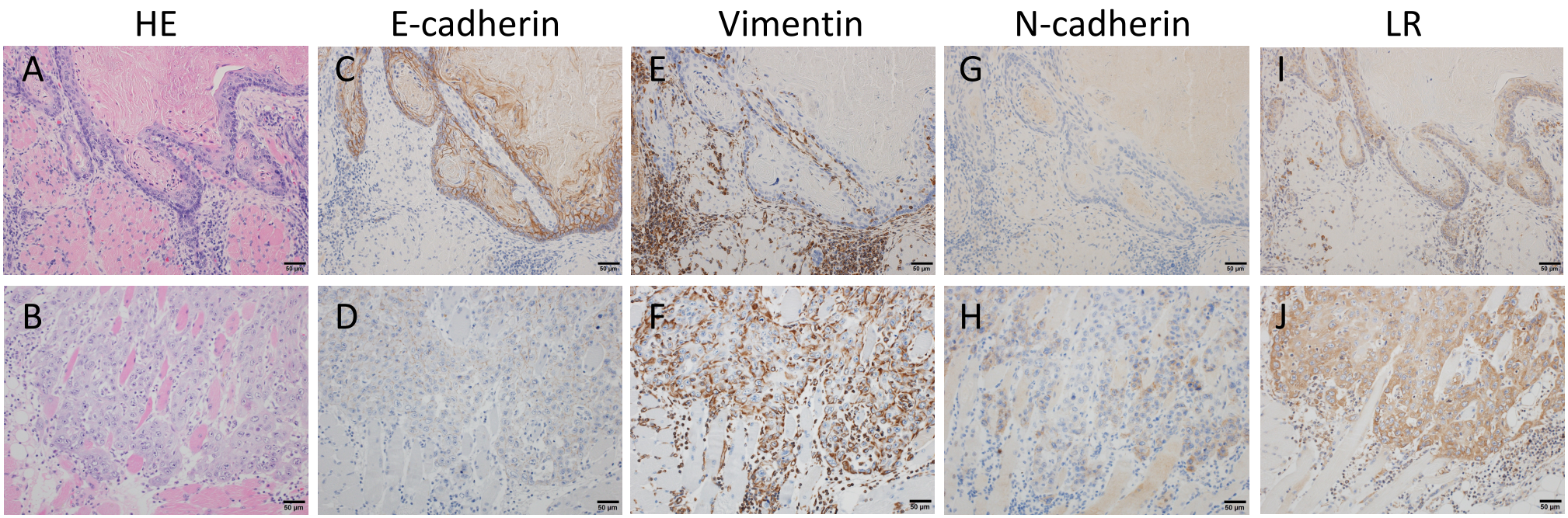
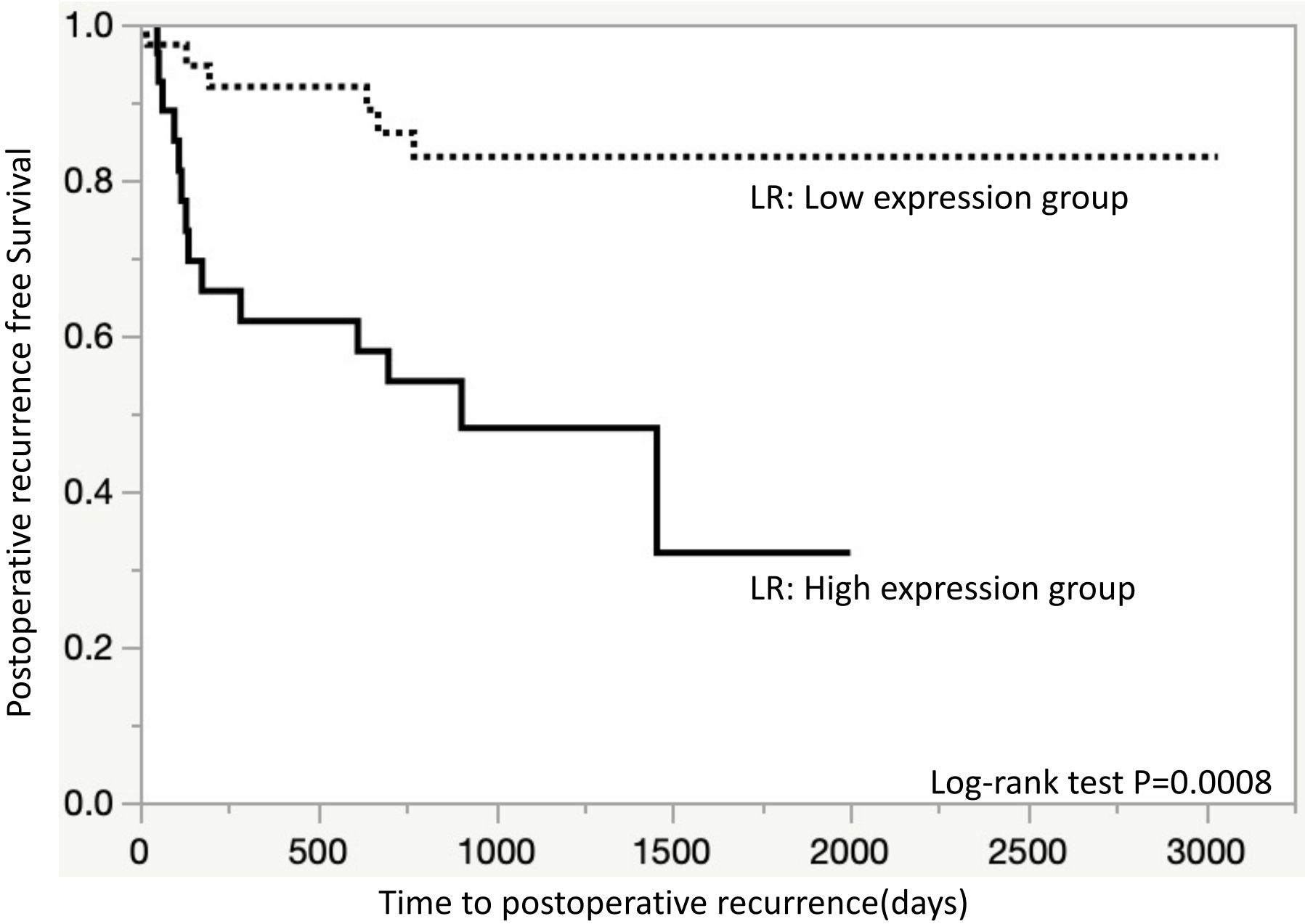
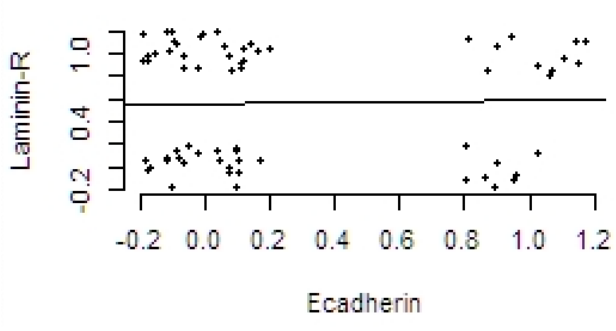


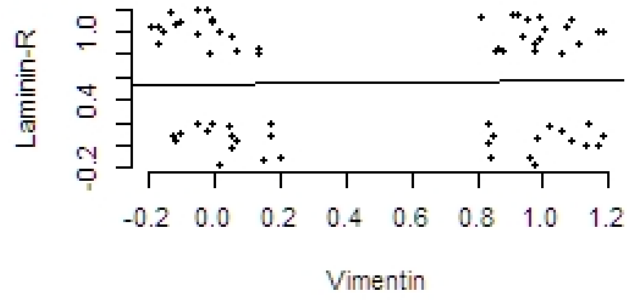
Figure 3


1.0
0.8
0.6
0.4
0.2
0.0
Postoperative recurrence free Survival
LR: Low expression group
LR: High expression group
Log-rank test P=0.0008
0
500
1000
1500
2000
2500
3000
Time to postoperative recurrence(days)

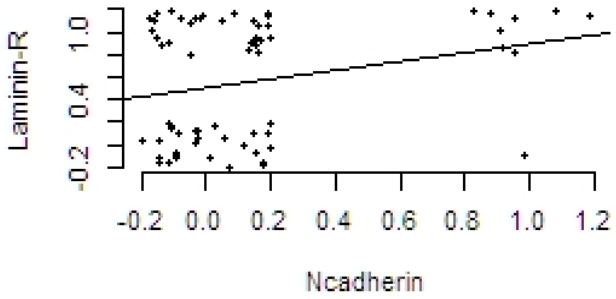
Supplemental Figure 1



Correlation=0.14935‡ P=0.369



Correlation=0.13341‡ P=0.4221



Correlation=0.42456‡ P=0.0089

‡: Polychoric correlation

Supplemental figure legend:

Supplemental Figure 1. Correlation analysis between LR and EMT markers was performed by employing polychoric correlation. The results of the analysis that a positive correlation was found between LR and N-cadherin (P = 0.0089). However, there was no correlation between LR and E-cadherin (P = 0.369) or vimentin (P = 0.4221).