Laminin-332 promotes the invasion of oesophageal squamous cell carcinoma via PI3K activation

Y Baba1,2, K-I Iyama2, K Hirashima1, Y Nagai1, N Yoshida1, N Hayashi1, N Miyanari1 and H Baba*1,1

1Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University 1-1-1 Honjo, Kumamoto City, Kumamoto 860-8556, Japan; 2Department of Surgical Pathology, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto City, Kumamoto 860-8556, Japan

Laminin-332 is major component of epithelial basement membrane, and has an important role in cell migration and tumour invasion. Recently, the phosphatidylinositol 3-kinase (PI3K) activation induced by laminin-332 during carcinogenesis or tumour invasion has been highlighted in skin squamous cell carcinoma. The expression of laminin-332 in 126 resected oesophageal squamous cell carcinoma (ESCC) specimens was immunohistochemically examined to determine its associations with the clinicopathological characteristics, and the effect of laminin-332 on the invasiveness and the PI3K activation was assessed in vitro experiments using ESCC cell lines (ESCCs). Sections with immunostaining signals in >30% cancer cells, which were observed in 55 of 126 cases, were judged to be positive for laminin-332. The positivity was significantly correlated with pTNM stage and poor prognosis. Inactivation of the PI3K pathway by laminin-332 blocking antibody suppressed the invasiveness of TE8 cell line, which secreted laminin-332 at high level and had high PI3K activity. The addition of the purified laminin-332 activated the PI3K pathway and increased the invasiveness of TE11 cell line, which secreted laminin-332 at lower level and had low PI3K activity. The deactivation of PI3K pathway using the PI3K inhibitor decreased the invasiveness of ESCCs and the secretion of laminin-332 in vitro. The expression of laminin-332 was one of the prognostic factors of ESCC. Laminin-332 could provide the autocrine positive-feedback loop through PI3K activation, contributing the invasive ability. Therefore, the inhibitor of PI3K pathway might be useful as the anticancer therapies for ESCC.

Keywords: laminin-332; PI3K pathway; oesophageal cancer; basement membrane; prognostic factor
data showing the relationship between laminin-332 and cancer cell invasiveness, using oesophageal squamous cancer cell lines (ESCCs).

The aims of this study are (1) to investigate the immunohistochemical expression of laminin-332 in ESCC and to evaluate the relationships between the clinicopathological characteristics and survival, and (2) to examine the biological role and the signalling pathway of laminin-332 using ESCCs.

MATERIALS AND METHODS

Patients

The present study involved 126 consecutive patients with ESCC who underwent the curative surgical resection at Kumamoto University Hospital from January 1997 to December 2006. All of these patients underwent an oesophagectomy with lymph node dissection. None of these patients underwent endoscopic mucosal resection, palliative resection, preoperative chemotherapy or radiotherapy, and none of them had synchronous or metachronous multiple cancers in other organs. Clinical data, including age, sex, tumour location, lymph node metastasis, T classification (Sobin and Wittekind, 2002), TNM stage (Sobin and Wittekind, 2002) and histological grading (Hamilton and Aaltonen, 2000), were available for all 126 patients. Patients were periodically (every 1–3 months) examined on an outpatient basis to make sure they did not have disease recurrence. The mean follow-up period for the 126 patients was 29.6 months (range, 1–110 months). Informed consent for the research was obtained from each patient. The study design was approved by the ethic review board of our university.

Immunohistochemistry

Sections of 5 μm in thickness were dewaxed in xylene and then heated to 132°C in an autoclave for 5 min in 10 mmol l⁻¹ citrate buffer (pH 6.3). The endogenous peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 60 min. The sections were immersed in 5% normal horse serum in PBS for 30 min, covered with mouse peroxidase activity was suppressed by a solution of 3% hydrogen peroxide in methanol for 60 min. The sections were immersed in 5% normal horse serum in PBS for 30 min, covered with mouse

ABC kit (Vector Laboratories, Peterborough, UK). The antigenic sites were demonstrated by reacting the sections with a mixture of 0.05% 3,3-diaminobenzidine tetrahydrochloride (Dojindo Chemicals, Kumamoto, Japan) in 0.05% Tris-HCl buffer, pH 7.6, containing 0.01% H2O2 for 5 min. The nuclei were stained with haematoxylin.

Sections with immunohistochemical staining in >30% of carcinoma cells at the invasive front were judged to be positive for laminin-332 as with the previous reports (Yamamoto et al, 2001; Fukai et al, 2005). The staining assessment was independently carried out by two of the authors (KI and YB) without any knowledge of either the clinical or survival data.

Cell culture

ESCCs were obtained from Cell Resource Center for Biomedical Research, Tohoku University, Japan. Cell cultures were grown in recommended medium with 10% fetal bovine serum (FBS) and incubated in 5% CO₂ at 37°C.

Antibodies and other reagents

Antibodies for western blot analysis were as follows: rabbit anti-phospho Akt, rabbit anti-Akt (Cell Signaling Technology, Danvers, USA), mouse anti-laminin γ2 chain (Chemicon), mouse anti-epidermal growth factor receptor (ZYMED Laboratories, San Francisco, USA), mouse anti-integrin β4 (Santa Cruz Biotechnology, Santa Cruz, USA). Anti-laminin γ2 antibody (Chemicon) was used as the blocking antibody as with previous article (Decline and Roussele, 2001). The purified human laminin-332 was purchased from Biodesign Internatinal, and the PI3K inhibitor Wortmannin was purchased from Santa Cruz Biotechnology.

Western blot analysis

Cultured cells were solubilised with the lysis buffer (Tris-HCl (pH7.4), 25 mm, NaCl 100 mm, EDTA 2 mm, TritonX 1% with 10 μg ml⁻¹ Aprotinin, 10 μg ml⁻¹ Leupeptin, 1 mm Na₃VO₄, 1 mm PMSF). To evaluate the secreted laminin-332 in the cell culture medium, each cell line was plated at 1 × 10⁶ cells per 100-mm culture dish in serum-free medium. Conditioned medium was collected at 24 h. 10 μg of each protein sample was mixed with 4 × sample buffer including 10% β-mercaptoethanol and boiled 5 min. Next, equal amount of samples were separated on 7.5% for the reduced laminin-332, 6.0% for the non-reduced laminin-332 or 12% (for others) SDS–polyacrylamide gels and transferred to PVDF membranes. The membranes were probed with each primary antibody overnight at room temperature, followed by a 1:1000 dilution of peroxidase-conjugated IgG antibody (Sigma, St Louis, USA). Detection was accomplished with an ECL reagent (Amersham, Buckinghamshire, UK).

To evaluate the relationship between the secretion of laminin-332 and the PI3K pathway, the confluent cells were incubated with the PI3K inhibitor (Wortmannin) at different concentration for 24 h, and the culture medium was collected. Equal amount of samples were separated on 7.5% SDS–polyacrylamide gel and transferred to PVDF membranes.

Cell-invasion assay

In vitro cell invasion assay was conducted using Biocat Matrigel Invasion Chambers (BD Biosciences Discovery Labware, Franklin, USA) according to the manufacturer’s protocols. After detaching the cells in 0.25% trypsin and counting, cells were diluted to 100 000 cells per ml in medium counting 0.5% FBS. A total of 500 μl of cells were then placed in quintuple in the top chamber of the insert above medium counting 10% FBS, and were incubated in 37°C, 5% CO₂ for 22 h. Next, the cells on the upper surface of the membrane were removed with a cotton swab, and the migrated cells on the underside of the membrane were fixed and stained using toluidine blue and counted in five random fields at ×200 magnification.

To examine the effects of the purified laminin-332, anti-laminin-332 blocking antibody and the PI3K inhibitor (Wortmannin) on the invasiveness of ESCCs, the purified laminin-332 (0.5 μg ml⁻¹), anti-laminin-332 blocking antibody (40 μg ml⁻¹) or the PI3K inhibitor (Wortmannin; 100 nm) was added into the top chamber. As controls, the purified albumin (0.5 μg ml⁻¹) or anti-IgG antibody (40 μg ml⁻¹) was used.

Statistical analysis

Laminin-332 expression in ESCC was assessed to identify any associations with clinicopathological parameters using χ²-test for unpaired samples. Statistical analysis was performed using SPSS software (version 13 for Windows; SPSS, Chicago, IL, USA) according to the manufacturer’s protocols. After detaching the cells in 0.25% trypsin and counting, cells were diluted to 100 000 cells per ml in medium counting 0.5% FBS. A total of 500 μl of cells were then placed in quintuple in the top chamber of the insert above medium counting 10% FBS, and were incubated in 37°C, 5% CO₂ for 22 h. Next, the cells on the upper surface of the membrane were removed with a cotton swab, and the migrated cells on the underside of the membrane were fixed and stained using toluidine blue and counted in five random fields at ×200 magnification.

To examine the effects of the purified laminin-332, anti-laminin-332 blocking antibody and the PI3K inhibitor (Wortmannin) on the invasiveness of ESCCs, the purified laminin-332 (0.5 μg ml⁻¹), anti-laminin-332 blocking antibody (40 μg ml⁻¹) or the PI3K inhibitor (Wortmannin; 100 nm) was added into the top chamber. As controls, the purified albumin (0.5 μg ml⁻¹) or anti-IgG antibody (40 μg ml⁻¹) was used.

Statistical analysis

Laminin-332 expression in ESCC was assessed to identify any associations with clinicopathological parameters using χ²-test for unpaired samples. Statistical analysis was performed using Mann–Whitney’s U-test for unpaired samples. Statistical analysis was performed using SPSS software (version 13 for Windows; SPSS, Chicago, IL, USA).
USA). A two-sided significance level of $P<0.05$ was used for all statistical analyses.

RESULTS

Immunohistochemical expression of laminin-332 in oesophageal squamous cell carcinomas

In normal oesophageal epithelium, laminin-332 γ2 chain was stained weakly in the cytoplasm of epithelial cells near basal layer. (Figure 1A and B) In some cancer tissues, the cytoplasm of cancer cells was stained for laminin-332 γ2 chain at levels much stronger than those in normal BM. Sections with immunostaining signals in >30% of carcinoma cells, which were observed in 55 (44%) cases, were judged to be positive for laminin-332 γ2 chain expression (Figure 1C and D).

The relationships between laminin-332 expression and clinicopathological characteristics are summarised in Table 1. The expression of laminin-332 was significantly correlated to TNM stage ($P=0.036$). The patients with laminin-332-positive ESCC had a higher tendency to have lymph node metastasis and deep invasion than those with negative; however, the differences were not statistically significant ($P=0.14$ and $P=0.15$, respectively). On the other hand, there were no significant relationships between laminin-332 expression and age, sex, tumour location and histological grading.

Patients with laminin-332-positive tumours had a significantly shorter disease-free survival and overall cancer-specific survival than patients with laminin-332 negative tumours ($P=0.0139$ and $P=0.0345$, respectively; Figure 2). In univariate analysis, the significant prognostic factors for predicting both the disease-free survival and overall cancer-specific survival were histological grading, T classification, TNM stage, lymph node metastasis and laminin-332 expression. In multivariate analysis, laminin-332 expression was the independent prognostic factors for predicting the disease-free survival, but not for the overall cancer-specific survival (Table 2).

| Characteristic | Total ($n=126$) | Negative ($n=71$) | Positive ($n=55$) | $P$-value |
|---------------|----------------|-------------------|-------------------|-----------|
| Mean age      |                | 63.7 ± 5.3        | 62.8 ± 4.9        | 0.81      |
| Sex           |                |                   |                   |           |
| Male          | 106            | 59                | 47                |           |
| Female        | 20             | 12                | 8                 |           |
| Tumour location |               |                   |                   | 0.62      |
| Upper         | 27             | 13                | 14                |           |
| Middle        | 57             | 33                | 24                |           |
| Lower         | 42             | 25                | 17                |           |
| Histological grading | |                   |                   | 0.75      |
| Well          | 51             | 29                | 22                |           |
| Moderate      | 48             | 26                | 22                |           |
| Poorly        | 27             | 16                | 11                |           |
| T classification |           |                   |                   | 0.15      |
| T1            | 63             | 39                | 24                |           |
| T2            | 24             | 15                | 9                 |           |
| T3            | 39             | 17                | 22                |           |
| N status      |                |                   |                   | 0.14      |
| N0            | 62             | 39                | 23                |           |
| N1            | 64             | 32                | 32                |           |
| Stage         |                |                   |                   | 0.036     |
| I             | 47             | 30                | 16                |           |
| II A          | 15             | 9                 | 6                 |           |
| II B          | 31             | 20                | 11                |           |
| III           | 33             | 12                | 22                |           |

N status indicates lymph node metastasis status. $P$-values were calculated by using $\chi^2$ and Fisher’s exact tests.

Figure 1 Immunohistochemical staining of laminin-332. Original magnification: x 200 (A,C,D) x 400 (B). (A, B) Expression of laminin-332 in normal oesophageal epithelium. Laminin-332 is expressed faintly in the cytoplasm of epithelial cells in the basal layer. (C) Positive staining of laminin-332 of ESCC is indicated. Laminin-332 is clearly shown in the cytoplasm of ESCC. (D) Negative staining of laminin-332 of ESCC is indicated.
Expression of laminin-332 in oesophageal cancer cell lines

A western blot analysis showed that all five ESCC cell lines (TE1, TE8, TE9, TE10, TE11) secreted laminin-332 γ2 chain into the conditioned medium, but the secreted level of TE11 was lowest (Figure 3A). In the reduced sample (upper panel), the 155-kDa band revealed the uncleaved laminin-332 γ2 chain, whereas the 105-kDa band revealed the cleaved laminin-332 γ2 chain. In the nonreduced sample (lower panel), the upper band 155-kDa band revealed the uncleaved laminin-332 γ2 chain, whereas the lower band revealed the laminin-332 heterotrimer including the uncleaved laminin-332 γ2 chain, whereas the lower band revealed the laminin-332 heterotrimer including the cleaved laminin-332 γ2 chain.

Expression of EGFR and integrin β4 in oesophageal cancer cell lines

All five ESCCs expressed EGFR at the protein level. But the band intensity was different between each ESCC, and showed expression at the highest level in TE8 and at the lowest level in TE1 (Figure 3B). All five ESCCs expressed integrin β4 at the protein level at the approximately same level (Figure 3B).

PI3K activation in oesophageal cancer cell lines

We examined the activity of PI3K pathway by using the phosphorylated Akt (p-Akt) antibody by western blot analysis. The band intensity of p-Akt showed expression at higher level in TE8, TE9 and TE10 than in TE1, TE11 (Figure 3C).

Laminin-332-blocking antibody inhibit PI3K activity and invasion in TE10

The effect of the laminin-332-blocking antibody on PI3K pathway was tested in vitro using TE10, which secreted laminin-332 at the highest level among cell lines and had high PI3K activity in vitro. After the incubation with the laminin-332-blocking antibodies at different concentration for 24 h, the cells were solubilised. The laminin-332-blocking antibody suppressed the activation of PI3K pathway at the concentration of > 40 μg ml⁻¹ (Figure 4A). The similar results were achieved by using TE8 and TE9 (data not shown). Next, we investigated whether the laminin-332-blocking antibody have an effect on the invasiveness of TE10 in vitro using the Matrigel-coated invasion chamber. The number of invaded cells decreased to an approximately half with the laminin-332-blocking antibody (40 μg ml⁻¹) by comparison with non-treated or the IgG antibody (40 μg ml⁻¹). The PI3K inhibitor (Wortmannin; 100 nm) decreased the number of invaded cells to an approximately quarter by comparison with non-treatment (Figure 4B).

Laminin-332 promotes invasion via PI3K activation in TE11

The effect of the purified laminin-332 on PI3K pathway was examined in vitro using TE11, which secreted laminin-332 at the lowest level. After the incubation with the purified laminin-332 at different concentration for 6 h, the cells were solubilised. The laminin-332 activated the PI3K pathway at the concentration of laminin-332 (Figure 5A). The number of invaded cells increased significantly with the purified laminin-332 (0.5 μg ml⁻¹) by comparison with non-treatment or control-purified albumin (0.5 μg ml⁻¹); however the addition of the PI3K inhibitor (Wortmannin; 100 nm) decreased the number of invaded cells significantly (Figure 5B).

Effect of PI3K inhibitor on the secretion of laminin-332 in vitro

The relationship between the secretion of laminin-332 and PI3K pathway was also investigated. The PI3K inhibitor (Wortmannin) decremented the activity of PI3K pathway in a dose-dependent manner, and the amount of the secreted laminin-332 into the medium decreased in proportion to the activity of PI3K pathway (Figure 6).

DISCUSSION

In the present study, the laminin-332 expression in cancer cells at the invasive front was seen in 44% of ESCC, and ESCC patients...
with its positivity had poorer prognosis than patients with its negativity. This result was consistent with previous reports about ESCC (Yamamoto et al., 2001; Fukai et al., 2005) and suggested that laminin-332 at the invasive front of ESCC might have an important role in the process of tumour invasion.

Laminin-332 has been reported to have potent cell migration-promoting activity. Miyazaki and co-workers showed that both...
Laminin-332 in oesophageal cancer

Y Baba et al

soluble and deposited laminin-332 could stimulate the migration of human normal epithelial cells and cancer cells in vitro (Miyazaki et al, 1993; Kariya and Miyazaki, 2004). Laminin-332 has been reported to interact with integrin α3β1 or α6β4 and activate many signal mediators such as PI3K, focal adhesion kinase, protein kinase C, Rac, ERK, JNK and nuclear factor κB, leading to enhanced cell migration and invasion (Nguyen et al, 2000; Kariya and Miyazaki, 2004; Nikolopoulos et al, 2005). In particular, the importance of PI3K activation induced by laminin-332 during carcinogenesis or tumour invasion has been recently suggested (Marinkovich, 2007; Waterman et al, 2007). Waterman and co-workers demonstrated that by in vivo model of human skin keratinocytes, the binding of laminin-332 to α6β4 integrin associated and drove tumorgenesis and invasion thorough PI3K activation in skin squamous cell carcinoma (Waterman et al, 2007). Furthermore, the interaction between laminin-332 /β2 chain III domain and EGFR has been suggested to have a role in PI3K activation via the action of PVK kinase (Schenk et al, 2003; Guo et al, 2006; Marinkovich, 2007). Briefly, the interaction of laminin-332 with α6β4 integrin and EGFR is involved in promoting PI3K activation and tumour invasion. To our knowledge, as there has been no article showing the relationship between laminin-332 and PI3K activation in ESCC, we focused on the relationship between the PI3K activation and invasiveness of ESCCs in the current study.

PI3K is a major signalling component downstream of growth factor receptor tyrosine kinase, and the PI3K pathway regulates various cellular processes, such as proliferation, growth, apoptosis and cytokoskeleton rearrangement (Cantley, 2002). The aberrant activation of the PI3K pathway has been widely implicated in several types of carcinomas (Vivanco and Sawyers, 2002; Fresno Varo et al, 2004). In our in vitro study, the PI3K activity was assessed at higher level in TE8, TE9 and TE10 than in TE1 and TE11. As TE8, TE9 and TE10 secreted laminin-332 at higher level and also expressed EGFR and integrin /β4, which were interacted with laminin-332, and TE11 secreted laminin-332 at very low level, the levels of PI3K activity in these ESCCs were understandable. Similarly in TE1, as this cell line expressed EGFR at very low level, the secreted laminin-332 might be unable to induce intracellular signal transduction in vitro. In fact, the addition of the purified laminin-332 did not activate the PI3K pathway in TE1 in vitro (data not shown). The laminin-332-blocking antibody deactivated the PI3K pathway, leading to suppress the invasiveness of TE10, which secreted laminin-332 at high level and had high PI3K activity. On the contrary, the addition of the purified laminin-332 activated the PI3K pathway, leading to increase the invasiveness of TE11, which secreted laminin-332 at lower level and had weak PI3K activity; however, the accession of the PI3K inhibitor may decrease the invasiveness. These results suggest that laminin-332 could enhance the invasiveness of ESCCs through the PI3K activation.

In our in vitro study, the deactivation of PI3K pathway using PI3K inhibitor decreased the secretion of laminin-332, hence the PI3K activation was considered to be required for the secretion of laminin-332. Laminin-332 might provide the autocrine positive-feedback loop via the PI3K activation, contributing the invasive ability. These results suggest that the inhibitor of PI3K pathway could be useful as the anticancer therapy for ESCC (Luo et al, 2003). As to the downstream of PI3K pathway, further studies are needed.

In conclusion, the increased laminin-332 immunoreactivity is one of the prognostic factors of ESCC. Laminin-332 could provide the autocrine positive-feedback loop through the PI3K activation, contributing the invasive ability, hence the inhibitor of PI3K pathway might be useful as the anticancer therapies for ESCC.

REFERENCES

Beck K, Hunter I, Engel J (1990) Structure and function of laminin: anatomy of a multidomain glycoprotein. FASEB J 4: 148 – 160
Cantley LC (2002) The phosphoinositide 3-kinase pathway. Science 296: 1655 – 1657
Carter WG, Kaur P, Gil SG, Gahr PJ, Wayner EA (1990) Distinct functions for integrins α3β1 or α6β4 in epithelial basement membranes. Cell 65: 599 – 619
Decline F, Rousselle P (2001) Keratinocyte migration requires α3β1 integrin-mediated interaction with the laminin 5 gamma2 chain. J Cell Sci 114: 811 – 823
Engle J (1992) Laminins and other strange proteins. Biochemistry 31: 10643 – 10651
Enzinger PC, Mayer RJ (2003) Esophageal cancer. N Engl J Med 349: 2241 – 2252
Fresno Vara JA, Casado E, De Castro J, Cejas P, Belda-Iniesta C, González-Barón M (2004) PI3K/Akt signalling pathway and cancer. Cancer Treat Rev 30: 193 – 204
Fukai Y, Masuda N, Kato H, Fukuchi M, Miyazaki T, Nakajima M, Soluda M, Kuwano H, Nakajima T (2005) Correlation between Laminin-5 /β2 chain and Epidermal growth factor receptor expression in esophageal squamous cell carcinomas. Oncology 69: 71 – 80
Guo W, Pylayeva Y, Pepe A, Yoshioa T, Muller WJ, Inghirami G, Gianocotti FG (2006) Beta 4 integrin amplifies ErbB2 signaling to promote mammary tumorgenesis. Cell 126: 489 – 502
Hamilton SR, Aaltonen LA (2000) World Health Organization classification of tumours. Tumours of the Digestive System. Lyon: IARC Press
Kariya Y, Miyazaki K (2004) The basement membrane protein laminin-5 acts soluble cell motility factor. Exp Cell Res 297: 508 – 520
Koshikawa N, Moriyma K, Takamura H, Mizushima H, Nagashima Y, Yanoma S, Miyazaki K (1999) Overexpression of laminin /β2-chain monomer in invading gastric carcinoma cells. Cancer Res 59: 5596 – 5601
Luo J, Manning BD, Cantley LC (2003) Targeting the PI3K – Akt pathway in human cancer: rationale and promise. Cancer Cell 4: 257 – 262
Marinkovich MP (2007) Laminin 332 in squamous-cell carcinoma. Nature Rev Cancer 7: 370 – 380
Matta M, Soini Y, Paakko P, Salo S, Tryggvason K, Autio-Harmainen H (1999) Expression of the laminin γ2 chain in different histological types of lung carcinoma. A study of immunohistochemistry and in situ hybridization. J Pathol 188: 361 – 368

Miyazaki K (2006) Laminin-5 (laminin-332) Unique biological activity and role in tumor growth and invasion. Cancer Sci 97: 91 – 98

Miyazaki K, Kikkawa Y, Nakamura A, Yasumitsu H, Umeda M (1993) A large cell-adhesive scatter factor secreted by human gastric carcinoma cells. Proc Natl Acad Sci USA 90: 508 – 520

Nguyen BP, Gil SG, Carter WG (2000) Deposition of laminin 5 by keratinocytes regulates integrin adhesion and signaling. J Biol Chem 275: 31896 – 31907

Nikolopoulos SN, Blaikie P, Yoshioka T, Guo W, Puri C, Tacchetti C, Giancotti FG (2005) Targeted deletion of the integrin beta4 signaling domain suppresses laminin-5-dependent nuclear entry of mitogen-activated protein kinases and NF-kappaB, causing defects in epidermal growth and migration. Mol Cell Biol 25: 6090 – 6102

Ono Y, Nakanishi Y, Ino Y, Niki T, Yamada T, Yoshimura K, Saikawa M, Nakajima T, Hirohashi S (1999) Clinicopathologic significance of laminin γ2 chain in squamous cell carcinoma of the tongue: immunohistochemical analysis of 67 lesions. Cancer 85: 2315 – 2321

Pyke C, Salo S, Ralfikiaer E, Romer J, Dano K, Tryggvason K (1995) Laminin-5 is a marker of invading cancer cells in some human carcinomas and its coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. Cancer Res 55: 4132 – 4139

Rousselie P, Lunstrum GP, Keene DR, Burgeson RE (1991) Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 114: 567 – 576

Ryan MC, Christiano AM, Enqvall E, Wewer UM, Miner JH, Sanes JR, Burgeson RE (1996) The functions of laminins: lessons from in vivo studies. Matrix Biol 15: 369 – 381

Schenk S, Hintermann E, Bilban M, Koshikawa N, Hojilla C, Khokha R, Quaranta V (2003) Binding to EGF receptor of a laminin-5 EGF-like fragment liberated during MMP-dependent mammary gland involution. J Cell Biol 161: 197 – 209

Sobin LH, Wittekind Ch (2002) TNM classification of malignant tumors, 6th ed New York: John Wiley&Sons Inc

Soini Y, Maatta M, Salo S, Tryggvason K, Autio-Harmainen H (1996) Expression of laminin γ2 chain in pancreatic adenocarcinoma. J Pathol 180: 290 – 294

Timpl R (1989) Structure and biological activity of basement membrane proteins. Eur J Biochem 180: 487 – 502

Vivanco J, Sawyers CL (2002) The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer 2: 489 – 501

Waterman EA, Sakai N, Nguyen NT, Horst BA, Veitch DP, Dev CN, Ortiz-Urda S, Khavari PA, Marinkovich MP (2007) A laminin–collagen complex drives human epidermal carcinogenesis through phosphoinositol-3-kinase activation. Cancer Res 67: 4264 – 4270

Yamamoto H, Itoh F, Iku S, Hosokawa M, Imai K (2001) Expression of the γ2 chain of laminin-5 at the invasive front is associated with recurrence and poor prognosis in human esophageal squamous cell carcinoma. Clin Cancer Res 7: 896 – 900

Yurchenco PD, Schittny JC (1990) Molecular architecture of basement membranes. FASEB J 4: 1577 – 1590