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LAMB3 is associated with disease progression and cisplatin cytotoxic sensitivity in head and neck squamous cell carcinoma

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ABSTRACT

Objectives: Laminin subunit beta-3 (LAMB3) is a major component of the basement membrane zone. In our study, we investigated the role of LAMB3 in head and neck squamous cell carcinoma (HNSCC) progression and its clinical implication as a prognostic biomarker.

Materials and methods: A retrospective analysis of 100 patients with HNSCC who had undergone curative surgery from 1999 to 2011 was performed. We evaluated LAMB3 expression by immunohistochemistry and its associations with clinicopathological characteristics and survival. For functional *in vitro* analyses, cell proliferation, migration, and invasion and western blot assays were performed following LAMB3 suppression. In addition, the role of LAMB3 in cisplatin-induced cytotoxicity was clarified by measuring cell proliferation.

Results: LAMB3 expression was up-regulated in HNSCC cell lines and patient tissues. High LAMB3 expression was significantly associated with positive lymph node metastasis (odds ratio: 6.316; $P < 0.001$) and poor prognosis in patients with HNSCC. LAMB3 suppression reduced cell migration/invasion via down-regulation of epithelial-to-mesenchymal transition-associated proteins (Vimentin and Slug). Moreover, LAMB3 suppression increased cisplatin cytotoxicity in HNSCC cells.

Conclusion: Our findings indicate that LAMB3 may be used as a prognostic biomarker in HNSCC and support that LAMB3 silencing could induce the sensitivity of anti-cancer drugs such as cisplatin.

Introduction

Head and neck squamous cell carcinoma (HNSCC) has the sixth highest cancer incidence worldwide, representing approximately 6% of solid tumors [1], and approximately 650,000 new cases are diagnosed every year [2]. Tobacco, alcohol, and high-risk human papillomavirus are considered risk factors, leading to malignant transformation [3,4]. Despite advances in multimodalities involving surgery, radiotherapy, and chemoradiotherapy, HNSCC has been associated with one of the lowest overall survival rates among all cancer types in the last three decades [5]. This is likely because of a lack of understanding of the specific molecular targets and molecular regulatory pathways that predict HNSCC progression.

Laminins are large extracellular glycoproteins composed of three different polypeptide chains (α, β, and γ), and different combinations of these chains generate 15 different laminin isoforms [6]. Of the many laminin isoforms, laminin-332 (formerly laminin-5) is distinctive in both structure and behavior [7]. Laminin-332, a major component of the basement membrane of

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skin and other epithelial tissues, regulates epithelial cell migration in epithelial regeneration and repair processes [8]. Many reports have indicated that laminin-332 is related to tumor invasiveness in various cancer types [9–11]. LAMA3, LAMB3, and LAMC3 encode the α3, β2, and γ2 subunits, respectively, of the trimeric basement membrane protein laminin-332 [12]. LAMB3, which is reported to be resistant to proteolytic processing, is processed by both matrilysin and MT-1-MMP [13,14], and its cleavage increases carcinoma cell migration. LAMB3 expression affects multiple malignant phenotypes of gastric cancer cell lines and may play an important role in gastric carcinogenesis (8). An important role of LAMB3 in regulating metastatic progression in lung adenocarcinoma was also reported [7]. However, the clinical significance and molecular mechanism of LAMB3 expression in HNSCC are not well understood.

The aim of this study was to investigate the functional significance of LAMB3 in HNSCC progression using *in vitro* functional assays and tissue microarray expression analyses in different HNSCC cell lines and patient cohorts. Furthermore, we investigated whether targeting LAMB3 exerts a better effect with platinum-based chemotherapy as a therapeutic strategy for HNSCC.

Materials and methods

HNSCC patients

We retrospectively evaluated 100 patients with HNSCC who had undergone curative surgery (primary resection and appropriate cervical lymph node dissection according to disease stage) at the Department of Otolaryngology-Head and Neck Surgery of Chungnam National University Hospital from April 1999 to December 2011. The survival and biology of patients with HPV are very distinct from those without HPV. Therefore, we analyzed after patients with HPV positive were excluded because very few patients were HPV positive in our study population. A total of 100 patients were eventually enrolled in this study. All patients in our study were initially undergone curative surgery and post-operative radiotherapy was performed according to NCCN guideline. This study was approved by the Institutional Review Board of Chungnam National University College of Medicine (Jung-gu Daejeon, Korea), and the requirement to obtain informed consent was waived. All experiments related to human tissue were performed in accordance with our institutional guidelines.

Tissue microarray construction

Formalin-fixed, paraffin-embedded tumor blocks were collected, and tissue microarrays were generated as described previously [15]. Sections were cut from each donor block and stained with hematoxylin and eosin to identify the tumor area. A small tissue core (two cylinders per patient, 2 mm in diameter) was removed from the donor block using a tissue chip microarray (Beecher Instruments, Silver Spring, MD, USA) and was transferred to a recipient paraffin block. Histologic sections (5 μm thickness) were cut from the recipient paraffin block using standard techniques.

Immunohistochemistry (IHC) and scoring system

IHC staining using an anti-LAMB3 antibody was performed using a 3,3′-diaminobenzidine peroxidase substrate kit according to the manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA). At least two experienced pathologists analyzed the slides under a light microscope (100 × magnification) while blinded to the clinical patient information. The results were classified using two parameters according to a modified method described previously [16]: LAMB3 staining extent score (0, no staining; 1, <35%; 2, 35–75%; 3, >75% positive cells) and staining intensity score (0, no staining; 1, weak; 2, moderate; 3, strong staining). By multiplying the staining extent score by the staining intensity score, we obtained the IHC staining grade (range: 0–9). For statistical comparisons, specimens with an IHC staining grade <3 versus ≥3 were included in the low versus high LAMB3 groups, respectively.

Cell lines and reagents

The human HNSCC cell lines SNU1041 (hypopharynx), SNU1076 (larynx), and FaDu (hypopharynx) were purchased from the Korean Cell Line Bank (Seoul, South Korea). Primary human fibroblast cultures, kindly donated by Professor J.H. Lee (Chungnam National University, Daejeon, South Korea) was used as normal epithelial cells. The SNU1041, SNU1076, and FaDu cell lines were cultured in RPMI 1640 medium (Gibco, Grand Island, NY, USA). All cell lines were supplemented with 10% fetal bovine serum and 100 U/mL penicillin–streptomycin (Gibco). All cell lines were routinely maintained at 37 °C under an atmosphere of humidified air with 5% CO_2. Cisplatin was purchased from Sigma-Aldrich.

RNA isolation and reverse-transcription PCR

Total cellular RNA was extracted from lysed cells using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. The total RNA was then reverse transcribed and amplified using primers specific for LAMB3 and glyceraldehyde 3-phosphate dehydrogenase, as described previously [17]. The primer sequences were as follows: LAMB3-F: 5′-CCA AGC CTG AGA CCT ACT GC-3′/LAMB3-R: AAG CTG GAA TCT CCT GTC CA-3′, and GAPDH-F: 5′-ACC CAG AAG ACT GTG GAT GG-3′/GAPDH-R:F: 5′-TTC TAG ACG GCA GGT CAG GT-3′.The PCR products were separated by electrophoresis on a 2% agarose gel containing ethidium bromide.

Transient transfection

Cells were seeded at 2×10^5/well in six-well plates and then cultured overnight to achieve 60–70% confluence. Transient transfection was performed using Lipofectamine RNAi MAX reagent (Invitrogen) following the manufacturer's standard protocol. LAMB3 siRNA (sense: 5′-GUG UGU GCA AGG AGC AUG U(dTdT)-3′; antisense: 5′-ACA UGC UCC UUG CAC ACA C(dTdT)-3′, or negative control siRNAs (#SN-1003, Bioneer) were acquired from Bioneer (Daejeon, Korea).

Cell proliferation assay

Cells were seeded at 1×10^4/well in 96-well plates. After 24 h, the cells were treated with control or LAMB3 siRNA for 48 h and 72 h, and SNU1041 and SNU1076 cell viabilities were measured using the cell proliferation reagent WST-1 (Roche Diagnostics Corporation, Indianapolis, IN, USA) as described previously [18]. The formazan product was measured quantitatively at 450 nm using an enzyme-linked immunosorbent assay reader.

Cell migration and invasion (transwell) assay

Transwell chambers (24-well; Costar, Cambridge, MA, USA) were used to examine cell migration and invasion as described previously [18]. Briefly, transwell membranes were coated with Matrigel for 6 h at 37 °C for the invasion assay and without Matrigel for the migration assay. Control or LAMB3 siRNA-transfected cells (5×10^5 in 100 μL serum-free medium) were added to the upper chamber.

Next, 700 μl medium containing 10% fetal bovine serum were added to the lower chamber. The chamber was incubated for 48 h in 5% CO_2 at 37 °C. Finally, non-attached cells were removed using a cotton swab, and the cells attached in the lower chamber (invading cells) were stained with crystal violet and counted in four representative fields under a light microscope (200 × magnification).

Western blot analysis

Cells were lysed in lysis buffer containing 150 mM NaCl, 1.0% Nonidet P-40 (NP40), 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris, pH 8.0, and a protease inhibitor cocktail (Roche Applied Science, Vienna, Austria, pH 7.4). Electrophoresis was performed as described previously [19]. The following primary anti-human antibodies were used for Western blot analysis: anti-LAMB3(1:1000; OriGene Technologies Inc. Rockville, MD, USA) anti-Slug, anti-Vimentin, anti-β-actin (1:1000; Cell Signaling Technology Inc, Danvers, MA, USA), and anti-E-cadherin, (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Following incubation with the corresponding horseradish peroxidase-conjugated secondary antibodies (1:5000; Santa Cruz Biotechnology), immunoreactive bands were visualized by enhanced chemiluminescence(ECL) detection.

Cisplatin treatment and cell viability assay

Cells were seeded at 1×10^4/well in 96-well plates. The following day, cells were treated with negative control or LAMB3 siRNA for 48 h and then with cisplatin at different concentrations (0, 5, 10, 30, and 50 μM). After 48 h, cell viability was measured using the cell proliferation reagent WST-1 (Roche Diagnostics Corporation). The formazan product was quantitatively measured at 450 nm using an enzyme-linked immunosorbent assay reader.

Statistical analysis

All statistical analyses were performed using SPSS for Windows statistical software (ver. 20.0; SPSS Inc., Chicago, IL, USA). Pearson's chi-squared or Fisher's exact test was used to analyze the relationships between LAMB3 expression and clinicopathologic parameters. Factors determined to be significant in the univariate analyses were evaluated together in multivariate logistic regression analyses to adjust for other factors. Survival curves were constructed using the Kaplan–Meier method and compared using the log-rank test. All *in vitro* experiments were repeated three times, and statistical significance was analyzed using two-sided Student's t-tests. The data are presented as means ± standard deviation, and a P value < 0.05 was deemed to indicate statistical significance ($^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$).

Results

LAMB3 expression in HNSCC tissues

To determine the clinical relevance of LAMB3 expression in human HNSCC, IHC staining of LAMB3 was performed on 100 paraffin-embedded samples harvested from patients with histologically confirmed HNSCC. Representative IHC images are demonstrated in Fig. 1A. LAMB3 was mostly expressed in the cytoplasm of cancer cells. LAMB3 staining was detected in 65 of 100 (65%) HNSCC specimens but was variable. LAMB3 staining was classified as low in 43 cases (43%) and high in 57 cases (57%).

In addition, we assessed whether there was a difference in LAMB3 expression between normal and tumor tissues derived from the same HNSCC patient. As shown in Fig. 1B, tumor tissues had markedly higher LAMB3 protein levels than did normal tissues. The results indicated that LAMB3 overexpression may be related to carcinogenesis in HNSCC.

LAMB3 expression is independently correlated with lymph node metastasis in HNSCC patients

The demographic data of our study population are shown in Supplementary Table 1. We evaluated the correlations between LAMB3 expression and clinicopathological characteristics. Among the various parameters described in Table 1, lymph node metastasis, American Joint Committee on Cancer stage, and tumor site were significantly associated with the LAMB3 expression status. Then, the above-mentioned covariates of interest were modeled together using multivariate logistic regression analyses to adjust for other factors. As revealed in Table 2, tumors with high LAMB3 expression showed a significantly increased odds of lymph node metastasis, of more than six-fold (odds ratio = 6.316; 95% confidence interval 2.420–16.483; $P < 0.001$).

LAMB3 expression is associated with a poor prognosis in HNSCC

To examine the potential correlation between high LAMB3 expression and survival of HNSCC patients, disease-free survival (DFS) and overall survival (OS) curves were calculated using the Kaplan–Meier method and compared using the log-rank test. As shown in Fig. 2A and B, patients with high LAMB3 expression showed significantly lower DFS and OS rates than did those with low LAMB3 expression ($P < 0.001$). The 5-year DFS rate was 40.7% for patients with high LAMB3 expression and 87.3% for those with low LAMB3 expression. The 5-year OS rate was 43.9% for patients with high LAMB3 expression and 82.0% for those with low LAMB3 expression. We also analyzed the correlation between LN stage and survival of HNSCC patients, that showing significant association (Supplementary Fig. 2). In addition, as shown in Supplementary Fig. 3, patients with both high LAMB3 expression and positive LN status showed significantly lower 5-year DFS (35.5% vs 49.5% vs 93.8%) and OS (32.7% vs 67.3% vs 88.1%) rate than did those with high LAMB3 expression or positive LN only or those with both low LAMB3 expression and negative LN ($P < 0.001$). This result suggests that LAMB3 may have an effect in the survival of LN positive HNSCC patinets. Collectively, these data suggest that LAMB3 represents a potential molecular biomarker for the prediction of HNSCC aggressiveness and prognosis.

LAMB3 expression in HNSCC cell lines

We performed *in vitro* functional studies to investigate the role of LAMB3 in HNSCC cell lines. We examined the LAMB3 mRNA and protein levels in a panel of HNSCC cell lines (FaDu, SNU1041, and SNU1076) and in normal human fibroblasts and human keratinocytes (HaCaT) for comparison. The expression levels of LAMB3 were significantly up-regulated in all three HNSCC cell lines compared with the fibroblasts and HaCaT cell (Fig. 3A and B). The results indicate that overexpression of LAMB3 is correlated with tumorigenesis in HNSCC cell lines.

LAMB3 promotes migration and invasion, but not proliferation, of HNSCC cells

To investigate the functional significance and mechanism of action of LAMB3 in HNSCC, we first examined the effect of LAMB3 on cell proliferation. SNU1041 and SNU1076 cell lines were transiently transfected with negative control siRNA and LAMB3 siRNA for 0 h, 24 h, 48 h and 72 h, and cell proliferation was detected by

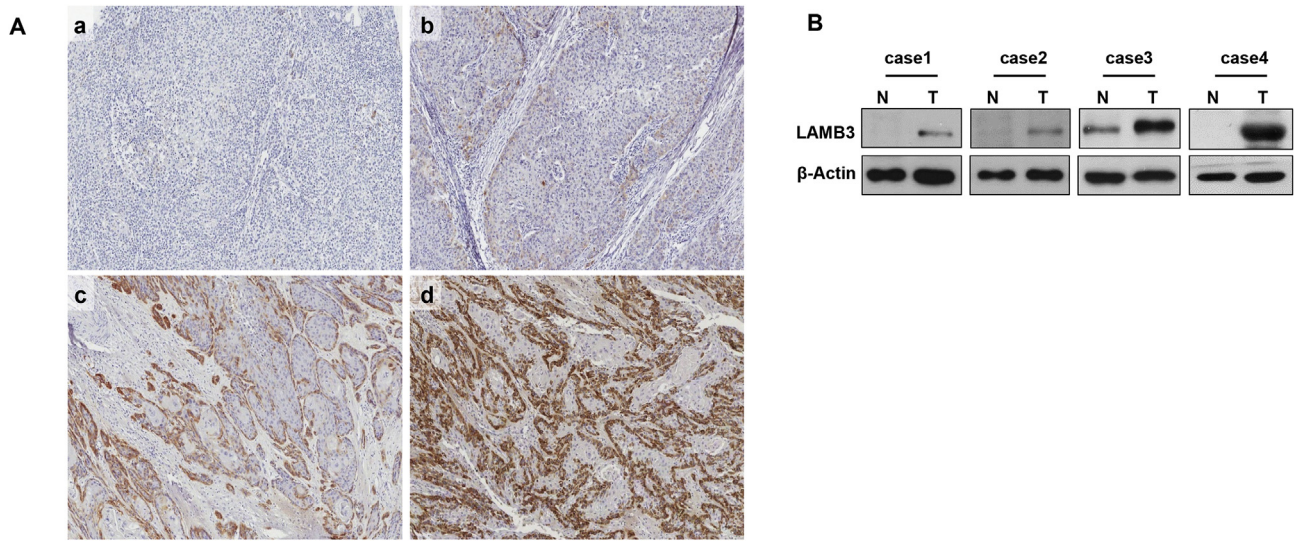

Fig. 1. LAMB3 expression in head and neck squamous cell carcinoma (HNSCC) clinical specimens. (A) LAMB3 expression as determined by immunohistochemistry. Representative immunohistochemical images representing (a) no staining, (b) weak staining, (c) moderate staining, and (d) strong staining intensities in cancer tissues from 104 HNSCC patients (100 × magnification). (B) The tissue samples obtained from HNSCC patients were examined by Western blot analysis using an anti-LAMB3 antibody.

Table 1
Association between LAMB3 expression and various clinicopathological features of 100 HNSCC patients.

Variables	No. of patients	LAMB3 expression		
		Low	High	*P*
Age (year)				1.000
<65	50	22	28	
≥65	50	21	29	
Gender				0.229
Male	87	35	52	
Female	13	8	5	
T stage				0.102
I + II	57	29	28	
III + IV	43	14	29	
LN metastasis				0.000*
No	50	33	17	
Yes	50	10	40	
AJCC stage				0.000*
I + II	37	26	11	
III + IV	63	17	46	
Histological grade				0.228
Well	30	17	13	
Moderate	50	19	31	
Poor	20	7	13	
Tumor site				0.044*
Oral cavity	39	22	17	
Oropharynx	12	3	9	
Hypopharynx	9	1	8	
Larynx	40	17	23	

LN, lymph node; AJCC, American Joint Committee on Cancer.
*$P < 0.05$ between the two categories for a given variable.

Table 2
Multinomial logistic regression for the association of LAMB3 expression with T classification, lymph node metastasis, and AJCC stage.

Factor	β	*P* value	Exp(β)	95% CI
Positive LN metastasis	1.843	0.000*	6.316	(2.420, 16.483)
Tumor site (reference: oral cavity)		0.625		
Oropharynx	0.633	0.441	1.883	(0.377, 9.400)
Hypopharynx	1.370	0.246	3.934	(0.390, 39.724)
Larynx	0.120	0.814	1.128	(0.414, 3.072)
Constant	−0.777	0.037	0.460	

Exp (β) indicates odd ratio; CI, confidence interval; LN, lymph node.
*$P < 0.05$ between the two categories for a given variable.

the WST-1 assay. As shown in Supplementary Fig. 1, LAMB3 knockdown had no significant effect on the proliferation of HNSCC cell lines. To determine the effects of LAMB3 on cell migration and invasion, which have been recognized as key steps in tumor metastasis, SNU1041 and SNU1076 cells were transiently transfected with LAMB3 siRNA or with negative control siRNA for 48 h. The cells were then allowed to migrate in Transwell chambers (cell migration) or in chambers coated with Matrigel (cell invasion). Despite no effect on cell proliferation, LAMB3 knockdown considerably suppressed the migration and invasion of SNU1041 and SNU1076 cells (Fig. 3C–F). These results suggest that LAMB3 positively affected the migration and invasion of the HNSCC cells.

LAMB3 promotes epithelial-to-mesenchymal transition (EMT)-related protein expression

An association between EMT and cell invasion has been demonstrated in cancer progression. We examined epithelial and mesenchymal markers, including E-cadherin, Vimentin, and Slug, by Western blot analysis. LAMB3 knockdown induced a significant increase in E-cadherin and decrease in Vimentin and Slug levels in SNU1041 and SNU1076 cells (Fig. 3G). These findings suggest that LAMB3 induces the migration and invasion of HNSCC cells via EMT activation.

LAMB3 suppression increases cisplatin cytotoxicity in HNSCC cells

Cells that undergo EMT acquire properties associated with cancer stem cells, which are related to chemoresistance [20]. We hypothesized that LAMB3 affects chemoresistance and aimed to determine the role of LAMB3 silencing in cisplatin efficacy in SNU1041 and SNU1076 cells. Both cell lines were treated with various concentrations of cisplatin for 48 h, and cell viability was assessed by the WST-1 assay. As shown in Fig. 4A and B, LAMB3 suppression showed more marked cisplatin-induced cytotoxic effects compared with the negative control siRNA. These data suggest that LAMB3 acts as an oncogenic driver in HNSCC by promoting EMT-associated invasion, and LAMB3 suppression increases chemosensitivity.

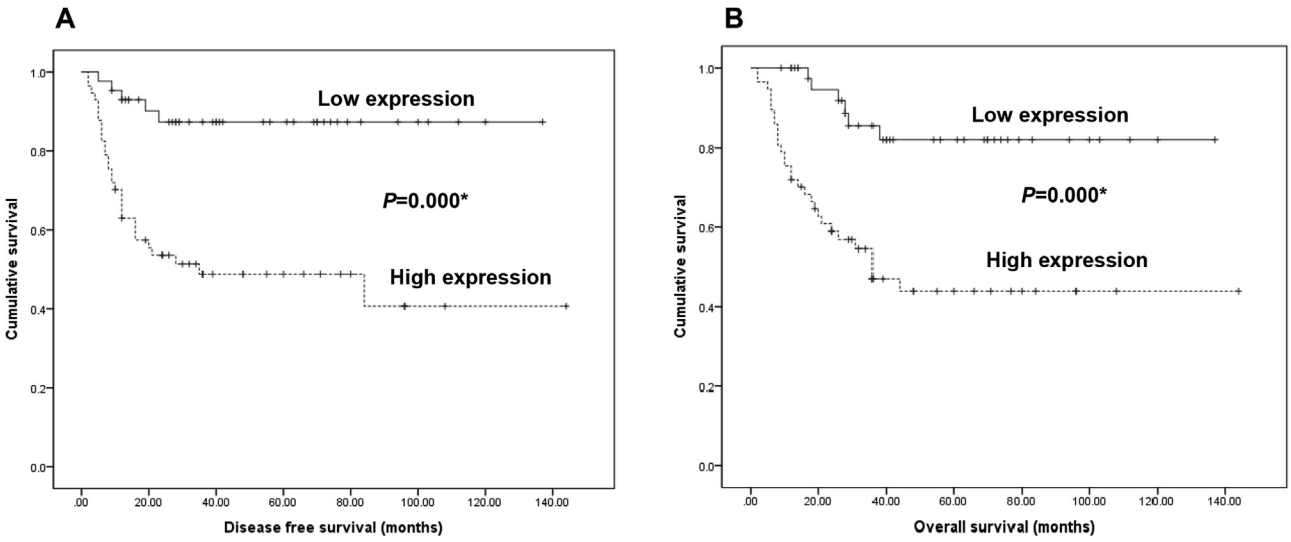

Fig. 2. Kaplan–Meier curves for (A) disease-free survival (DFS) and (B) overall survival (OS) based on the LAMB3 protein level in tumor tissues as determined by Western blot analysis.

Discussion

Tumor initiation and progression may involve an array of molecular mechanisms that underlie the complexity and heterogeneity of cancer. At least three important classes of genes play key roles in tumor initiation: proto-oncogenes, tumor suppressor genes, and genes involved in DNA repair mechanisms [21]. Cancer metastasis requires multiple cellular events, including cytoskeletal alterations, disruption of cell-to-cell adhesive contacts, increased motility and invasiveness, entry and survival in the circulation, and spread into new tissue [22]. To identify genes involved in HNSCC carcinogenesis, we analyzed transcriptomic profiles in non-tumor and tumor tissues from HNSCC patients using next-generation sequencing technology. One of the validated hits was LAMB3.

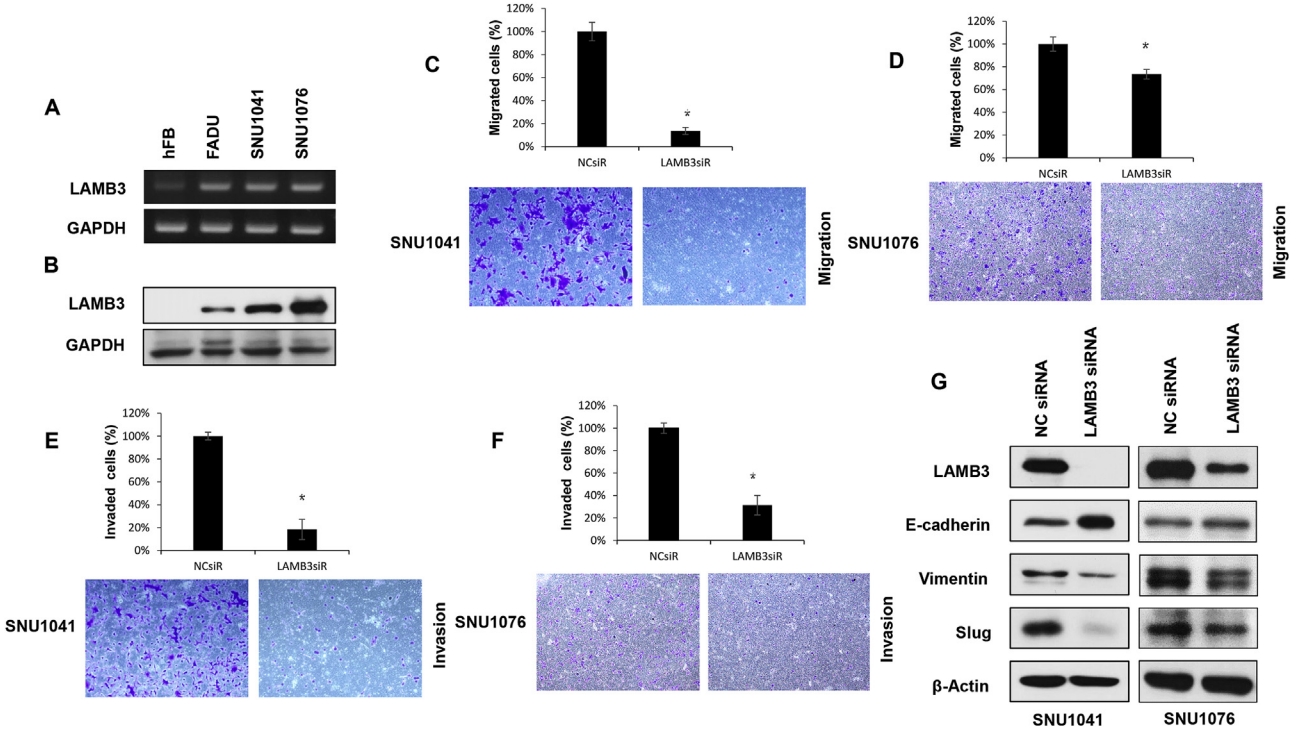

Fig. 3. LAMB3 expression and migration, invasion and EMT-related protein expression by LAMB3 in HNSCC cell lines. (A) Cell lysates were prepared from human fibroblasts (hFBs) and three HNSCC cell lines (FaDu, SNU1041, and SNU1076) were examined by reverse-transcription PCR analysis (B) Western blot analysis using an anti-LAMB3 antibody. SNU1041 (C, E) and SNU1076 (D, F) cell lines were transiently transfected with LAMB3 siRNA or negative control siRNA for 48 h. Cells were allowed to migrate for 48 h in Transwell chambers (cell migration) or in chambers coated with Matrigel (cell invasion). To quantify migration and invasion, stained cells were counted under a light microscope. The data represent the means ± standard deviation of three independent experiments. $^*P < 0.05$. (G) SNU1041 and SNU1076 cell lines were transiently transfected with LAMB3 siRNA or negative control siRNA for 48 h. After transfection, the expression of LAMB3 and EMT-related proteins, including E-cadherin, Vimentin, and Slug, was evaluated by Western blot analysis. Each figure is representative of three independent experiments.

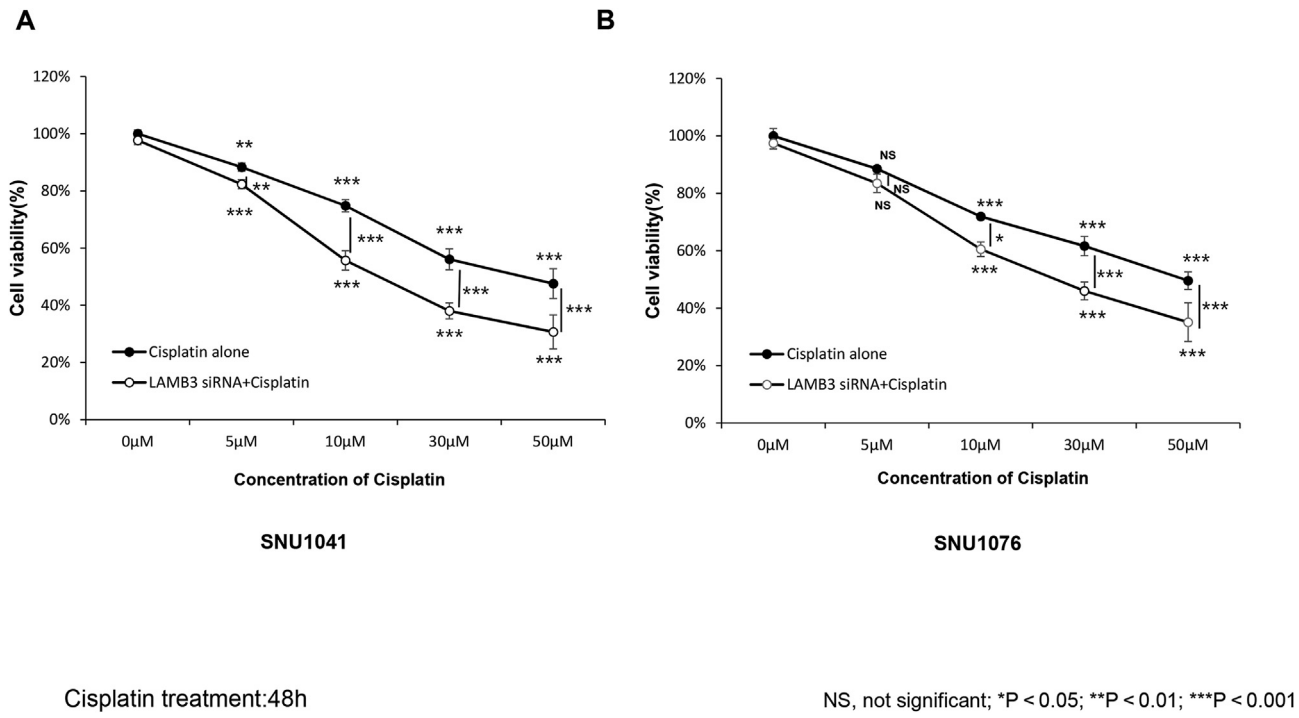

Fig. 4. LAMB3 suppression increases cisplatin cytotoxicity in HNSCC cells. Following exposure of the indicated cell lines to various concentrations of cisplatin for 48 h, cell viability was measured by the WST-1 proliferation assay. LAMB3 siRNA-transfected cells demonstrated marked cisplatin-induced cytotoxic effects compared with control siRNA-transfected cells in SNU1041(A) and SNU1076(B). The data represent the means ± standard deviation of three independent experiments. NS, not significant; *P < 0.05; * *P < 0.01; * * *P < 0.001.

LAMB3 encodes one of the three subunits of laminin-332, a protein component of the extracellular matrix. Laminin-332 has been well studied in tumorigenesis at the biological and molecular signaling levels [12,23]. In squamous cell carcinoma, laminin-332 promotes tumorigenesis via interactions with cell surface receptors such as epidermal growth factor receptor, integrins, and syndecan 1 [24]. LAMB3, belonging to the laminin family of basement membrane proteins, was previously found overexpressed in papillary thyroid carcinoma [25]. However, the molecular mechanism of aberrant LAMB3 expression in HNSCC is not well understood. To determine the clinical relevance of LAMB3 expression in HNSCC, we evaluated all parts of the head and neck in our study cohort and demonstrated a correlation between high LAMB3 expression and lymph node metastasis in HNSCC patients. Furthermore, we found that LAMB3 expression is associated with a poor prognosis in HNSCC patients. The above results indicate that LAMB3 expression represents a promising marker predictive of prognosis in patients with HNSCC.

Kwon et al. found that LAMB3 was overexpressed in many cancer types [8]. In our study, we found that both the mRNA and protein levels of LAMB3 were overexpressed in HNSCC cell lines. We hypothesized that LAMB3 overexpression may be involved in HNSCC tumorigenesis. A previous study demonstrated that LAMB3 functions as an oncogene to influence cell proliferation [26,27].

However, in our study, LAMB3 suppression has no effect on the proliferation of HNSCC cells, in contrast to a finding from a previous report. These results suggest that LAMB3 participates in cellular events other than proliferation in HNSCC. We also reported evidence supporting a biological link between LAMB3 and cell migration/invasion, which are essential for HNSCC tumor progression and metastasis. In our study, LAMB3 down-regulation increased E-cadherin expression and decreased the expression of Vimentin and Slug, suggesting Slug-dependent E-cadherin regulation and EMT induction in HNSCC cells. This evidence was accompanied by reductions in migration and invasion induced by LAMB3 suppression, in agreement with the findings by Kinoshita et al. that LAMB3 is directly regulated by miR-218, while LAMB3 silencing significantly inhibits cell migration and invasion [5].

Drug resistance is a major obstacle in cancer chemotherapy. Many solid tumors initially respond to chemotherapeutic drugs, but most will recur as a result of drug resistance [28]. Cisplatin, one of the most well-known chemotherapeutic drugs, is currently used for the treatment of numerous cancers including head and neck, lung, bladder, and testicular cancers [29]. It is a cytotoxic drug that kills cancer cells by damaging DNA, inhibiting DNA synthesis and mitosis, and inducing apoptosis. Intriguingly, we found that LAMB3 silencing increased the chemosensitivity of HNSCC cell lines to cisplatin, although LAMB3 silencing alone did not have an effect on cell viability.

Heterogeneity of the study cohort is one of the limitations of this study. In addition, we excluded the patients infected by HPV due to their rarity. Further robust studies using homogenous large cohort are mandatory.

In conclusion, our findings suggest that LAMB3 may be an independent predictor of clinical prognosis that is associated with responses to platinum-based chemotherapy in HNSCC. In the future, the combination of a LAMB3-targeting drug with conventional treatment modalities may be an effective strategy to overcome drug resistance, leading to improved therapies for HNSCC patients with a better and more durable clinical response.

Disclaimers

The authors indicate no financial disclosures.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejso.2018.10.543.

References

[1] Cognetti DM, Weber RS, Lai SY. Head and neck cancer: an evolving treatment paradigm. Cancer 2008;113:1911–32.
[2] Duray A, Demoulin S, Hubert P, et al. Immune suppression in head and neck cancers: a review. Clin Dev Immunol 2010. 2010:701657.
[3] Arora S, Aggarwal P, Pathak A, et al. Molecular genetics of head and neck cancer (Review). Mol Med Rep 2012;6:19–22.
[4] Leemans CR, Braakhuis BJ, Brakenhoff RH. The molecular biology of head and neck cancer. Nat Rev Canc 2011;11:9–22.
[5] Kinoshita T, Hanazawa T, Nohata N, et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion through targeting laminin-332 in head and neck squamous cell carcinoma. Oncotarget 2012;3:1386–400.
[6] Patarroyo M, Tryggvason K, Virtanen I. Laminin isoforms in tumor invasion, angiogenesis and metastasis. Semin Canc Biol 2002;12:197–207.
[7] Wang XM, Li J, Yan MX, et al. Integrative analyses identify osteopontin, LAMB3 and ITGB1 as critical pro-metastatic genes for lung cancer. PloS One 2013;8, e55714.
[8] Kwon OH, Park JL, Kim M, et al. Aberrant up-regulation of LAMB3 and LAMC2 by promoter demethylation in gastric cancer. Biochem Biophys Res Commun 2011;406:539–45.
[9] Young AL, Bailey EE, Colaco SM, et al. Anti-laminin-332 mucous membrane pemphigoid associated with recurrent metastatic prostate carcinoma: hypothesis for a paraneoplastic phenomenon. Eur J Dermatol: EJD 2011;21:401–4.
[10] Tripathi M, Nandana S, Yamashita H, et al. Laminin-332 is a substrate for hepsin, a protease associated with prostate cancer progression. J Biol Chem 2008;283:30576–84.
[11] Tsuruta D, Kobayashi H, Imanishi H, et al. Laminin-332-integrin interaction: a target for cancer therapy? Curr Med Chem 2008;15:1968–75.
[12] Marinkovich MP. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nat Rev Canc 2007;7:370–80.
[13] Udayakumar TS, Chen ML, Bair EL, et al. Membrane type-1-matrix metalloproteinase expressed by prostate carcinoma cells cleaves human laminin-5 beta3 chain and induces cell migration. Cancer Res 2003;63:2292–9.
[14] Remy L, Trespeuch C, Bachy S, et al. Matrilysin 1 influences colon carcinoma cell migration by cleavage of the laminin-5 beta3 chain. Cancer Res 2006;66:11228–37.
[15] Sauter G, Simon R, Hillan K. Tissue microarrays in drug discovery. Nat Rev Drug Discov 2003;2:962–72.
[16] Chang JW, Jung SN, Kim JH, et al. Carboxyl-terminal modulator protein positively acts as an oncogenic driver in head and neck squamous cell carcinoma via regulating akt phosphorylation. Sci Rep 2016;6:28503.
[17] Hsu SM, Lin YC, Chang JW, et al. Intramolecular interactions of a phenyl/perfluorophenyl pair in the formation of supramolecular nanofibers and hydrogels. Angew Chem 2014;53:1921–7.
[18] Chang JW, Gwak SY, Shim GA, et al. EZH2 is associated with poor prognosis in head-and-neck squamous cell carcinoma via regulating the epithelial-to-mesenchymal transition and chemosensitivity. Oral Oncol 2016;52:66–74.
[19] Taniuchi K, Nakagawa H, Hosokawa M, et al. Overexpressed P-cadherin/CDH3 promotes motility of pancreatic cancer cells by interacting with p120ctn and activating rho-family GTPases. Cancer Res 2005;65:3092–9.
[20] Knezevic J, Pfefferle AD, Petrovic I, et al. Expression of miR-200c in claudin-low breast cancer alters stem cell functionality, enhances chemosensitivity and reduces metastatic potential. Oncogene 2015;34:5997–6006.
[21] Tysnes BB, Bjerkvig R. Cancer initiation and progression: involvement of stem cells and the microenvironment. Biochim Biophys Acta 2007;1775:283–97.
[22] Mierke CT, Rosel D, Fabry B, et al. Contractile forces in tumor cell migration. Eur J Cell Biol 2008;87:669–76.
[23] Guess CM, Quaranta V. Defining the role of laminin-332 in carcinoma. Matrix Biol: J Int Soc Matrix Biol 2009;28:445–55.
[24] Ortiz-Urda S, Garcia J, Green CL, et al. Type VII collagen is required for Ras-driven human epidermal tumorigenesis. Science 2005;307:1773–6.
[25] Aldred MA, Huang Y, Liyanarachchi S, et al. Papillary and follicular thyroid carcinomas show distinctly different microarray expression profiles and can be distinguished by a minimum of five genes. J Clin Oncol: Official J Am Soc Clin Oncol 2004;22:3531–9.
[26] Yamamoto N, Kinoshita T, Nohata N, et al. Tumor suppressive microRNA-218 inhibits cancer cell migration and invasion by targeting focal adhesion pathways in cervical squamous cell carcinoma. Int J Oncol 2013;42:1523–32.
[27] Calaluce R, Bearss DJ, Barrera J, et al. Laminin-5 beta3A expression in LNCaP human prostate carcinoma cells increases cell migration and tumorigenicity. Neoplasia 2004;6:468–79.
[28] Chang X, Monitto CL, Demokan S, et al. Identification of hypermethylated genes associated with cisplatin resistance in human cancers. Cancer Res 2010;70:2870–9.
[29] Dasari S, Tchounwou PB. Cisplatin in cancer therapy: molecular mechanisms of action. Eur J Pharmacol 2014;740:364–78.