PEER-REVIEWED SCIENTIFIC AND PRACTICAL JOURNAL RESEARCH'n PRACTICAL MEDICINE JOURNAL Vol. 4. №4 2017

Research'n Practical Medicine Journal. 2017, v.4, №4, p. 73-78

REVIEW

DOI: 10.17709/2409-2231-2017-4-4-8

LAMININS IN COLORECTAL CANCER: EXPRESSION, FUNCTION, PROGNOSTIC POWER AND MOLECULAR MECHANISMS

S.A.Rodin[1,2], D.V.Maltseva[1]

1. SRC Bioclinicum, 2 bld. 85 Ugreshskaya str., Moscow, 115088, Russia
2. Karolinska Institutet, 17177 Stockholm, Sweden

Abstract

Extracellular matrix (ECM) proteins are a major component of the tumor stroma. Laminins emerge as one of the main families of ECM proteins with signaling properties. Apart from the structural function, laminins and products of their degradation affect survival and differentiation of cancer cells, motility of cancer and stromal cells, angiogenesis, invasion into distant organs, and other aspects of cancer development. Here, we discus expression of laminins in colorectal cancer (CRC), studying of laminin functions in *in vitro* and *in vivo* models of CRC, and using laminins as prognostic markers of CRC. Recently, we have reported a new approach to assessing prognostic power using classifiers constructed from sets of laminin genes. The method allows for accurate prognosis of CRC and provides additional information that may suggest possible molecular mechanisms of laminin function in CRC progression.

Keywords:
laminins, colorectal cancer, prognostic markers of cancer

For citation
Rodin S.A., Maltseva D.V. Laminins in colorectal cancer: expression, function, prognostic power and molecular mechanisms. Research'n Practical Medicine Journal. 2017; 4(4): 73-78. DOI: 10.17709/2409-2231-2017-4-4-8

For correspondence
Sergey Rodin, PhD, senior researcher, Department of medical biochemistry and biophysics, Karolinska Institutet, senior researcher at SRC Bioclinicum
Address: Scheelesvag 2, Plan 4:B1, 17177 Stockholm. E-mail: sergey.rodin@ki.se

Information about funding. This study has been supported by the Russian Science Foundation (grant RSF № 17-14-01338).
Conflict of interest. All authors report no conflict of interest.

The article was received 15.10.2017, accepted for publication 30.11.2017

РЕЦЕНЗИРУЕМЫЙ НАУЧНО-ПРАКТИЧЕСКИЙ ЖУРНАЛ ИССЛЕДОВАНИЯ И ПРАКТИКА В МЕДИЦИНЕ
Том 4. №4
2017

Исследования и практика в медицине 2017, т.4, №4, с. 73-78

ОБЗОР

DOI: 10.17709/2409-2231-2017-4-4-8

ЛАМИНИНЫ В КОЛОРЕКТАЛЬНОМ РАКЕ: ЭКСПРЕССИЯ, ФУНКЦИИ, ПРОГНОСТИЧЕСКАЯ ЗНАЧИМОСТЬ И МОЛЕКУЛЯРНЫЙ МЕХАНИЗМ ДЕЙСТВИЯ

С.А.Родин[1,2], Д.В.Мальцева[1]

1. ООО НТЦ «БиоКлиникум», 115088, Россия, Москва, ул. Угрешская, д. 2, стр. 85 «IQ-Park»
2. Каролинский институт, 17177, Стокгольм, Швеция

Резюме

Белки внеклеточного матрикса (ВНМ) представляют собой одну из важнейших составляющих опухолевой стромы. В отличии от других молекул ВНМ, ламинины являются семейством белков с ярко выраженными сигнальными свойствами. Кроме организации клеток в пространстве, ламинины и продукты их деградации влияют на выживание и дифференцировку опухолевых клеток, миграцию опухолевых и стромальных клеток, ангиогенез, инвазию опухолевых клеток и другие процессы развития рака. В этой работе представлен анализ литературных данных по экспрессии ламининов в опухолях при колоректальном раке (КРР), изучению роли ламининов в развитии КРР в *in vivo* и *in vitro* экспериментах и использованию ламининов как прогностических маркеров КРР. Недавно, мы применили новый подход, использующий классификаторы на основе экспрессии групп ламининовых генов, для изучения прогностической значимости ламининов при КРР. Этот метод позволил дать более точный прогноз развития КРР в пациентах и предложить возможный молекулярный механизм действия ламининов при КРР.

Ключевые слова:
колоректальный рак, ламинины, прогностические маркеры рака

Оформление ссылки для цитирования статьи
Родин С.А., Мальцева Д.В. Ламинины в колоректальном раке: экспрессия, функции, прогностическая значимость и молекулярный механизм действия. Исследования и практика в медицине. 2017; 4(4): 73-78. DOI: 10.17709/2409-2231-2017-4-4-8

Для корреспонденции
Родин Сергей Алексеевич, к.б.н., старший научный сотрудник отделения изучения белков внеклеточного матрикса факультета медицинской биохимии и биофизики Каролинского института, ведущий научный сотрудник ООО НТЦ «БиоКлиникум»
Адрес: 115088 Россия, Москва, ул. Угрешская, д. 2, стр. 85 «IQ-Park». E-mail: sergey.rodin@ki.se

Информация о финансировании. Работа выполнена при поддержке Российского научного фонда (грант РСФ № 17-14-01338).
Конфликт интересов. Все авторы заявляют об отсутствии конфликта интересов.

Статья поступила 15.10.2017 г., принята к печати 30.11.2017 г.

INTRODUCTION

Colorectal cancer (CRC) affects colon and/or rectum and is the second or the third (depending on gender) most common malignancy in humans. The individual tumors are very different in the genetic/epigenetic mutations involved, the metastatic potential, the heterogeneity of cancer cell populations, and the transcription profiles [1]. Therefore, there is a need for development of prognostic and predictive strategies for efficient treatment of CRC [1]. Also, there are patients, especially at the late stages of CRC, that are resistant to existing treatment protocols warranting development of new approaches.

Tumors do not consist of the neoplastic cells only, but also cancer-associated fibroblasts, immune cells, vessels, extracellular matrix (ECM), and more [2]. Two major families of ECM, which are abundantly present in tumors, are collagens and laminins. Laminins are heterotrimeric glycoproteins that consist of one α, one β, and one γ chains. Five α, four β, and three γ laminin chains and 16 trimers (laminin isoforms) have been described in the human body to date [3]. Laminins are named after their chain composition, e. g. laminin-332 (LN-332) consists of α3, β3, and γ2 chains. Some laminin isoforms are capable of polymerization, e. g. as a part of basement membranes (BM) [3]. BMs are thin layers of ECM proteins that consist of two interconnected polymers of laminins and collagens interconnected by nidogens with additional ECM proteins and growth factors attached [4]. The membranes isolate epithelial or endothelial cells from underlying stroma and are in contact with almost all organized cells in the human body. The blood and lymphatic vessels, the gut, and the tumors themselves contain BMs [3]. Dissemination of cancer cells to metastatic sites implies traversing of up to four BMs making it an important part of cancer development. In BMs, laminins are in direct contact with cellular surface interacting with various receptors such as integrins, sulfated glycolipids, dystroglycan, Lutheran receptor, 67 kDa laminin receptor, MCAM, and others [3]. Importantly, laminins have been shown to trigger signaling events inside the cells via interaction with the cellular receptors affecting survival, differentiation, polarization, and migration of the cells. It has been shown that the majority of steps of cancer development are affected by laminins at certain extent [5, 6]. Laminins have been used as prognostic markers of various cancer types including CRC [5]. Importantly, a therapeutic strategy targeting tumor-specific interaction between LN-332 and Syndecan 1 using monoclonal antibodies has been developed in order to inhibit squamous cell carcinoma proliferation and dissemination [7]. Hence, studying laminin functions in cancer is useful both for development of prognostic and therapeutic strategies.

Expression of laminin chains in the normal gut and CRC

Expression of all 5 known laminin α chains has been detected in the developing and mature human small intestine [8]. Expression of α4 is very low and mostly attributed to the mesenchymal tissues. Laminin α1 chain is mostly expressed at the early stages of the intestine development and expression level in the mature gut is low, but localized in the epithelial fraction that is believed to be the source of cells for the neoplastic transformation. Interestingly, there are evidences that α1 laminins are involved in the progression of CRC (see below). Laminin α2, α3, and α5 chains are responsible for the maintenance and function of the mature small intestine suggesting the expression of LN-511, LN-521, LN-211, LN-221, LN-311, and, importantly, LN-332.

Among all laminin isoforms, LN-332 and its components have been most often associated with cancer development and poor prognosis for the patients [9]. Several studies have shown expression of a LN-332 component, γ2 laminin chain, in CRC tumors and, especially, at the invasive front [10–12]. Fukazawa et al. [13] demonstrated expression of another component of LN-332, β3 laminin chain, in tissue specimens from CRC patients. Immunostaining of tissue specimens from CRC patients has shown expression of α5 laminin (detected by 4C7 antibodies), β1, and γ1 chains and, surprisingly, almost no expression of β2 and α2 laminin chains [14]. Our analysis of existing microarray data of CRC tumors revealed significant expression of α5 and α4 laminin chains (Galatenko et al., in press).

Laminins in *in vitro* assays and xenograft models of CRC

Corroborating with the analysis of tissue specimens from CRC patients, colon carcinoma cell line LIM1215 has been shown to express LN-511 and to adhere to it in EGF-dependent manner. The adhesion is mediated by interaction of LN-511 with multiple integrin receptors, primarily integrin α6β4 [15]. Combination of EGF treatment and LN-511 substratum is also responsible for increased motility of LIM1215 cells [16] suggesting LN-511 involvement in the progression and metastasis of CRC.

Ectopic overexpression of *LAMC2* gene, which encodes laminin γ2 chain, in HCT8 and HCT116 CRC cells led to increased rate of proliferation and enhanced migration and invasion compared with that of the vector-transduced control cells [17]. De Arcangelis et al. [18] introduced a α1 laminin expression vector into HT-29 human colonic cancer cells and injected the

cells into the nude mice (xenograft model). The induction of ectopic laminin α1 expression led to a significant increase in tumor growth and to an increase in recruitment of tumor and vascular cells into the tumors in comparison with that in xenograft models based on unmodified HT-29 cells. Disruption of laminin α1 expression in CaCo2 epithelial colorectal adenocarcinoma cells that are normally express the laminin chain blocks the tumor growth in the xenograft models of CRC [18]. The in vivo experiments suggest that the influence of laminin α1 chain on the CRC tumor progression is important and reminiscent of the normal gut development.

Laminins as prognostic markers of CRC

Early studies of laminin predictive and prognostic power used single laminin chains as biomarkers of the outcome in the patients. Thus, prognostic implications of laminin γ2 expression have been thoroughly studied on several cohorts of CRC patients. One group has demonstrated positive γ2 laminin cytoplasm staining in 96% of studied CRC tumors (89 out of 93) [10]. Univariate analysis has identified LN-332 expression as a statistically significant marker of the outcome of the disease. However, multivariate analysis has failed to confirm that expression of laminin γ2 chain is an independent marker of CRC. A later study [11] utilized a cohort of 103 CRC patients and has confirmed by both uni- and multivariate analyses the predictive power of laminin γ2 chain expression. But, the method of quantification chosen by the authors does not allow reliable absolute quantification of the expression. Shinto et al. [12] quantified laminin γ2-positive cells in different areas of 120 individual CRC tumors and evaluated the area-specific prognostic significance of the expression. Only expression of laminin γ2 chain at the invasive front of the tumor has been identified as an independent prognostic marker in uni- and multivariate analyses, while the expression in the center of the tumors has failed to correlate with the outcome of the disease. Huang et al. performed a meta-analysis of publically available datasets of microarray data of patients with CRC and found out that expression of γ2 correlated with poor clinical outcomes in terms of overall survival, disease-specific survival, disease-free survival, and recurrence-free survival [17]. Since laminin γ2 chain has been shown to enhance migration of cancer cells, the results are in concert with the probable molecular mechanism of γ2 action.

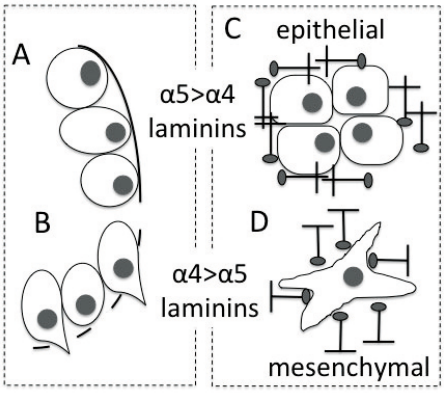

Figure 1. Two possible molecular mechanisms of laminin involvement in progression of CRC. (A. and B.) Basement membrane (BM) permeability hypothesis. A. Low ratio of α4 to α5 laminin chain expression levels leads to formation of hard-to-traverse BMs that might contain cancer cells inside the primary tumor. B. High ratio of α4 to α5 laminin chain expression levels leads to formation of easy-to-traverse BMs that might facilitate cancer cell escape from the primary tumor. (C. and D.) Epithelial-mesenchymal transition hypothesis. C. Low ratio of α4 to α5 laminin chain expression levels shifts plastic cancer cells to a more epithelial phenotype. LN-511/521 are represented as cross-shaped. D. High ratio of α4 to α5 laminin chain expression levels shifts plastic cancer cells to a more mesenchymal phenotype. LN-411/421 are represented as T-shaped.

Another component of LN-332, β3 laminin chain, has been shown to be useful in predicting clinical outcomes of CRC in patients in the stage dependent manner [13]. While no significant difference in expression of β3 laminin chain in patients with good and bad prognosis at Stage II of CRC was detected, among patients with Stage III high expression of β3 correlated with poorer prognosis. Importantly, expression of the laminin chain has also a predictive power for Stage III CRC patients, because multivariate analysis has shown that β3-low patients benefited from adjuvant chemotherapy and β3-high patients did not.

Proteomic analysis of secretomes from HCT116 CRC line and its more metastatic derivative revealed that expression of β1 laminin chain correlated with aggressiveness of the cells [19]. The authors also demonstrated using ELISA that expression of laminin β1 chain was significantly higher in patients with CRC in comparison with that in healthy individuals. But the study lacks statistical power as samples from only 19 patients and 47 healthy individuals were analyzed and the conclusion warrants additional verification. If proven right, the method might be useful in the clinic for diagnostics of CRC.

We have applied a new approach to estimate cumulative predictive power of the entire laminin family (Galatenko et al., in press). The estimation was based on analysis of publically available datasets of microarray data of CRC patients using construction classifiers based on expression of laminin genes. Our data suggest that predictive power of the whole laminin family is higher than that of any individual chains, but it is achieved already with some pairs and triples of the genes. Analysis of the triplets and their

weights in the classifiers has revealed that high ratio of laminin α4 to α5 expression levels correlates with poorer prognosis in CRC patients. Application of the gene signatures method allows for not only more accurately predict the outcomes of CRC in patients, but also hypothesize the molecular mechanism of laminin action in the progression of CRC.

Possible molecular mechanisms of laminin involvement in progression of CRC

One possible mechanism of molecular action might be associated with BMs produced by cancer cells themselves. It has been earlier shown that leukocytes have an increased ability to traverse α4-rich BMs in comparison with α5-rich BMs [20, 21]. Since molecular mechanisms of penetration through a BM are similar for leukocytes and cancer cells, laminin α4-low/α5-high expressing tumors produce hard-to-traverse BMs. It is possible that α5-rich BMs are impregnable for cancer cells thus the tumors restrict their own progression and dissemination (Fig. 1A). In case of laminin α4-high/α5-low expression, cancer BMs are easy to traverse that is the first step of the tumor progression (Fig. 1B).

Earlier, it has been shown that increased level of LN-411 and decreased level of LN-511 are associated with epithelial to mesenchymal transition (EMT) [22]. Importantly, LN-411 reduces adhesion of the cancer cells to LN-511, suggesting that high α4/α5 laminin expression ratio will lead to complete replacement of α5 laminins by α4-containg ones and will drive plastic tumor cells to a more mesenchymal and aggressive phenotype (Fig. 1C and D).

Both possible mechanisms of laminin involvement in progression of CRC are just hypotheses and need experimental confirmation. The BM permeability hypothesis could be tested by quantification of circulating tumor cells in patients and xenograft models based on CRC lines with various α4/α5 laminin expression ratio. Also, analysis of xenograft models based on non aggressive CRC lines (such as RKO and SW-480) with ectopic overexpression of *LAMA4* gene, which encodes laminin α4 chain, might be instrumental for testing of the hypothesis. The EMT hypothesis can be tested by simple treatment of CRC cells with purified laminin isoforms LN-511/521 and LN-411/421 to detect expression of markers of EMT.

CONCLUSION

Colorectal cancer is a serious healthcare problem that warrants development of new prognostic and therapeutic approaches. Application of the gene signature method allows for not only more precise prognosis of CRC outcomes in patients, but also allows proposing possible molecular mechanisms of CRC progression and metastasis. Laminins are involved in progression of CRC in patients at different stages. Knowledge on the molecular mechanisms of laminin involvement in CRC might be useful for development of new therapeutic approaches.

References

1. Cunningham D, Atkin W, Lenz HJ, Lynch HT, Minsky B, Nordlinger B, Starling N. Colorectal cancer. Lancet. 2010 Mar 20;375 (9719):1030–47. DOI: 10.1016/S0140–6736 (10)60353–4

2. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol. 2006;1:119–50. DOI: 10.1146/annurev.pathol.1.110304.100224

3. Domogatskaya A, Rodin S, Tryggvason K. Functional diversity of laminins. Annu Rev Cell Dev Biol. 2012;28:523–53. DOI: 10.1146/annurev-cellbio-101011–155750

4. Yurchenco PD. Basement membranes: cell scaffoldings and signaling platforms. Cold Spring Harb Perspect Biol. 2011 Feb 1;3 (2). pii: a004911. DOI: 10.1101/cshperspect.a004911

5. Qin Y, Rodin S, Simonson OE, Hollande F. Laminins and cancer stem cells: Partners in crime? Semin Cancer Biol. 2017 Aug;45:3–12. DOI: 10.1016/j.semcancer.2016.07.004

6. Patarroyo M, Tryggvason K, Virtanen I. Laminin isoforms in tumor invasion, angiogenesis and metastasis. Semin Cancer Biol. 2002 Jun;12 (3):197–207. DOI: 10.1016/S1044–579X (02)00023–8

7. Tran M, Rousselle P, Nokelainen P, Tallapragada S, Nguyen NT, Fincher EF, Marinkovich MP. Targeting a tumor-specific laminin domain critical for human carcinogenesis. Cancer Res. 2008 Apr 15;68 (8):2885–94. DOI: 10.1158/0008–5472.CAN-07–6160

8. Teller IC, Auclair J, Herring E, Gauthier R, Ménard D, Beaulieu JF. Laminins in the developing and adult human small intestine: relation with the functional absorptive unit. Dev Dyn. 2007; 236 (70): 1980–90. DOI: 10.1002/dvdy.21186

9. Marinkovich, M. P. Tumour microenvironment: laminin 332 in squamous-cell carcinoma. Nat Rev Cancer. 2007 May; 7 (5): 370–80. DOI: 10.1038/nrc2089

10. Lenander C, Habermann JK, Ost A, Nilsson B, Schimmelpenning H, Tryggvason K, Auer G. Laminin-5 gamma 2 chain expression correlates with unfavorable prognosis in colon carcinomas. Anal Cell Pathol. 2001; 22 (4): 201–9.

11. Aoki S, Nakanishi Y, Akimoto S, Moriya Y, Yoshimura K, Kitajima M, et al. Prognostic significance of laminin-5 gamma2 chain expression in colorectal carcinoma: immunohistochemical analysis of 103 cases. Dis Colon Rectum. 2002 Nov;45 (11):1520–7. DOI: 10.1097/01.DCR.0000029593.41892.62

12. Shinto E, Tsuda H, Ueno H, Hashiguchi Y, Hase K, Tamai S, et al. Prognostic implication of laminin-5 gamma 2 chain expres-

sion in the invasive front of colorectal cancers, disclosed by area-specific four-point tissue microarrays. Lab Invest. 2005 Feb;85 (2):257–66. DOI: 10.1038/labinvest.3700199
13. Fukazawa S, Shinto E, Tsuda H, Ueno H, Shikina A, Kajiwara Y, et al. Laminin beta3 expression as a prognostic factor and a predictive marker of chemoresistance in colorectal cancer. Jpn J Clin Oncol. 2015 Jun;45 (6):533–40. DOI: 10.1093/jjco/hyv037
14. Hewitt RE, Powe DG, Morrell K, Balley E, Leach IH, Ellis IO, Turner DR. Laminin and collagen IV subunit distribution in normal and neoplastic tissues of colorectum and breast. Br J Cancer. 1997; 75 (2): 221–9.
15. Pouliot N, Connolly LM, Moritz RL, Simpson RJ, Burgess AW. Colon cancer cells adhesion and spreading on autocrine laminin-10 is mediated by multiple integrin receptors and modulated by EGF receptor stimulation. Exp Cell Res. 2000 Dec 15;261 (2):360–71. DOI: 10.1006/excr.2000.5065
16. Pouliot N, Nice EC, Burgess AW. Laminin-10 mediates basal and EGF-stimulated motility of human colon carcinoma cells via alpha (3)beta (1) and alpha (6)beta (4) integrins. Exp Cell Res. 2001 May 15; 266 (1): 1–10. DOI: 10.1006/excr.2001.5197
17. Huang D, Du C, Ji D, Xi J, Gu J. Overexpression of LAMC2 predicts poor prognosis in colorectal cancer patients and promotes cancer cell proliferation, migration, and invasion. Tumour Biol. 2017 Jun;39 (6):1010428317705849. DOI: 10.1177/1010428317705849
18. De Arcangelis A,, Lefebvre O, Méchine-Neuville A, Arnold C, Klein A, Rémy L, et al. Overexpression of laminin alpha1 chain in colonic cancer cells induces an increase in tumor growth. Int J Cancer. 2001 Oct 1;94 (1):44–53. DOI: 10.1002/ijc.1444
19. Lin Q, Lim HS, Lin HL, Tan HT, Lim TK, Cheong WK, et al. Analysis of colorectal cancer glyco-secretome identifies laminin β-1 (LAMB1) as a potential serological biomarker for colorectal cancer.Proteomics. 2015 Nov;15 (22):3905–20. DOI: 10.1002/pmic.201500236
20. Sixt M, Engelhardt B, Pausch F, Hallmann R, Wendler O, Sorokin LM. Endothelial cell laminin isoforms, laminins 8 and 10, play decisive roles in T cell recruitment across the blood-brain barrier in experimental autoimmune encephalomyelitis. J Cell Biol. 2001 May 28;153 (5):933–46.
21. Kenne E, Soehnlein O, Genové G, Rotzius P, Eriksson EE, Lindbom L. Immune cell recruitment to inflammatory loci is impaired in mice deficient in basement membrane protein laminin alpha4. J Leukoc Biol. 2010 Sep;88 (3):523–8. DOI: 10.1189/jlb.0110043
22. Takkunen M, Ainola M, Vainionpää N, Grenman R, Patarroyo M, García de Herreros A, et al. Epithelial-mesenchymal transition downregulates laminin alpha5 chain and upregulates laminin alpha4 chain in oral squamous carcinoma cells. Histochem Cell Biol. 2008 Sep;130 (3):509–25. DOI: 10.1007/s00418–008–0443–6

Information about authors:

Sergey Rodin, PhD, senior researcher, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, senior researcher at SRC Bioclinicum. E-mail: sergey.rodin@ki.se

Diana V. Maltseva, PhD, Deputy Director for Science, SRC Bioclinicum. E-mail: d.maltseva@bioclinicum.com

Информация об авторах:

Родин Сергей Алексеевич, к.б.н., с.н.с. отделения изучения белков внеклеточного матрикса факультета медицинской биохимии и биофизики Каролинского института, в.н.с. ООО НТЦ «БиоКлиникум». E-mail: sergey.rodin@ki.se

Мальцева Диана Васильевна, к.х.н., заместитель генерального директора по науке ООО НТЦ «БиоКлиникум». E-mail: d.maltseva@bioclinicum.com