Revising the Role of Integrin Subunit $\beta 4$ Expression in Colon Cancer Progression and Survival: A Retrospective Study

Eva Rademaker (✉ e.rademaker@lumc.nl)

Leids Universitair Medisch Centrum <https://orcid.org/0000-0002-9083-2550>

Esther Bastiaannet

Leids Universitair Medisch Centrum

Jan Oosting

Leids Universitair Medisch Centrum

Neeltje G. Dekker-Ensink

Leids Universitair Medisch Centrum

Peter J. K. Kuppen

Leids Universitair Medisch Centrum

Noel F. C. C. De Miranda

Leids Universitair Medisch Centrum

Gerrit-Jan Liefers

Leids Universitair Medisch Centrum

Research article

Keywords: Integrin $\beta 4$, $\beta 4$ subunit, biomarker, colon cancer, invasion, hemidesmosome.

Posted Date: August 7th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52129/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Integrin subunit $\beta 4$ ($\beta 4$) has been proposed to play an important role in colon cancer progression through its involvement in hemidesmosome disassembly processes and tumor cell migration. However, no consensus has yet been achieved regarding its association with clinical outcomes, particularly in colon cancer. The aim of this study was to reveal the relation between $\beta 4$ expression and clinicopathological features and colon cancer outcomes.

Methods: Expression of $\beta 4$ was assessed by immunohistochemistry in a large cohort of 651 colon cancer patients, the largest colon cancer cohort so far. Chi-square tests were used to study the association between $\beta 4$ expression and clinicopathological features while its relation with disease-free survival was assessed by Cox proportional hazard models. Furthermore, a potential relation between levels of *ITGB4* expression and patient outcomes was also investigated with the TCGA dataset.

Results: No association between $\beta 4$ expression and disease-free survival was encountered ($P = 0.767$), which disputes the role of $\beta 4$ as a biomarker of malignant behavior in colon cancer. Strikingly, loss of $\beta 4$ expression was instead associated with advanced pathological tumor (pT) stage of the primary tumor. Only 17.9% of the pT1 tumors displayed weak $\beta 4$ expression level versus 28.1% of pT4 tumors, and 25.0% of the pT1 tumors had a high expression level versus 8.6% of the pT4 tumors ($P = 0.012$). Additionally, no relation was observed between $\beta 4$ expression and colon cancer stage ($P = 0.138$), tumor differentiation grade ($P = 0.097$) or mismatch repair (MMR) status ($P = 0.878$).

Conclusions: Contradictory reports have suggested opposite roles for $\beta 4$ expression in (colon) cancer progression. In the present large cohort of colon cancer patients we found that $\beta 4$ expression was not associated with worse clinical prognosis and tumor differentiation grade, but decreased with advanced pathological tumor stage. Future studies should establish whether loss of $\beta 4$ expression promotes invasive characteristics of colon cancer cells.

Introduction

Integrins are transmembrane receptors consisting of two subunits (α and β) that bind to extracellular matrix (ECM) components. Integrin $\alpha 6\beta 4$ is commonly expressed at the basal surface of intestinal epithelial cells where it interacts with its ligand laminin, at the basement membrane.(1) The integrin $\beta 4$ subunit ($\beta 4$) has exceptional characteristics compared to other integrin subunits, because of its long cytoplasmic tail containing 1017 amino acids that interacts directly with the intermediate filament plectin and thereby the cell's cytoskeleton.(2-4) This association forms a stable adhesive structure denominated hemidesmosome.

Acquisition of migratory and invasive properties by cancer cells is a hallmark of carcinogenesis and the loss of hemidesmosome-mediated adhesion can be an important enabler.(3) Several signaling pathways can affect hemidesmosome stability, namely through phosphorylation of the cytoplasmic tail of $\beta 4$.(5-7) As a consequence, the hemidesmosome structure is disrupted and $\alpha 6\beta 4$ is mobilized from this complex,

thereby enabling $\alpha 4$ to trigger the activation of the PI3K and Ras-MAPK signaling pathways.(8, 9) In turn, their activation drives a number of oncogenic features in cancer cells including proliferation, migration, and resistance to apoptosis.(10)

Previous studies have shown that higher expression of $\alpha 4$ is associated with malignant behavior and poor clinical prognosis in bladder cancer(11), cervical cancer with a squamous histology,(12) squamous cell carcinomas of the head and neck,(13) non-small cell lung cancer of squamous subtype,(14) pancreatic cancer,(15) thyroid cancer,(16) and basal-like breast cancer.(17) Furthermore, $\alpha 6\alpha 4$ deficiency was demonstrated to lead to higher apoptotic rates, *in vivo*, of human breast carcinoma cells, thereby confirming the close link between this integrin and cell survival processes.(18)

However, no consensus has yet been achieved regarding the potential role of $\alpha 4$ in colorectal cancer progression. Several studies proposed that $\alpha 4$ is upregulated in carcinomas in comparison with normal mucosa or adenomas while others have found no relevant associations, or even diminished expression of $\alpha 4$ in invasive lesions.(19-25) Mishra and colleagues demonstrated that circulating tumor cells, as expected, had less adhesive properties, but also lower $\alpha 4$ expression as compared to the primary tumor. (26)

It is expected that the cellular localization of this integrin also has profound impact on cell behavior as a basal, membranous localization is typical of normal colonic epithelium while diffuse $\alpha 4$ expression might correspond to an oncogenic pattern.(27, 28) Accordingly, the expression levels and localization of $\alpha 4$ have been associated to cellular differentiation: membranous expression of $\alpha 4$ was shown to be increased in well and moderately differentiated carcinomas, whereas largely absent in poorly differentiated tumors. (22, 24, 29) On the other hand, increased $\alpha 4$ expression but with a diffuse localization has also been reported in poorly differentiated tumors.(21) For several cancer types, a poorly differentiated histology associates with more aggressive behavior, which is somewhat contradictory to the proposed oncogenic role of $\alpha 4$ overexpression in some studies.(30) This apparent contradiction, together with the distinct roles for $\alpha 4$ according to its cellular localization and distinct isoforms of both the $\alpha 6$ and $\alpha 4$ subunit may partly explain the conflicting reports on this integrin.(22, 31) When looking into clinical parameters, specifically in colorectal cancer, the association between clinical outcomes and level of $\alpha 4$ expression are also debatable: whereas one study did not find any association between $\alpha 4$ expression and tumor stage, another study reported that $\alpha 4$ expression levels positively correlated with clinical stage.(21, 23)

To elucidate the relation between the expression of this integrin subunit $\alpha 4$ and both clinical outcome and malignant features of the colon tumor, we evaluated its expression in tissues derived from 653 colon cancer patients.

Methods

Patient cohort

The patient cohort was previously described by Reimers and colleagues.(32) Patient data was anonymized in line with the national ethics guidelines (Code for Proper Secondary Use of Human Tissue, Dutch Federation of Medical Scientific Societies) and the analysis was done according to the code of conduct for responsible use. In brief, it comprises 1026 patients diagnosed with a colon tumor at time of diagnosis and who underwent resection between 2002 and 2008. Tissue microarrays (TMAs) were constructed with TMA Master (3DHistech Ltd). They were comprised of three 1.0 mm diameter cores, extracted from archival formalin-fixed paraffin-embedded tumor tissues under supervision of a pathologist. After immunohistochemical analysis, 653 patients were determined to be represented by good-quality, evaluable TMA cores.

Immunohistochemistry

Four μ m TMA sections were deparaffinized in xylene for three consecutive steps of 15 minutes after which slides were washed in decreasing concentrations of ethanol (100%-70%-50%). Endogenous peroxidase activity was blocked by using a solution of 0,3% hydrogen peroxide diluted in methanol (Merck Millipore, Burlington, MA, USA) for 20 minutes. Heat-induced antigen retrieval was performed in boiling citrate buffer (10 mM, pH 6.0) for 10 minutes in a microwave. After washing with PBS-Tween (0,2%), all slides were incubated overnight with anti-integrin α 4 rabbit monoclonal antibodies, diluted 1:400 in PBA-BSA (1%) (D8P6C, Cell Signaling, USA). The next day, slides were washed three times in PBS-Tween and incubated for one hour with poly-horseradish peroxidase solution (Immunologic, The Netherlands). DAB+ chromogen (DAKO, Agilent Technologies, USA) was used for chromogenic development and counterstaining was done with hematoxylin (Thermo Fisher Scientific, USA).

Immunodetection of α 4 was scored as absent (0), low (1), and high (2). Scorings from tissue cores belonging to the same tumor were averaged and categorized according to the following: 0 – 0.49 (weak), 0.5 – 1.49 (intermediate), 1.5 – 2 (high). Immunohistochemistry was performed on all slides at once, to avoid inter-assay variation. Tonsil tissue served as positive control. Simultaneously with the IHC-procedure for detecting the integrin expression, a negative tonsil control underwent the same procedure except that the primary antibodies was replaced with PBS-BSA.

Statistical analysis

Chi-squared tests were used to study the relation between clinicopathological and tumor characteristics and level of α 4 expression. Missing values were included as missing indicator. Cox proportional hazard models with the lowest group (α 4-weak) as reference were used to investigate whether there was an association between the intensity of α 4 in the tumor tissue and disease-free survival. Here, disease-free survival was defined as the period of the patient in which no tumor or metastases associated with colon cancer were observed during the follow-up time, which ended at the last date of follow-up (1st of January 2012) or at the date of death. Analyses were done with IBM SPSS Statistics version 25 and statistical tests were two-sided and considered significant if $P < 0.05$.

TCGA analysis

To analyze the gene expression level of *ITGB4*, The Cancer Genome Atlas (TCGA) dataset for colorectal adenocarcinoma was used. We selected samples where both RNAseq data (caselist = 'coadread_tcga_rna_seq_v2_mrna'), and information on disease-free survival were available. Two groups were made based on the median level of expression, which resulted in the first group with low *ITGB4* expression (expression < median) and a second group with high *ITGB4* expression (expression > median). The groups consisted of N < median = 166 and N > median = 165 patients.

Results

To investigate whether there was an association between disease-free survival in colon cancer patients and $\alpha 4$ expression in tumor tissues, the expression of this molecule was determined in 653 colon cancers. $\alpha 4$ expression was solely detected in cancer cells and not in stromal cells (Figure 1A-C). According to the levels of $\alpha 4$ expression, tumor samples were categorized into weak ($\alpha 4$ -weak, n = 132, Figure 1A), intermediate ($\alpha 4$ -intermediate, n = 404, Figure 1B), and high expression ($\alpha 4$ -high, n = 117, Figure 1C) groups. Two patients without information on tumor stage were excluded from further analyses, resulting in a cohort of 651 patients.

First, the association between $\alpha 4$ expression groups and disease-free survival was investigated. Median survival time was 6.77 years (95% CI 5.24-8.31) and there were 324 events. There were no differences in disease-free survival among the three groups according to $\alpha 4$ expression ($P = 0.767$, Figure 1D), with an HR of 0.938 (95% CI 0.715-1.231; $P = 0.647$) for the intermediate expression as compared to weak expression, and HR 0.878 (95% CI 0.618-1.247; $P = 0.468$) for the high expression as compared to weak expression. This observation was confirmed by making use of the TCGA dataset where RNA expression levels of *ITGB4* did not associate with disease-free survival (Figure 1E) in a cohort of 331 patients.

When looking into other parameters, no association could be observed between $\alpha 4$ expression and clinical cancer stage ($P = 0.138$, Table 1), differentiation grade ($P = 0.097$, Table 1), or the mismatch repair (MMR) status of tumors ($P = 0.878$, Table 1). However, we did observe that at advanced pathological tumor (pT) stage, pT4, tumors generally expressed lower levels of $\alpha 4$ (weak expression level of 28.1% in the pT4 tumors versus 17.9% in pT1 tumors). This might suggest a relation between this integrin expression and invasion of the primary tumor ($P = 0.012$, Table 1, Figure 1F).

To investigate whether there was a difference in $\alpha 4$ expression between cancer and normal tissues, we assessed the expression of this integrin in 28 samples of healthy colonic mucosa. No differences could be observed in terms of expression levels or cellular localization of this integrin (e.g. membranous, diffuse).

Table 1: relation between integrin $\alpha 4$ expression on the tumor and clinicopathological characteristics (Chi-squared tests).

		β4 weak	β4 intermediate	β4 high	
	N	N(%)	N(%)	N(%)	<i>P-value</i>
Cancer stage					<i>0.138</i>
Stage I	91	17 (18.7)	50 (54.9)	24 (26.4)	
Stage II	264	46 (17.4)	173 (65.5)	45 (17.0)	
Stage III	195	48 (24.6)	119 (61.0)	28 (14.4)	
Stage IV	101	21 (20.8)	60 (59.4)	20 (19.8)	
Differentiation status					<i>0.097</i>
Good differentiated	54	15 (27.8)	28 (51.9)	11 (20.4)	
Moderately differentiated	419	83 (19.8)	272 (64.9)	64 (15.3)	
Poorly differentiated	127	22 (17.3)	75 (59.1)	30 (23.6)	
MMR status					<i>0.259</i>
MMR proficient	581	117 (20.1)	361 (62.1)	103 (17.7)	
MMR deficient	54	11 (20.4)	32 (59.3)	11 (20.4)	
Tumor stage					<i>0.012</i>
pT1	28	5 (17.9)	16 (57.1)	7 (25.0)	
pT2	77	13 (16.9)	43 (55.8)	21 (27.3)	
pT3	418	78 (18.7)	262 (62.7)	78 (18.7)	
pT4	128	36 (28.1)	81 (63.3)	11 (8.6)	

Discussion

Integrins play a fundamental role in cell migration and tumor invasion. Integrin β6β4 has been proposed to be involved in such processes, namely by contributing to hemidesmosome disassembly and PI3K and Ras signaling activation, leading to malignant characteristics like proliferation, migration and apoptosis resistance.(2, 8, 10) Previous studies have failed to reach a consensus about a potential association between β4 expression and the aggressive phenotype invasive behavior of colorectal cancer cells.(19-25) Therefore, we sought to investigate the prognostic role of β4 expression in a large cohort of colon cancer patients.

Assessment of expression of $\alpha 4$ in a cohort of 651 colon cancers did not reveal any association between expression level and patient disease-free survival. However, high expression of this integrin was less frequent in tumors that had penetrated through the colonic wall (pT4). Also, we did not find an association between $\alpha 4$ expression and tumor grade, although it was previously proposed that cellular differentiation impacted the expression of this integrin. In colorectal cancer, differentiation is tightly linked to the mismatch repair status of tumors which could constitute an important confounder when performing such analysis. However, we also demonstrated that no differences in $\alpha 4$ expression can be observed when comparing mismatch repair-deficient or -proficient cancers.

This is the first study looking at a large (n=651) colon cancer cohort to explore the prognostic value of $\alpha 4$ expression. Two recent studies associated $\alpha 4$ expression by immunohistochemistry to patient outcome. Zhang and colleagues described that higher $\alpha 4$ expression was associated to worse patient survival.(23) However, the authors could not discriminate differences between tumors stages. In contrast to our study, that study did demonstrate that $\alpha 4$ expression was significantly higher in tumor tissues when compared to normal colon, whereas we did not observe any difference between tumor and healthy colon tissue. Another study evaluated the expression of $\alpha 4$ in relation to tumor budding features in the tissues derived from 232 stage II colorectal cancer patients.(28) Tumor budding is a known poor prognostic factor in stage II CRC but the authors demonstrated that by incorporating information on $\alpha 4$ expression, the prognostic significance of tumor budding increased considerably: tumor buds that displayed higher expression of $\alpha 4$ were associated with worse patient prognosis. Nevertheless, the overall expression of $\alpha 4$ alone was not predictive for clinical outcome, in line with our study. We have not attempted to replicate these observations, as we considered that scoring of tumor budding would not be reliable on a TMA. Sordat and colleagues also described that $\alpha 4$ expression was found decreased in carcinomas, particularly at tumors buds, when compared to adenomatous and healthy tissue thereby arguing against a role for the overexpression of this protein in invasive and migratory processes.(24) One could also propose that loss of $\alpha 4$ expression would promote hemidesmosome disassembly and migratory properties of cancer cells. This could explain the observation that this protein is less frequently expressed at advanced pT stages in our cohort.

In part, the conflicting reports regarding the role of $\alpha 4$ in colorectal cancer may be explained by the existence of different isoforms of this protein and of its dimer partner ($\alpha 6$). (31, 33) A $\alpha 4$ isoform that lacks its cytoplasmic domain ($\alpha 4^{ctd-}$) was described to be expressed in normal colonic mucosa but largely absent in cancer cells.(22) Also, a distinction can be made between two $\alpha 6$ subunits, where the $\alpha 6A$ isoform is generally more frequent than the $\alpha 6B$ isoform in colon and lung tumors.(33, 34) The different possibilities of integrin heterodimer formation involving $\alpha 4$ could thus confound the association of $\alpha 4$ expression with clinical prognosis in colon tumors.

Limitations with the current study include the use of a TMA for the evaluation of $\alpha 4$ expression which could complicate the identification of heterogeneous patterns of expression, for instance, between tumor core and invasive front. However, this is partially compensated by the fact that each tumor was represented by three different cores distributed in separate TMAs as well as by the inclusion of a large

number of samples. Secondly, different isoforms of integrin $\alpha 4$ were shown to exert distinct functions while the current immunohistochemical detection did not allow the discrimination of those. The same applies for the possibility that different β subunits can form a complex with $\alpha 4$.

Conclusions

In sum, this is the largest study investigating the relation between $\alpha 4$ integrin expression and colon cancer-related clinical outcomes and shows no association. The apparent lack of an association with disease free survival does not exclude a role for the $\alpha 4$ integrin, as we did find a lower expression at advanced stages of invasion which argues against the hypothesis that higher $\alpha 4$ levels relate to the acquisition of invasive properties by cancer cells.

List Of Abbreviations

$\alpha 4$ Integrin $\alpha 4$ subunit

$\alpha 4^{\text{ctd-}}$ $\alpha 4$ isoform lacking its cytoplasmic domain

ECM Extracellular matrix

MMR Mismatch repair

pT Pathological tumor

TCGA The Cancer Genome Atlas

TMA Tissue microarray

Declarations

Ethics approval and consent to participate

Not applicable.

Data from the Cancer Registry are fully anonymized prior to being made available to researchers, so data cannot be traced back to the individual patient. Therefore, no informed consent was required from the included patients and there was no need for approval of the medical ethical committee.

Consent for publication

Not applicable

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant number 852832). The funding body had no role in study design and collection, analysis, interpretation of data or writing the manuscript.

Authors' contributions

Conceptualization: ER EB ND JO JM PK NM GL

Methodology: ER EB JO PK NM GL

Software: IBM SPSS Statistics version 25

Validation: ER EB JO NM GL

Formal analysis: ER EB JO NM GL

Investigation: ER EB JO NM GL

Resources: ER EB ND JO PK NM GL

Data curation: ER EB ND JO PK NM GL

Writing – original draft: ER NM GL

Writing – review and editing: ER EB ND JO PK NM GL

Visualization: ER EB JO PK NM GL

Supervision: ER EB ND JO PK NM GL

Project administration: ER

Acknowledgements

The authors thank the LUMC pathologist Hans Morreau for assistance in setting up the TMA.

References

1. Mercurio AM. Laminin receptors: achieving specificity through cooperation. *Trends Cell Biol.* 1995;5(11):419-23.
2. Wilhelmsen K, Litjens SH, Sonnenberg A. Multiple functions of the integrin alpha6beta4 in epidermal homeostasis and tumorigenesis. *Mol Cell Biol.* 2006;26(8):2877-86.
3. Litjens SH, de Pereda JM, Sonnenberg A. Current insights into the formation and breakdown of hemidesmosomes. *Trends Cell Biol.* 2006;16(7):376-83.

4. Streuli CH, Akhtar N. Signal co-operation between integrins and other receptor systems. *Biochem J.* 2009;418(3):491-506.
5. Guo W, Pylayeva Y, Pepe A, Yoshioka T, Muller WJ, Inghirami G, et al. Beta 4 integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. *Cell.* 2006;126(3):489-502.
6. Mariotti A, Kedeshian PA, Dans M, Curatola AM, Gagnoux-Palacios L, Giancotti FG. EGF-R signaling through Fyn kinase disrupts the function of integrin alpha6beta4 at hemidesmosomes: role in epithelial cell migration and carcinoma invasion. *J Cell Biol.* 2001;155(3):447-58.
7. Trusolino L, Bertotti A, Comoglio PM. A signaling adapter function for alpha6beta4 integrin in the control of HGF-dependent invasive growth. *Cell.* 2001;107(5):643-54.
8. Shaw LM, Rabinovitz I, Wang HH, Toker A, Mercurio AM. Activation of phosphoinositide 3-OH kinase by the alpha6beta4 integrin promotes carcinoma invasion. *Cell.* 1997;91(7):949-60.
9. Mainiero F, Murgia C, Wary KK, Curatola AM, Pepe A, Blumemberg M, et al. The coupling of alpha6beta4 integrin to Ras-MAP kinase pathways mediated by Shc controls keratinocyte proliferation. *EMBO J.* 1997;16(9):2365-75.
10. Guo W, Giancotti FG. Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol.* 2004;5(10):816-26.
11. Grossman HB, Lee C, Bromberg J, Liebert M. Expression of the alpha6beta4 integrin provides prognostic information in bladder cancer. *Oncol Rep.* 2000;7(1):13-6.
12. Carico E, French D, Bucci B, Falcioni R, Vecchione A, Mariani-Costantini R. Integrin beta 4 expression in the neoplastic progression of cervical epithelium. *Gynecol Oncol.* 1993;49(1):61-6.
13. Nagata M, Noman AA, Suzuki K, Kurita H, Ohnishi M, Ohyama T, et al. ITGA3 and ITGB4 expression biomarkers estimate the risks of locoregional and hematogenous dissemination of oral squamous cell carcinoma. *BMC Cancer.* 2013;13:410.
14. Stewart RL, West D, Wang C, Weiss HL, Gal T, Durbin EB, et al. Elevated integrin alpha6beta4 expression is associated with venous invasion and decreased overall survival in non-small cell lung cancer. *Hum Pathol.* 2016;54:174-83.
15. Gleason B, Adley B, Rao MS, Diaz LK. Immunohistochemical detection of the beta4 integrin subunit in pancreatic adenocarcinoma. *J Histochem Cytochem.* 2005;53(6):799-801.
16. Kitajiri S, Hosaka N, Hiraumi H, Hirose T, Ikehara S. Increased expression of integrin beta-4 in papillary thyroid carcinoma with gross lymph node metastasis. *Pathol Int.* 2002;52(7):438-41.
17. Lu S, Simin K, Khan A, Mercurio AM. Analysis of integrin beta4 expression in human breast cancer: association with basal-like tumors and prognostic significance. *Clin Cancer Res.* 2008;14(4):1050-8.
18. Lipscomb EA, Simpson KJ, Lyle SR, Ring JE, Dugan AS, Mercurio AM. The alpha6beta4 integrin maintains the survival of human breast carcinoma cells in vivo. *Cancer Res.* 2005;65(23):10970-6.
19. Stallmach A, von Lampe B, Matthes H, Bornhoft G, Riecken EO. Diminished expression of integrin adhesion molecules on human colonic epithelial cells during the benign to malign tumour transformation. *Gut.* 1992;33(3):342-6.

20. Lohi J, Oivula J, Kivilaakso E, Kiviluoto T, Frojzman K, Yamada Y, et al. Basement membrane laminin-5 is deposited in colorectal adenomas and carcinomas and serves as a ligand for alpha3beta1 integrin. *APMIS*. 2000;108(3):161-72.
21. Falcioni R, Turchi V, Vitullo P, Navarra G, Ficari F, Cavaliere F, et al. Integrin Beta-4 expression in colorectal-cancer. *Int J Oncol*. 1994;5(3):573-8.
22. Ni H, Dydensborg AB, Herring FE, Basora N, Gagne D, Vachon PH, et al. Upregulation of a functional form of the beta4 integrin subunit in colorectal cancers correlates with c-Myc expression. *Oncogene*. 2005;24(45):6820-9.
23. Zhang W, Zhang B, Vu T, Yuan G, Zhang B, Chen X, et al. Molecular characterization of pro-metastatic functions of beta4-integrin in colorectal cancer. *Oncotarget*. 2017;8(54):92333-45.
24. Sordat I, Bosman FT, Dorta G, Rousselle P, Aberdam D, Blum AL, et al. Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. *J Pathol*. 1998;185(1):44-52.
25. Li M, Jiang X, Wang G, Zhai C, Liu Y, Li H, et al. ITGB4 is a novel prognostic factor in colon cancer. *J Cancer*. 2019;10(21):5223-33.
26. Mishra DK, Scott KL, Wardwell-Ozgo JM, Thrall MJ, Kim MP. Circulating tumor cells from 4D model have less integrin beta 4 expression. *J Surg Res*. 2015;193(2):745-53.
27. Sanvito F, Vivoli F, Gambini S, Santambrogio G, Catena M, Viale E, et al. Expression of a highly conserved protein, p27BBP, during the progression of human colorectal cancer. *Cancer Res*. 2000;60(3):510-6.
28. Slik K, Blom S, Turkki R, Valimaki K, Kurki S, Mustonen H, et al. Combined epithelial marker analysis of tumour budding in stage II colorectal cancer. *J Pathol Clin Res*. 2019;5(1):63-78.
29. Chao C, Lotz MM, Clarke AC, Mercurio AM. A function for the integrin alpha6beta4 in the invasive properties of colorectal carcinoma cells. *Cancer Res*. 1996;56(20):4811-9.
30. Steinberg SM, Barkin JS, Kaplan RS, Stablein DM. Prognostic indicators of colon tumors. The Gastrointestinal Tumor Study Group experience. *Cancer*. 1986;57(9):1866-70.
31. Beaulieu JF. Integrin alpha6beta4 in colorectal cancer. *World J Gastrointest Pathophysiol*. 2010;1(1):3-11.
32. Reimers MS, Bastiaannet E, Langley RE, van Eijk R, van Vlierberghe RL, Lemmens VE, et al. Expression of HLA class I antigen, aspirin use, and survival after a diagnosis of colon cancer. *JAMA Intern Med*. 2014;174(5):732-9.
33. Dydensborg AB, Teller IC, Groulx JF, Basora N, Pare F, Herring E, et al. Integrin alpha6Bbeta4 inhibits colon cancer cell proliferation and c-Myc activity. *BMC Cancer*. 2009;9:223.
34. Patriarca C, Alfano RM, Sonnenberg A, Graziani D, Cassani B, de Melker A, et al. Integrin laminin receptor profile of pulmonary squamous cell and adenocarcinomas. *Hum Pathol*. 1998;29(11):1208-15.

Figures

Figure 1

integrin $\alpha 4$ expression on the tumor, relation with disease-free survival and differentiation grade. A-C: integrin $\alpha 4$ expression in three different TMA cores, showing the difference in expression between weak (1A), intermediate (1B) and high (1C) $\alpha 4$ expression in tumor cells (anti-integrin $\alpha 4$ monoclonal antibodies, x10). D: survival curve for disease-free survival reported in months for all groups with $\alpha 4$ -weak used as reference group. E: relation between disease-free patient survival in months and $\alpha 4$ expression based on RNA expression levels derived from the TCGA dataset. N < median = 166, N > median = 165. F: relation between tumor stage (pT1-4) and $\alpha 4$ expression on the tumor.