Laminin-5 $\gamma 2$ chain expression correlates with unfavorable prognosis in colon carcinomas

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Expression of the $\gamma 2$ chain at the invasive front of different tumors has indicated an important role for laminin-5 in cell migration during tumor invasion and tissue remodeling. As there is considerable need for reliable invasion and prognostic markers we evaluated the correlation of laminin-5 $\gamma 2$ chain expression with clinicopathologic parameters and patient survival in 93 primary colon carcinomas. Epithelial cells of normal mucosa were consistently negative for staining. In contrast, positive cytoplasmic staining was observed in 89 tumors (96%). Twenty-four (26%) cases were scored as sparse, 34 (37%) as moderate, and 31 (33%) as frequent $\gamma 2$ chain expression. There was a significant association of laminin-5 $\gamma 2$ chain expression and local invasiveness of colon carcinomas according to Dukes stage (A-C) ($p = 0.001$) and tumor budding ($p < 0.001$). A statistical significance could also be noted in decreasing tumor differentiation ($p < 0.001$) and correlation to tumor size ($p = 0.032$). No correlation was observed to tumor site. Univariate analysis identified laminin-5 ($p = 0.010$), tumor differentiation ($p = 0.006$) and Dukes grade ($p < 0.001$) as significant variables in predicting prognosis. However, by multivariate analyses, this study could not demonstrate that laminin-5 $\gamma 2$ chain expression is an independent predictive factor for survival.

The results indicate that laminin-5 $\gamma 2$ chain expression is up-regulated during the progression of human colon cancer and that it plays a role in the aggressiveness of these tumors. Demonstration of laminin-5 $\gamma 2$ chain positivity also facilitates detection of individual cells or minor cell clusters invading the surrounding stroma.

Figures on http://www.esacp.org/acp/2001/22-4/lenander.htm.

Keywords: Laminin-5 $\gamma 2$ chain, colon carcinoma, immunohistochemistry

1. Introduction

Colon cancer is one of the most common malignant diseases in the western world, ranked as the second leading cause of death from cancer. The incidence ranges from 25–40 per 100 000 population and the risk of developing colon tumors begins in the fourth decade of life and increases with age. The mean age at presentation is 60–65 years. Approximately two thirds of the patients will undergo a bowel resection with curative intent. Nevertheless, more than 50% of these patients are expected to die of the disease. This suggests that a significant proportion of micrometasases are present at the time of surgery which can be fatal for the clinical outcome and underline the importance to find reliable prognostic predictors to select patients for adequate additional treatment as a complement to surgery.

Tumor development and formation of metastases is a cascade of complex and poorly understood events. In this process, the cells must gain the ability to invade which is the characteristic of malignancy. Transition from an *in situ* tumor to initiation of invasive growth requires that tumor cells detach from adhesive interactions in the epithelium, penetrate the basement membrane, dissolve the extracellular matrix and migrate in the underlying interstitial stroma. At this point, the tumor cells gain access to lymphatic and blood

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vessels for further dissemination. Within the intestine, the basal membrane separates the epithelial cells from the underlying connective tissue, and colon carcinomas have been shown to be characterized by loss of an intact basal membrane [1]. Hereby, one of the most important steps in tumor cell invasion to achieve dissemination is to permeate the 20–100 nm thick basement membrane composed by collagen type IV, laminin, heparan sulphate proteoglycan and nidogen, among others [2].

Laminins, a major component in basement membranes, belong to a family of glycoproteins organized as a heterotrimer composed of one heavy α chain and two light chains designated β and γ, all originating from different genes. At present, there are 11 different subunit chains known that form at least twelve isoforms [3–7]. These different laminin structures are associated with cell adhesion, migration, proliferation, growth and differentiation, as a true structural component of the basal membrane, reflecting their diverse function and tissue specific involvement [8–14]. One of these isoforms laminin-5, previously termed kalinin, nicein, epiligrin or ladsin, has been localized to anchoring filaments involved in formation of the hemidesmosomes and by this, serves as an important adhesion component for epithelial cells positioned on the basement membrane [15–17]. Laminin-5 is composed of the $\alpha 3, \beta 3$ and $\gamma 2$ chains each encoded by separate genes, LAMA3 (18q), LAMB3 (1q 32) and LAMC2 (1q 25–31), respectively. Mutations in any of these genes is now known to cause the severe skin blistering disease Herlitz junctional epidermiolysis bullosa (HEJB) [18–22]. Although it is known that laminin-5 expression is up-regulated in coloncarcinomas, no investigations have been presented demonstrating whether these genes are up-regulated and/or amplified in colorectal cancer.

Cell–matrix interactions play an essential role in the structural morphology and functional differentiation of the tissue where cell–basal lamina interactions are mediated by a group of transmembrane proteins known as integrins. Integrins are composed of two noncovalent subunits, α and β, both which contribute to the binding of matrix proteins. For example, Knox et al. demonstrated in prostate tissue that the basal cells are attached to the basal membrane through interactions between the laminin receptor $\alpha 6 \beta 1$ to form focal adhesions and the $\alpha 6 \beta 4$ integrin has shown to be associated with formation of hemidesmosomes [23]. In contrast, Orian and Rousseau et al. could not demonstrate a true hemidesmosome in the intestine because they lacked several hemidesmosome associated proteins. Here they do not seem to function as stable anchorage devises and therefore the authors suggest a role in the epithelial cell migration to the tip of the villi [24]. Other groups have also shown that interaction of laminin-5 and integrins is essential for adhesion of epithelial cells to the basement membranes [11] and also by promoting cell migration [12,25]. Additional important factors in these interactions are proteases as tissue-type plasminogen (tPA) and metalloprotease-2 (MMP2) expressed by stromal cells [26–28]. However, these proteases have tissue specific patterns explaining their diverse functions in different tissue [29]. Gianelli et al. showed that the laminin-5 $\gamma 2$ chain is the primary target of MMP2 cleavage, providing cell migration during tissue modulation and tumor invasion [30]. Further, previous studies found that Laminin-5 is only expressed in small clusters of undifferentiated cancer cells just ahead the invasive front of the lesion, known as tumor budding [31–33].

Against the background that Laminin-5 has been proposed as a marker of invading cancer cells [32], as well as a predictor of microinvasion [34], and that tumor budding has been suggested as a useful prognostic factor in adenocarcinoma of colorectal cancer [35]. The aim of this study was to investigate the diagnostic and prognostic impact of Laminin-5 $\gamma 2$ chain expression in colon carcinomas.

2. Materials and methods

2.1. Tissue samples

Files from a total of 93 consecutively chosen patients resected for colon adenocarcinomas at Ersta Hospital between 1993 and 1996 were examined. The study was limited to patients classified as Dukes stage A-C, i.e., only cases presenting local invasive tumors at surgery were included, excluding those with distant metastases. Thus, all patients entered into the study underwent curative surgery. There were 52 women (56%) and 41 men (44%); the mean age was 74 years (range 51–92). The clinicopathologic features are summarized in Table 1. All cases were selected by original histopathological diagnosis and the original hematoxylin/eosin (HE) glass slides were reexamined by two pathologists (Å.Ö. and G.A.) to confirm the original diagnosis as well as the representativity of the sections used for histochemical studies before immunohistochemical staining was performed. Samples were

Table 1
Correlations between clinicopathologic parameters and laminin-5 $\gamma 2$ chain expression in 93 colon carcinomas*

Parameter	Overall	Laminin-5 expression**				
		0	1	2	3	p value
Dukes stage						
A	20 (21)	2 (10)	9 (45)	6 (30)	3 (15)	0.001
B	52 (56)	1 (2)	12 (23)	24 (46)	15 (29)	
C	21 (23)	1 (5)	3 (14)	4 (19)	13 (62)	
Tumor differentiation						
Well	23 (25)	2 (9)	9 (39)	9 (39)	3 (13)	<0.001
Moderate	55 (59)	2 (4)	13 (24)	23 (41)	17 (31)	
Poor	15 (16)		2 (13)	2 (13)	11 (74)	
Tumor size						
$\leqslant$ 2cm	12 (13)	2 (17)	4 (33)	3 (25)	3 (25)	0.032
2–4 cm	22 (24)	2 (9)	6 (27)	9 (41)	5 (23)	
$\geqslant$ 4cm	59 (63)		14 (24)	22 (37)	23 (39)	
Tumor localization						
Right colon	51 (55)	3 (6)	15 (30)	16 (31)	17 (33)	0.477
Left colon	42 (45)	1 (2)	9 (22)	18 (43)	14 (33)	
Tumor budding						
0	3 (3)	1 (33)	2 (67)			<0.001
1	18 (20)	2 (11)	10 (56)	4 (22)	2 (11)	
2	43 (46)		8 (19)	22 (51)	13 (30)	
3	29 (31)	1 (3)	4 (14)	8 (28)	16 (55)	
Tumor cell dissociation						
0	22 (23)	3 (14)	15 (68)	4 (18)		<0.001
1	36 (39)	1 (3)	8 (22)	17 (47)	10 (28)	
2	23 (25)			11 (48)	12 (52)	
3	12 (13)		1 (8)	2 (17)	9 (75)	

*Data are given as number (%).
**For grading of laminin-5 expression see material and methods.

classified for histologic type [36] and Dukes stage according to standard criteria [37]. Evidence of tumor budding (small clusters of undifferentiated cancer cells just ahead of the invasive front) and tumor cell dissociation (single cancer cells scattered in the stroma) were documented by two of the authors (Å.Ö. and C.L.) on newly HE stained samples. Classification was based on the predominant Laminin-5 $\gamma 2$ chain staining pattern in the specimens. Tumor budding and tumor cell dissociation were divided into four groups according to the degree: 0 = none, 1 = mild, 2 = moderate and 3 = severe, according to Hase et al. [35].

2.2. Immunohistochemistry

Preparation and characterization of polyclonal antibodies raised in rabbit against a fusion protein containing the C-terminus of the laminin $\gamma 2$ chain (amino acid residues # 1017–1178) and GST were performed according to methods described earlier [25].

Representative blocks of formalin-fixed and paraffin-embedded sections from colon tumors were cut five μm thick and subjected to a standard horseradish peroxidase avidin–biotin-complex (ABC) technique. In brief, the sections were deparaffinized with xylene, rehydrated and microwave treated for 10 minutes at 500 W in 10 mM sodium citrate buffer (pH 6). After a brief rinse in Tris buffered saline (TBS), pH 7.6, the sections were treated with 0.5% hydrogen peroxidase in distilled water for 30 minutes to block endogenous peroxidase activity, followed by 1% bovine serum albumin (BSA) in TBS for 20 minutes to prevent unspecific staining. After incubation overnight at 4°C with the rabbit polyclonal antibody against the $\gamma 2$ chain of laminin-5 (1 : 500), a biotinylated antirabbit IgG (1 : 200) was applied for 30 minutes. After rinsing in TBS, the biotinylated secondary antibody and

horseradish peroxidase-conjugated antibiotin antibody (Vector Elite standard kit, cat. PK-6100) were applied to the sections according to the manufacturer's instructions. Peroxidase activity was visualized by applying diaminobenzidine tertrahydrochloride, 0.6 mg/ml with 0.03% H_2O_2, for 6 min. The slides were then slightly counterstained with hematoxylin, dehydrated and mounted. Parallel incubations to adjacent sections where the laminin-γ2 chain antibody was replaced with BSA served as negative controls.

Staining of the laminin-5 γ2 chain was categorized semi-quantitatively as follows: 0 = negative, no cells were positive, 1 = sparse, one or a few ($\leqslant$5%) cells were positive, 2 = moderate, a significant amount (>5%–$\leqslant$20%) of cells were positive, and 3 = frequent, an extensive amount (>20%) of cells were positive. The degree of staining was evaluated independently by two investigators (C.L. and G.A.) and any cases with discrepant scores were reevaluated by a third investigator (Å.Ö.). All evaluations were performed in a coded manner without knowledge of the clinical and pathological data of the patients. No mucinous cancers were included.

2.3. Statistical analysis

Five patients with more than one tumor and one that died of unknown cause were excluded for survival analysis. The follow-up interval ranged from 1 to 79 months past surgery. Of the 86 patients included in the survival study, 20 died of coloncancer (23%).

The pattern of associations between laminin-5 γ2 chain expression and clinicopathologic parameters was determined by using the gamma exact test. Survival curves were generated according to the Kaplan–Meier method and compared using the log rank test. Prognostic relevance of clinicopathologic variables was analyzed applying univariate and multivariate Cox proportional hazards models.

3. Results

Laminin-5 γ2 chain expression was examined by immunohistochemistry in a series of 93 primary human colon carcinomas. As previously described for colon carcinoma, positive signals were always cytoplasmic and only detected in cancer cells mainly localized to the invasive front [31,32] (Fig. 1, C and F). These laminin-5 γ2 chain positive cells showed strong correlation to the undifferentiated cancer cells judged as tumor budding cells from the HE-staining ($p < 0.001$) (Fig. 1, A and B). However, in some tumors graded as sparse laminin-5 γ2 chain positive, stained cells could be scattered at different parts of the tumor. We also analyzed the γ2 expression in lymph nodes invaded by cancer cells where we found the same frequent cytoplasmic staining of infiltrating carcinoma cells as seen in the budding cells at the invasive front of the tumor. Adjacent normal colon mucosal cells were consistently negative for staining.

Laminin-5 γ2 chain staining was positive in 89 tumors (96%). Four cases (4%) were scored as negative, twenty-four (26%) as sparsely, 38 (37%) as moderately, and 32 (31%) as frequently stained (Fig. 1, D–F). Correlation's between laminin-5 γ2 chain staining pattern and clinicopathologic features of the tumors are summarized in (Table 1). The number of laminin-5 γ2 positive cells showed a significant correlation to increasing Dukes stage ($p = 0.001$) and decreasing tumor differentiation ($p < 0.001$). There was also an association to tumor size ($p = 0.032$), however, no correlation was observed to tumor location ($p = 0.477$). Figure 2 shows the survival curve obtained with the Kaplan–Meier method indicating decreased survival as a function of increased numbers of laminin-5 γ2 chain expressing tumor cells. Univariate analysis identified laminin-5 ($p = 0.010$), tumor differentiation ($p = 0.006$) and Dukes grade ($p < 0.001$) as significant variables in predicting prognosis (Table 2). However, when a multivariable model was constructed with all the clinicopathological variables, only Dukes grades were identified as significant covariates. Neither laminin-5 γ2 chain staining, tumor grade, tumor budding nor tumor cell dissociation provided any independent prognostic information on survival.

4. Discussion

Figures on http://www.esacp.org/acp/2001/22-4/lenander.htm.

Earlier studies by Pyke et al. [31,32] as well as Sordat et al. [33] indicate that laminin-5 is a marker of invading cancer cells at the invasive front of the tumor. Both groups also claimed that these γ2 positive cells represent tumor budding cells which has been associated with a worse clinical outcome [35]. In addition, Skyldberg et al. [34] recently reported that laminin-5 γ2 chain expression can be used to predict the invasiveness of uterine cervical lesions. To our knowledge only two studies have evaluated the prognostic sig-

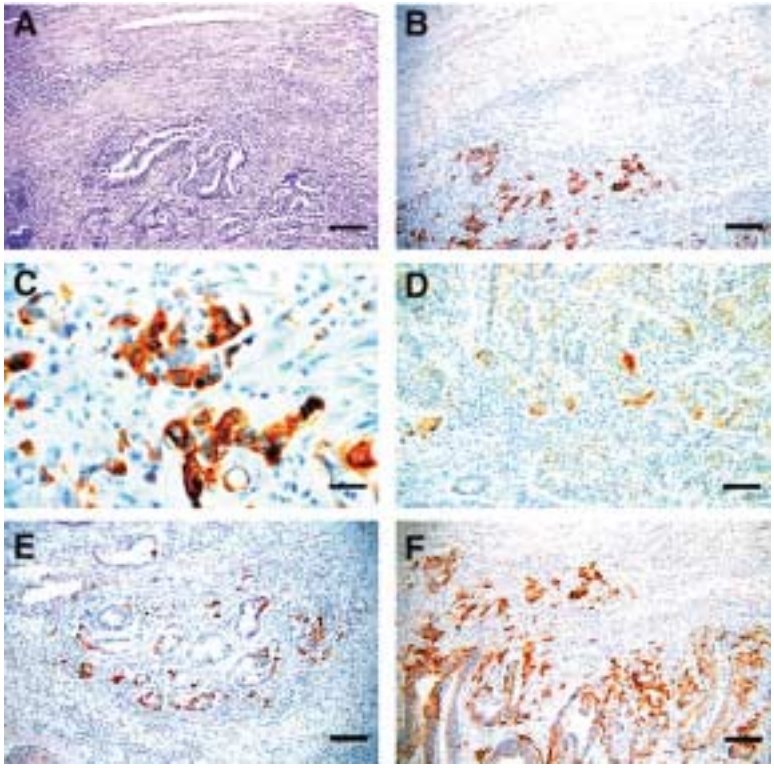

Fig. 1. Human colon adenocarcinoma stained by hematoxylin/eosin (H/E) (A) and immunohistochemistry for the γ2 chain of laminin-5 (B–F). (A) Marked tumor budding. (B) Performed on an adjacent section to A, showing strong staining for the γ2 chain in clearly budding cancer cells at the invasive front of the cancer. Note how clear the budding cells are visualized compared to H/E staining. (C) High magnification, demonstrating the strong cytoplasmic staining with the laminin-5 γ2 chain antibody in budding cancer cells. (D–F) Showing a colon tumor representing a sparse (D), moderate (E) and frequent (F) γ2 chain staining. Bars, 140 μm (A, B, E and F), 24 μm (C) and 36 μm (D). This figure can be viewed on http://www.esacp.org/acp/2001/22-4/lenander.htm.

nificance of the laminin-5 γ2 chain expression in tumors, Soini et al. in pancreatic adenocarcinomas [38] and Ono et al. in tongue cancer [39]. Therefore we wanted to investigate if the expression of the γ2 chain of laminin-5 could be used as a marker in patients suffering from colon carcinomas. In addition we wanted to study the relationship between tumor budding, tumor cell dissociation, differentiation grade, size, location and laminin-5 γ2 chain expression.

In the present study we found that laminin-5 γ2 chain is expressed in 96% of the malignant colon tumors investigated, indicating a high activity of laminin-5 synthesis in colon cancer tissue. This is in accordance with previous reports where 89–100% of the tumors were immunostained [31,32,39]. Only four out of 93 tumors were negative for staining, two classified as Dukes A, (one highly- and one moderately-differentiated) one as Dukes B (highly differentiated) and one as Dukes C (moderately differentiated). Furthermore, in the Dukes A group, only three patients (15%) were classified as grade 3 γ2 staining compared to 13 patients (62%) in the Dukes stage C group. We also found that laminin-5 γ2 staining correlated significantly with progression from Dukes grades A to C. This is in line with previous reports, that laminin-5 has a potent cell migration and tumor cell invasion promoting activity [12,30]. In agreement with earlier studies [31–34,38,39], laminin-5 γ2 expression was preferentially detected in cancer cells at the invasive front of the tumor, i.e., in budding tumor cells. Hase et al. [35] demonstrated that these tumor budding cells were associated with a worse clinical outcome. In line with our results, they also found that the incidence of severe budding increased with higher Dukes grades as well as decreasing tumor differentiation (data not shown). In addition, we found these parameters to be correlated to increasing γ2 chain staining. In agreement with our study, Ono et al. [39], studying squamous cell carcinomas of the tongue, verified a strong association of the γ2 staining to infiltrative growth and poorer tumor differentiation.

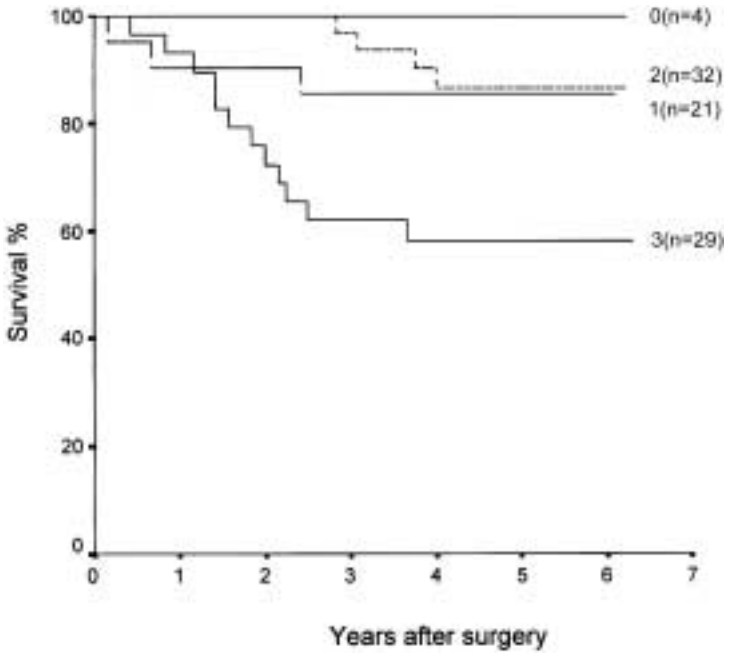

Fig. 2. Kaplan–Meier survival curve for 86 patients with colon adenocarcinoma related to the laminin-5 $\gamma 2$ chain expression 0–3 (log rank test: $p = 0.010$).

In our study, immunopositivity was preferentially located to the invasive front of the tumor except for grade 1 (sparse) stained cells. Twenty-four cases (26%) were found to belong to sparsely stained tumors and some of these laminin-5 $\gamma 2$ chain positive cells were seen as single cells with distinct cytoplasmic staining scattered throughout the tumor mass, i.e., not only at the invasive front (Fig. 1D). This phenomenon has not been described before in the evaluation of colon adenocarcinomas. Earlier investigators only stated that $\gamma 2$ chain immunoreactivity was almost exclusively confined to cancer cells and most prominent at the invasive front [31–33]. Since Pyke et al. [32] also demonstrated coexpression of the $\gamma 2$ chain of laminin-5 and the urokinase plasminogene activator (uPAR) in the budding cancer cells, it would be interesting to investigate the expression of uPAR in these scattered cells. Interestingly, they suggested that there must be a tight regulation of these proteins since they were not able to find any expression of these proteins in adjacent non budding cancer cells.

Sordat et al. [33] investigated 15 colon adenomas and found an enhanced $\gamma 2$ chain expression of the basement membrane at the periphery compared to normal colon mucosa. However, no cytoplasmic staining could be detected within the adenoma cells. As the $\gamma 2$ chain antibody used in our study only detected cytoplasmic antigens in epithelial cancer cells and the fact that laminin-5 is considered to be an early marker of invasiveness, it would be interesting to investigate the expression of this antibody in colon polyps where it might stain potentially malignant cells in the colon crypt.

Even though $\gamma 2$ chain expression was not an independent predictor of survival in a multivariate analysis in the present study, it was significantly correlated to tumor differentiation and size in the univariate analysis. In several multivariate analyses, tumor differentiation has shown to be independently prognostic of survival [40–43]. However, most studies find no significant adverse relation of tumor size to survival [41–46]. The relationship of the $\gamma 2$ chain positivity to tumor size in our study might reflect that large tumors have a higher potential to express extensive areas of invasive cells. Furthermore, our results verify that increased immunoreactivity of the $\gamma 2$ chain in colon adenocarcinoma correlates to the tumor budding cell phenotype, decreasing tumor cell dissociation and differentiation. Hase et al. [35] reported that patients with more undifferentiated cancers showed a higher incidence of tumor budding which is well in line with our observations. They also claimed that severe budding was seen in 21.8% in well- or moderately differentiated tumors, which differs from our study where we

Table 2
Univariate survival analysis

Variables	n	Categorical factors			
		Deaths	%	$\chi 2^*$	p-value
Dukes stage				16.86	<0.001
A	19	1	5		
B	48	8	16.7		
C	19	11	57.9		
Tumor differentiation				10.26	0.006
Well	20	1	5		
Moderate	53	13	24.5		
Poor	13	6	46.2		
Tumor localization				0.37	0.543
Right colon	46	10	21.7		
Left colon	40	10	25.0		
Laminin-5 $\gamma 2$ chain expression				11.39	0.010
0	4	1	25.0		
1	21	3	14.3		
2	32	4	12.5		
3	29	12	41.4		
Tumor budding				1.68	0.641
0	3	0	0		
1	15	4	26.7		
2	40	8	20.0		
3	28	8	28.6		
Tumor cell dissociation				7.03	0.070
0	19	1	5		
1	33	7	21.2		
2	22	7	31.8		
3	12	5	41.7		

*The log rank statistic (modified $\chi 2$).

found severe budding (2 and 3) in 73% of well- or moderately differentiated tumors and in 100% of tumors with poor differentiation (data not shown). In parallel, well- and moderately differentiated tumors showed high laminin-5 $\gamma 2$ chain expression (2 and 3) in 67%. We were not able to find any difference in survival, where Hase et al. found that the outcome was worse in tumors presenting severe budding in well- or moderately differentiated lesions.

In agreement with previous reports on carcinomas of epithelial origin of different anatomic sites [31–34, 38,39], the present study supports that laminin-5 $\gamma 2$ chain expression is a marker of local invasiveness and that it might be of prognostic importance. Furthermore, it confirms that positive laminin-5 $\gamma 2$ chain staining of cells facilitates identification of tumor budding and dissociated colon tumor cells.

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References

[1] A. Stallmach, D. Schuppan, J. Dax, C. Hanski and E.O. Riecken, Identification of laminin binding proteins in cell membranes of a human colon adenocarcinoma cell line, *Gut* **31** (1990), 70–76.

[2] P.D. Yurchenco and J.C. Schittny, Molecular architecture of basement membranes, *FASEB J.* **4** (1990), 1577–1590.

[3] H. Colognato and P.D. Yurchenco, Form and function: the laminin family of heterotrimers, *Dev. Dyn.* **218** (2000), 213–234.

[4] E. Engvall and U.M. Wewer, Domains of laminin, *J. Cell. Biochem.* **61** (1996), 493–501.

[5] R.E. Burgeson, M. Chiquet, R. Deutzmann, P. Ekblom, J. Engel, H. Kleinman, G.R. Martin, G. Meneguzzi, M. Paulsson and J. Sanes, A new nomenclature for the laminins, *Matrix Biol.* **14** (1994), 209–211.

[6] J.H. Miner, B.L. Patton, S.I. Lentz, D.J. Gilbert, W.D. Snider, N.A. Jenkins, N.G. Copeland and J.R. Sanes, The laminin alpha chains: expression, developmental transitions, and chromosomal locations of alpha1-5, identification of heterotrimeric laminins 8-11, and cloning of a novel alpha3 isoform, *J. Cell. Biol.* **137** (1997), 685–701.

[7] A. Iivanainen, T. Morita and K. Tryggvason, Molecular cloning and tissue-specific expression of a novel murine laminin gamma3 chain, *J. Biol. Chem.* **274** (1999), 14 107–14 111.

[8] W.G. Carter, M.C. Ryan and P.J. Gahr, Epiligrin, a new cell adhesion ligand for integrin alpha 3 beta 1 in epithelial basement membranes, *Cell* **65** (1991), 599–610.

[9] P. Rousselle, G.P. Lunstrum, D.R. Keene and R.E. Burgeson, Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments, *J. Cell. Biol.* **114** (1991), 567–576.

[10] P. Rousselle and M. Aumailley, Kalinin is more efficient than laminin in promoting adhesion of primary keratinocytes and some other epithelial cells and has a different requirement for integrin receptors, *J. Cell. Biol.* **125** (1994), 205–214.

[11] S.E. Baker, S.B. Hopkinson, M. Fitchmun, G.L. Andreason, F. Frasier, G. Plopper, V. Quaranta and J.C. Jones, Laminin-5 and hemidesmosomes: role of the alpha 3 chain subunit in hemidesmosome stability and assembly, *J. Cell. Sci.* **109** (1996), 2509–2520.

[12] K. Miyazaki, Y. Kikkawa, A. Nakamura, H. Yasumitsu and M. Umeda, A large cell-adhesive scatter factor secreted by human gastric carcinoma cells, *Proc. Natl. Acad. Sci. USA* **90** (1993), 11 767–11 771.

[13] H. Mizushima, Y. Miyagi, Y. Kikkawa, N. Yamanaka, H. Yasumitsu, K. Misugi and K. Miyazaki, Differential expression of laminin-5/ladsin subunits in human tissues and cancer cell lines and their induction by tumor promoter and growth factors, *J. Biochem. (Tokyo)* **120** (1996), 1196–1202.

[14] R. Chammas, D. Taverna, N. Cella, C. Santos and N.E. Hynes, Laminin and tenascin assembly and expression regulate HC11 mouse mammary cell differentiation, *J. Cell. Sci.* **107** (1994), 1031–1040.

[15] M.C. Ryan, R. Tizard, D.R. VanDevanter and W.G. Carter, Cloning of the LamA3 gene encoding the alpha 3 chain of the adhesive ligand epiligrin. Expression in wound repair, *J. Biol. Chem.* **269** (1994), 22 779–22 787.

[16] D.R. Gerecke, D.W. Wagman, M.F. Champliaud and R.E. Burgeson, The complete primary structure for a novel laminin chain, the laminin B1k chain, *J. Biol. Chem.* **269** (1994), 11 073–11 080.

[17] P. Kallunki, K. Sainio, R. Eddy, M. Byers, T. Kallunki, H. Sariola, K. Beck, H. Hirvonen, T.B. Shows and K. Tryggvason, A truncated laminin chain homologous to the B2 chain: structure, spatial expression, and chromosomal assignment, *J. Cell. Biol.* **119** (1992), 679–693.

[18] F. Vidal, C. Baudoin, C. Miquel, M.F. Galliano, A.M. Christiano, J. Uitto, J.P. Ortonne and G. Meneguzzi, Cloning of the laminin alpha 3 chain gene (LAMA3) and identification of a homozygous deletion in a patient with Herlitz junctional epidermolysis bullosa, *Genomics* **30** (1995), 273–280.

[19] L. Pulkkinen, A.M. Christiano, T. Airenne, H. Haakana, K. Tryggvason and J. Uitto, Mutations in the gamma 2 chain gene (LAMC2) of kalinin/laminin 5 in the junctional forms of epidermolysis bullosa, *Nat. Genet.* **6** (1994), 293–297.

[20] D. Aberdam, M.F. Galliano, J. Vailly, L. Pulkkinen, J. Bonifas, A.M. Christiano, K. Tryggvason, J. Uitto, E.H. Epstein, J.P. Ortonne et al., Herlitz's junctional epidermolysis bullosa is linked to mutations in the gene (LAMC2) for the gamma 2 subunit of nicein/kalinin (LAMININ-5), *Nat. Genet.* **6** (1994), 299–304.

[21] L. Pulkkinen, J. McGrath, T. Airenne, H. Haakana, K. Tryggvason, S. Kivirikko, G. Meneguzzi, J.P. Ortonne, A.M. Christiano and J. Uitto, Detection of novel LAMC2 mutations in Herlitz junctional epidermolysis bullosa, *Mol. Med.* **3** (1997), 124–135.

[22] J. Vailly, L. Pulkkinen, C. Miquel, A.M. Christiano, D. Gerecke, R.E. Burgeson, J. Uitto, J.P. Ortonne and G. Meneguzzi, Identification of a homozygous one-basepair deletion in exon 14 of the LAMB3 gene in a patient with Herlitz junctional epidermolysis bullosa and prenatal diagnosis in a family at risk for recurrence, *J. Invest. Dermatol.* **104** (1995), 462–466.

[23] J.D. Knox, A.E. Cress, V. Clark, L. Manriquez, K.S. Affinito, B.L. Dalkin and R.B. Nagle, Differential expression of extracellular matrix molecules and the alpha 6-integrins in the normal and neoplastic prostate, *Am. J. Pathol.* **145** (1994), 167–174.

[24] V. Orian-Rousseau, D. Aberdam, L. Fontao, L. Chevalier, G. Meneguzzi, M. Kedinger and P. Simon-Assmann, Developmental expression of laminin-5 and HD1 in the intestine: epithelial to mesenchymal shift for the laminin gamma-2 chain subunit deposition, *Dev. Dyn.* **206** (1996), 12–23.

[25] S. Salo, H. Haakana, S. Kontusaari, E. Hujanen, T. Kallunki and K. Tryggvason, Laminin-5 promotes adhesion and migration of epithelial cells: identification of a migration-related element in the gamma2 chain gene (LAMC2) with activity in transgenic mice, *Matrix Biol.* **18** (1999), 197–210.

[26] D.E. Kleiner and W.G. Stetler-Stevenson, Matrix metalloproteinases and metastasis, *Cancer Chemother. Pharmacol.* **43** Suppl (1999), S42–S51.

[27] H. Birkedal-Hansen, Proteolytic remodeling of extracellular matrix, *Curr. Opin. Cell. Biol.* **7** (1995), 728–735.

[28] W.T. Chen, Membrane proteases: roles in tissue remodeling and tumour invasion, *Curr. Opin. Cell. Biol.* **4** (1992), 802–809.

[29] L.E. Goldfinger, M.S. Stack and J.C. Jones, Processing of laminin-5 and its functional consequences: role of plasmin and tissue-type plasminogen activator, *J. Cell. Biol.* **141** (1998), 255–265.

[30] G. Giannelli, J. Falk-Marzillier, O. Schiraldi, W.G. Stetler-Stevenson and V. Quaranta, Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5, *Science* **277** (1997), 225–228.

[31] C. Pyke, J. Romer, P. Kallunki, L.R. Lund, E. Ralfkiaer, K. Dano and K. Tryggvason, The gamma 2 chain of

kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers, *Am. J. Pathol.* **145** (1994), 782–791.

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

[33] I. Sordat, F.T. Bosman, G. Dorta, P. Rousselle, D. Aberdam, A.L. Blum and B. Sordat, Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia, *J. Pathol.* **185** (1998), 44–52.

[34] B. Skyldberg, S. Salo, E. Eriksson, U. Aspenblad, B. Moberger, K. Tryggvason and G. Auer, Laminin-5 as a marker of invasiveness in cervical lesions, *J. Natl. Cancer. Inst.* **91** (1999), 1882–1887.

[35] K. Hase, C. Shatney, D. Johnson, M. Trollope and M. Vierra, Prognostic value of tumor "budding" in patients with colorectal cancer, *Dis. Colon Rectum* **36** (1993), 627–635.

[36] B.C. Morson and L.H. Sobin, Histological typing of intestinal tumours, World Health Organization, Geneva, 1976, p. 15.

[37] C.E. Dukes, The classification of cancer of the rectum, *J. Pathol. Bacteriol.* **35** (1932), 323–332.

[38] Y. Soini, M. Maatta, S. Salo, K. Tryggvason and H. Autio-Harmainen, Expression of the laminin gamma 2 chain in pancreatic adenocarcinoma, *J. Pathol.* **180** (1996), 290–294.

[39] Y. Ono, Y. Nakanishi, Y. Ino, T. Niki, T. Yamada, K. Yoshimura, M. Saikawa, T. Nakajima and S. Hirohashi, Clinocopathologic significance of laminin-5 gamma2 chain expression in squamous cell carcinoma of the tongue: immunohistochemical analysis of 67 lesions, *Cancer* **85** (1999), 2315–2321.

[40] R.K. Phillips, R. Hittinger, L. Blesovsky, J.S. Fry and L.P. Fielding, Large bowel cancer: surgical pathology and its relationship to survival, *Br. J. Surg.* **71** (1984), 604–610.

[41] P.H. Chapuis, O.F. Dent, R. Fisher, R.C. Newland, M.T. Pheils, E. Smyth and K. Colquhoun, A multivariate analysis of clinical and pathological variables in prognosis after resection of large bowel cancer, *Br. J. Surg.* **72** (1985), 698–702.

[42] B.D. Minsky, C. Mies, T.A. Rich, A. Recht and J.T. Chaffey, Potentially curative surgery of colon cancer: patterns of failure and survival, *J. Clin. Oncol.* **6** (1988), 106–118.

[43] J.D. Godwin and C.C. Brown, Some prognostic factors in survival of patients with cancer of the colon and rectum, *J. Chronic Dis.* **28** (1975), 441–454.

[44] A.H. Qizilbash, Pathologic studies in colorectal cancer. A guide to the surgical pathology examination of colorectal specimens and review of features of prognostic significance, *Pathol. Annu.* **17** (1982), 1–46.

[45] N. Wolmark, I. Cruz, C.K. Redmond, B. Fisher and E.R. Fisher, Tumor size and regional lymph node metastasis in colorectal cancer. A preliminary analysis from the NSABP clinical trials, *Cancer* **51** (1983), 1315–1322.

[46] J.S. Spratt, Jr. and H.J. Spjut, Prevalence and prognosis of individual clinical and pathologic variables associated with colorectal carcinoma, *Cancer* **20** (1967), 1976–1985.