ACADEMIC PRESS

Biochemical and Biophysical Research Communications 296 (2002) 73–77

BBRC

www.academicpress.com

Matrix metalloproteinase-2 is involved in A549 cell migration on laminin-10/11

J. Gu,* R. Nishiuchi, and K. Sekiguchi

Division of Protein Chemistry, Institute for Protein Research, Osaka University, 3-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Received 9 July 2002; accepted 10 July 2002

Abstract

We have reported that laminin-10/11 strongly promotes migration of A549 human lung carcinoma cells by activating the α3β1 integrin-dependent signaling pathway. To elucidate the mechanism involved, we investigated whether matrix metalloproteinases (MMPs) are involved in cell migration on laminin-10/11. Here, we demonstrate that laminin-10/11, but not fibronectin which does not greatly promote A549 cell movement, stimulated MMP-2 secretion ~3-fold. The cell migration-promoting activity of laminin-10/11 was down-regulated by an MMP inhibitor. In addition, cell motility was significantly increased when cells adhered to a mixture of fibronectin and laminin-10/11 with a concomitant decrease of focal contacts, compared with those adhering to fibronectin alone. The enhanced cell migration was partially suppressed by the MMP inhibitor. Furthermore, an anti-α3 integrin, but not an anti-α5 integrin, antibody induced the activated form of MMP-2. These data suggest that MMP-2 may play an important role in A549 cell migration on laminin-10/11 through an α3β1 integrin-dependent pathway.

Keywords: Laminin; Integrin; Migration; MMP-2

Matrix metalloproteinases (MMPs) are a family of structurally related zinc-dependent extracellular matrix (ECM)-degrading enzymes that are thought to play a crucial role in tumor cell invasion [1,2]. It has been shown that elevated levels of expression of different MMPs are associated with a metastatic stage in the progression of various types of tumors [3]. Their expression is induced not only by growth factors or cytokines, but also by ECM stimulation. It has been reported that integrin-mediated cell–ECM interactions may have a significant impact on the production of MMPs by tumor cells [4]. Integrin-mediated adhesion to laminin and antibody-induced clustering of α3β1 integrins enhance the secretion of MMP-2 in rhabdomyosarcoma and glioblastoma cells [5,6]. In moderately invasive melanoma cells, treatment with an anti-αvβ3 integrin antibody results in a concomitant increase in the invasiveness and expression and secretion of MMP-2 [7]. Although the mechanisms through which MMP-2 can promote cancer metastasis are not completely understood, it seems that MMP-2 is concentrated along the invasive edge of tumors and degrades the ECM components of the basement membrane thereby driving tumor invasion [8].

Laminins are the major components of basement membranes. Cells bind directly to laminins via a subset of integrins and other non-integrin receptors, such as α-dystroglycans [9,10]. All laminins are composed of α, β, and γ chains. The α5-containing laminins, LN-10 (α5β1γ1) and LN-11 (α5β2γ1), are widely expressed in fetal and adult tissues [11,12]. Recently, we purified LN-10/11 from the conditioned medium of A549 human lung carcinoma cells and found that the α3β1 integrin is the preferred receptor for LN-10/11 [13,14]. LN-10/11 is more potent than FN in preventing the apoptosis induced by serum depletion [15]. In addition, LN-10/11 is more active than FN in promoting cell migration and preferentially activates Rac, but not Rho, via the $p130^{Cas}$-CrkII-DOCK180 pathway. Cells adhering to FN develop stress fibers and focal contacts, whereas cells adhering to LN-10/11 do not suggest that LN-10/11

* Corresponding address. Fax: +81-6-6879-8619.
E-mail address: jgu@protein.osaka-u.ac.jp (J. Gu).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00831-8

and FN have distinct effects on integrin-mediated cell spreading and migration [16].

The purpose of this study was to determine whether MMPs contribute to cell migration on LN-10/11. Our results indicate that MMP-2 is involved in the promotion of cell migration on laminin-10/11.

Materials and methods

Cells and cell culture. A549 human lung adenocarcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 °C in a humidified atmosphere containing 5% CO_2.

Reagents and antibodies. A monoclonal antibody against paxillin was obtained from Transduction Laboratories (San Diego, CA). Monoclonal antibodies (mAbs) against α3 (3G8) and α5 (8F1) integrins were produced and characterized in our laboratory [17]. F-actin was detected by rhodamine-labeled phalloidin obtained from Molecular Probes (Eugene, Oregon). The FITC-conjugated rabbit secondary antibody to mouse IgG was purchased from Cappel (West Chester, PA).

Preparation of LN-10/11 and LN-5. LN-10/11 was purified to homogeneity from the conditioned media of A549 cells as previously described [13]. The 5D6 mAb, recognizing the human laminin α5 chain, was used for immunoaffinity chromatography. Silver staining and immunoblotting with a panel of mAbs specific for individual laminin α chains verified that the purified LN-10/11 lacked other laminin isoforms [17]. LN-5 was purified from the conditioned media of MKN45 cells by immunoaffinity chromatography using an anti-laminin γ2 chain antibody [18].

Gelatin zymography. The production of MMPs by A549 cells was analyzed by gelatin zymography as previously described [19]. Briefly, 24-well culture dishes were coated with LN-10/11 (5 nM), LN-5 (5 nM), or FN (20 nM) in PBS overnight at 4 °C and then non-specific binding sites were blocked with 1% BSA. The coating concentration of FN was 4-fold higher than that of LN-10/11 to attain comparable levels of cell-adhesive activity for A549 cells. A549 cells were replated on the coated culture dishes or non-coated dishes and incubated for 4 h in DMEM containing 10% FBS. After washing with serum-free DMEM, the cells on coated dishes were incubated for 48 h in DMEM containing 1% FBS and those on uncoated dishes were incubated for 48 h in serum-free DMEM, supplemented with or without 13 μg/ml antibodies. The conditioned media were concentrated to ~1/10 and then resolved in 10% SDS–polyacrylamide gels containing 1 mg/ml gelatin. The gels were washed three times with 2.5% Triton X-100 for 1 h, equilibrated with 10 mM Tris–HCl, pH 8.0, for 30 min, and then incubated for 20 h at 37 °C in 10 mM Tris–HCl, pH 8.0, 5 mM $CaCl_2$, and 1 nM $ZnCl_2$. The lytic bands were visualized by Coomassie brilliant blue R250 staining.

Cell motility assays. A549 cells were replated on 35-mm culture dishes (Corning Costar, Osaka, Japan) coated with LN-10/11 (5 nM), FN (20 nM) or a mixture of LN-10/11 (5 nM) and FN (20 nM) in serum-free DMEM and blocked with 1% BSA. Thirty minutes post-replating, the medium was changed to medium containing 1% FBS with or without an MMP inhibitor (MMP-2/MMP-9 inhibitor II from Calbiochem, La Jolla, CA). Cell migration was then monitored using a Zeiss S-25 inverted microscope. Video images were collected using a CCD camera at 20 min intervals using the Image-Pro software (Media Cybernetics, Silverspring, MD). The positions of the nuclei were tracked to quantify cell motility. Velocities were calculated in micrometers per 8 h using the same software.

Immunofluorescence microscopy. Glass coverslips were coated with LN-10/11 (5 nM), FN (20 nM) or a mixture of LN-10/11 (5 nM) and FN (20 nM) in PBS overnight at 4 °C and then blocked with 1% BSA. A549 cells were serum-starved overnight then replated on the coverslips by incubating for 2 h in DMEM containing 1% BSA. Cells were then fixed with 3.7% paraformaldehyde in PBS for 20 min and permeabilized with 0.5% Triton X-100 for 5 min. Focal adhesions were visualized by incubating cells with the mouse anti-paxillin antibody, followed by an incubation with a FITC-conjugated rabbit antibody specific for mouse IgG. Actin filaments were stained with rhodamine-conjugated phalloidin.

Results

Effects of LN-10/11 and FN on MMP-2 secretion

Signals transduced from the ECM to tumor cells could affect cell attachment, motility, and invasion. Furthermore, alterations in cell–matrix interactions are known to regulate MMP expression [20]. To investigate whether MMPs contributed to the cell migration promoted by LN-10/11, we examined the effects of FN, LN-10/11, and LN-5 on MMP secretion by A549 cells. LN-5 was included as a control ligand, which binds to α3β1 integrin. A549 cells were seeded onto culture dishes coated with FN, LN-10/11 or LN-5 and incubated for 48 h in the presence of 1% FCS. LN-10/11 and LN-5, but not FN, markedly stimulated MMP-2 secretion 2–3-fold (Fig. 1). None of these ECM proteins induced MMP-9 secretion.

Effect of an MMP inhibitor on cell migration promoted by LN-10/11

Since LN-10/11 was more active than FN in promoting A549 cell migration [16], it prompted us to investigate whether there were any changes in cell motility on substrates coated with a mixture of FN and LN-10/11. Interestingly, the cell migration on the substrates coated with a mixture of FN and LN-10/11 was significantly higher than the migration on the substrates coated by FN alone (Fig. 2), indicating that the effect of LN-10/11 is dominant over that of FN. To discover whether enhanced MMP-2 secretion by LN-10/11

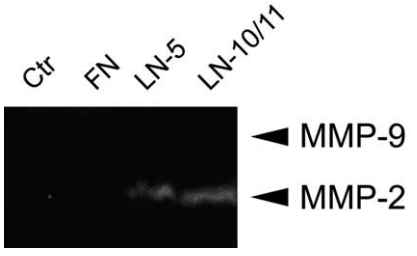

Fig. 1. Secreted levels of collagenase activity determined by gelatin zymography in response to ECM proteins. A549 cells were seeded onto plastic (control, Ctr), FN (20 nM)-, LN-5 (5 nM)-, and LN-10/11 (5 nM)-coated dishes and incubated in media containing 1% FCS for 48 h. Twenty-five microliters conditioned media was analyzed by zymogram analysis.

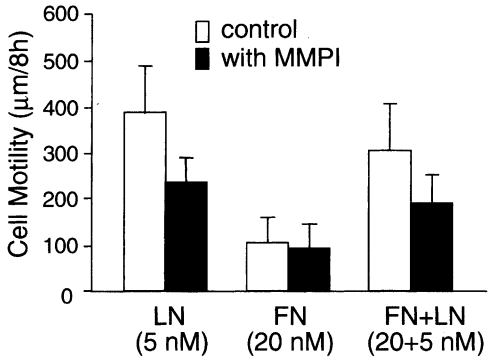

Fig. 2. An MMP inhibitor reduced the migration of A549 cells on LN-10/11. A549 cells were replated on dishes coated with FN (20 nM), LN-10/11 (5 nM) or a mixture of FN (20 nM) and LN-10/11 (5 nM) in the presence of 1% FBS with or without an MMP inhibitor (MMP-2/MMP-9 inhibitor II, 2.5 μM). Cell movements were monitored by time-lapse video microscopy. Cell motility was evaluated by velocity (μm/8 h) determined using the Image-Pro software as described in Materials and methods. Quantified cell motility is expressed as mean ± SD in triplicate assays.

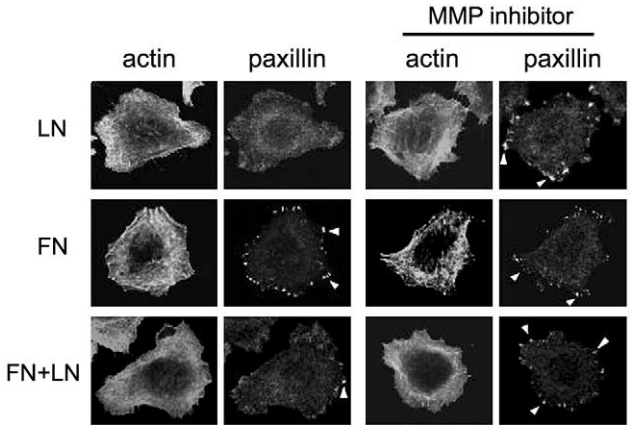

Fig. 3. An MMP inhibitor increased focal contact formation. A549 cells were allowed to spread on coverslips coated with FN, LN-10/11 or a mixture of LN-10/11 and FN for 2 h in DMEM containing 1% BSA with or without an MMP inhibitor. Cells were then stained with rhodamine–phalloidin to detect F-actin or an anti-paxillin antibody to detect focal adhesions. The arrowheads point to focal contacts.

contributed to the cell motility, the cells cultured on LN-10/11 were incubated with a specific MMP-2 inhibitor. As expected, the cell migration on LN-10/11 or the mixture of FN and LN-10/11 was significantly inhibited by the MMP inhibitor (Fig. 2).

MMP inhibitor induced focal contact formation

The interaction of cells with the ECM through the establishment of focal adhesion contacts, typically composed of integrins and various cytoplasmic proteins that link to the cytoskeleton, regulates integrin-mediated cell migration. Cells adhering to FN have been shown to develop stress fibers and focal contacts, whereas cells adhering to LN-10/11 did not [16]. To determine whether the MMP inhibitor had any effects on actin reorganization and the formation of focal contacts, cells cultured on LN-10/11, FN or the mixture of LN-10/11 and FN with or without the MMP inhibitor were stained with rhodamine–phalloidin to detect F-actin or an anti-paxillin antibody to detect focal adhesions. Treatment with the MMP inhibitor greatly enhanced focal contact formation on LN-10/11 (Fig. 3). In contrast, there were no apparent changes in the formation of focal contacts on FN after treatment with the MMP inhibitor. These results suggested that focal contact assembly may be compromised in cells spread on LN-10/11 and that the MMP inhibitor could rescue the ability of cells on LN-10/11 to assemble these structures. Consistent with these results, the cells adhering to the mixture of LN-10/11 and FN substrates showed significantly fewer focal contacts compared with the cells adhering to FN alone (Fig. 3). These results support previous observations that LN-10/11 and FN have distinct effects on integrin-mediated cell spreading and migration [16], and that α3β1 integrin transduces a signal that transdominantly inhibits the cytoskeletal organization induced by other integrins [21].

An anti-α3 integrin, but not anti-α5 integrin, antibody promotes the production of activated MMP-2

Since integrins have been linked to involvement in the signal transduction for MMP secretion [4], we examined the effects of antibodies to α3β1 integrin, a receptor for LN-10/11 and LN-5, and anti-α5β1 integrin, a receptor for FN, on MMP secretion by A549 cells. As shown in Fig. 4, an activated form of MMP-2 was induced by the anti-α3 integrin antibody, but not by the anti-α5-integrin antibody. In contrast, neither the anti-α3 nor anti-α5 integrin antibodies activated MMP-9. These data support the hypothesis that α3β1 integrin-mediated signals transduced from LN-10/11 specifically up-regulate MMP-2 activation.

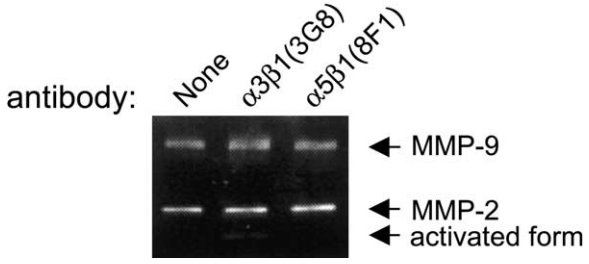

Fig. 4. Effects of anti-integrin antibodies on MMP-2 secretion. A549 cells in serum-free media were incubated in the presence of 13 μg/ml control IgG, anti-α3 or α5-integrin antibodies for 48 h. Conditioned media were concentrated and then analyzed by gelatin zymography as described in Materials and methods.

Discussion

We have previously shown that LN-10/11 and FN have distinct effects on cell spreading and migration. LN-10/11 induces the formation of a protrusive structure, whereas FN enhances the formation of actin stress fibers and focal contacts [16]. In the present study, we have explored the mechanisms for the cell migration-promoting activity of LN-10/11 and found that MMP-2 secretion/activation is involved in the cell migration and focal contact formation on LN-10/11. The secretion of MMP-2 by A549 cells was greatly enhanced when cells were adhering to LN-10/11, but not to FN, which is less potent in promoting cell migration. Consistent with these results, an MMP inhibitor increased focal adhesion formation and reduced cell migration on the LN-10/11 substrate. Furthermore, an activated form of MMP-2 was observed when A549 cells were treated with an anti-α3 integrin, but not an anti-α5 integrin, antibody, indicating that LN-10/11 promotes cell migration by up-regulation of MMP-2.

Migration of cells through tissues involves not only adhesive and signaling interactions of cells with the ECM but also requires the ability of cells to degrade components of the ECM. The latter activity is a role for MMPs. In fact, it has been reported that signaling events induced by α3β1 integrin may be involved in glioma and rhabdomyosarcoma invasion as a result of modulation of MMP expression [5,6]. In those experiments, antibodies to α3β1 integrin stimulated the production of MMP-2 and matrigel invasion. Similarly, up-regulation of MMP-2 was also observed in MDA-MB-231 cells treated not only with an antibody to α3β1 integrin but also with antibodies to CD63 or CD9 tetraspanins which associate with α3β1 integrin [22]. This is in agreement with our observation that MMP-2 is up-regulated in A549 cells adhering to LN-10/11 or cultured with a function blocking anti-α3β1 integrin antibody. It also seems likely that α3β1 has different functions in different organs. For example, immortalized keratinocytes from wild-type mice express MMP-9, but MMP-9 is not expressed in similarly derived cells deficient in α3β1 integrin [23]. Taken together, these data suggest that ligand engagement by α3β1 integrin is an important regulator of the MMP activity required for cell motility.

Cell migration can be viewed as a process regulated by counterbalanced signals that control the rate of motility by several mechanisms. Regulation of the strength of cell adhesion is one mechanism and optimal or inhibitory degrees of cell adhesion can regulate the speed of motility [24]. In fact, extensive formation of focal adhesions results in the reduction of the cell migration [25]. In the present study, increased migration was correlated with a decrease in focal contact formation in the cells adhering to substrates coated with the mixture of FN and LN-10/11. Conversely, the decreased migration of MMP inhibitor-treated cells was accompanied by an increase in focal contact formation in the cells adhering to LN-10/11. These results were supported by a study in which MMP inhibitor treatment stabilized focal adhesion contacts in fibroblasts [26], although the underlying mechanism is not clear.

Proteolysis is required to initiate and sustain migration, but excessive proteolysis may degrade matrix signals and receptors, thereby disrupting cell–matrix interactions and inhibiting migration. Keratinocytes can migrate on native collagen but not on a collagenase-resistant collagen matrix [27]. Similarly, in fibroblasts, the binding of FN fragments, but not intact FN, to α5β1 integrin leads to the induction of MMP-1 synthesis [28]. In related experiments, it has been reported that MMP-2 and/or MT1-MMP cleave the LN-5 γ2 chain, stimulating the migration of breast, liver, and colon carcinoma cells [29–31]. The migration on LN-5, another potent ligand for α3β1 integrin, is blocked by the MMP inhibitor BB-94 [30,31]. Since LN-10/11 is another pro-migratory substrate, it remains to be examined whether MMP-2 cleaves LN-10/11 for promoting cell migration.

In conclusion, we have shown that MMP-2 secretion/activation is involved in the cell migration and focal contact formation of A549 cells on LN-10/11. The present study supports the hypothesis that α3β1 integrin transdominantly regulates the signaling events induced by other integrins (e.g., α5β1 integrin) and provides insight into the molecular mechanisms of ECM-regulated cellular behaviors.

References

[1] L.M. Coussens, Z. Werb, Matrix metalloproteinases and the development of cancer, Chem. Biol. 3 (1996) 895–904.
[2] W.G. Stetler-Stevenson, S. Aznavoorian, L.A. Liotta, Tumor cell interactions with the extracellular matrix during invasion and metastasis, Annu. Rev. Cell Biol. 9 (1993) 541–573.
[3] J. Westermarck, V.M. Kahari, Regulation of matrix metalloproteinase expression in tumor invasion, FASEB J. 13 (1999) 781–792.
[4] J. Ivaska, J. Heino, Adhesion receptors and cell invasion: mechanisms of integrin-guided degradation of extracellular matrix, Cell Mol. Life Sci. 57 (2000) 16–24.
[5] S.K. Chintala, R. Sawaya, Z.L. Gokaslan, J.S. Rao, Modulation of matrix metalloprotease-2 and invasion in human glioma cells by α3β 1 integrin, Cancer Lett. 103 (1996) 201–208.
[6] S. Kubota, H. Ito, Y. Ishibashi, Y. Seyama, Anti-α3 integrin antibody induces the activated form of matrix metalloprotease-2 (MMP-2) with concomitant stimulation of invasion through matrigel by human rhabdomyosarcoma cells, Int. J. Cancer 70 (1997) 106–111.
[7] R.E. Seftor, E.A. Seftor, K.R. Gehlsen, W.G. Stetler-Stevenson, P.D. Brown, E. Ruoslahti, M.J. Hendrix, Role of the α v β 3 integrin in human melanoma cell invasion, Proc. Natl. Acad. Sci. USA 89 (1992) 1557–1561.
[8] W.T. Chen, J.Y. Wang, Specialized surface protrusions of invasive cells, invadopodia and lamellipodia, have differential MT1-MMP, MMP-2, and TIMP-2 localization, Ann. NY Acad. Sci. 878 (1999) 361–371.

[9] H. Colognato, P.D. Yurchenco, Form and function: the laminin family of heterotrimers, Dev. Dyn. 218 (2000) 213–234.
[10] F. Montanaro, M. Lindenbaum, S. Carbonetto, α-Dystroglycan is a laminin receptor involved in extracellular matrix assembly on myotubes and muscle cell viability, J. Cell Biol. 145 (1999) 1325–1340.
[11] L.M. Sorokin, F. Pausch, M. Frieser, S. Kroger, E. Ohage, R. Deutzmann, Developmental regulation of the laminin α5 chain suggests a role in epithelial and endothelial cell maturation, Dev. Biol. 189 (1997) 285–300.
[12] J.H. Miner, R.M. Lewis, J.R. Sanes, Molecular cloning of a novel laminin chain, α 5, and widespread expression in adult mouse tissues, J. Biol. Chem. 270 (1995) 28523–28526.
[13] Y. Kikkawa, N. Sanzen, K. Sekiguchi, Isolation and characterization of laminin-10/11 secreted by human lung carcinoma cells. Laminin-10/11 mediates cell adhesion through integrin α3 β1, J. Biol. Chem. 273 (1998) 15854–15859.
[14] Y. Kikkawa, N. Sanzen, H. Fujiwara, A. Sonnenberg, K. Sekiguchi, Integrin binding specificity of laminin-10/11: laminin-10/11 are recognized by α3β1, α6β1 and α6β4 integrins, J. Cell Sci. 113 (2000) 869–876.
[15] J. Gu, A. Fujibayashi, K.M. Yamada, K. Sekiguchi, Laminin-10/11 and fibronectin differentially prevent apoptosis induced by serum removal via phosphatidylinositol 3-kinase/Akt- and MEK1/ERK-dependent pathways, J. Biol. Chem. 277 (2002) 19922–19928.
[16] J. Gu, Y. Sumida, N. Sanzen, K. Sekiguchi, Laminin-10/11 and fibronectin differentially regulate integrin-dependent, Rho and Rac activation via p130(Cas)-CrkII-DOCK180 pathway, J. Biol. Chem. 276 (2001) 27090–27097.
[17] H. Fujiwara, Y. Kikkawa, N. Sanzen, K. Sekiguchi, Purification and characterization of human laminin-8. Laminin-8 stimulates cell adhesion and migration through α3β1 and α6β1 integrins, J. Biol. Chem. 276 (2001) 17550–17558.
[18] Y. Fukushima, T. Ohnishi, N. Arita, T. Hayakawa, K. Sekiguchi, Integrin α3β1-mediated interaction with laminin-5 stimulates adhesion, migration and invasion of malignant glioma cells, Int. J. Cancer 76 (1998) 63–72.
[19] S. Kubota, T. Mitsudomi, Y. Yamada, Invasive human fibrosarcoma DNA mediated induction of a 92 kDa gelatinase/type IV collagenase leads to an invasive phenotype, Biochem. Biophys. Res. Commun. 181 (1991) 1539–1547.
[20] S.D. Shapiro, Matrix metalloproteinase degradation of extracellular matrix: biological consequences, Curr. Opin. Cell Biol. 10 (1998) 602–608.
[21] K.M. Hodivala-Dilke, C.M. DiPersio, J.A. Kreidberg, R.O. Hynes, Novel roles for α3β1 integrin as a regulator of cytoskeletal assembly and as a trans-dominant inhibitor of integrin receptor function in mouse keratinocytes, J. Cell Biol. 142 (1998) 1357–1369.
[22] T. Sugiura, F. Berditchevski, Function of α3β1–tetraspanin protein complexes in tumor cell invasion. Evidence for the role of the complexes in production of matrix metalloproteinase 2 (MMP-2), J. Cell Biol. 146 (1999) 1375–1389.
[23] C.M. DiPersio, M. Shao, L. Di Costanzo, J.A. Kreidberg, R.O. Hynes, Mouse keratinocytes immortalized with large T antigen acquire α3β1 integrin-dependent secretion of MMP-9/gelatinase B, J. Cell Sci. 113 (2000) 2909–2921.
[24] S.P. Palecek, J.C. Loftus, M.H. Ginsberg, D.A. Lauffenburger, A.F. Horwitz, Integrin–ligand binding properties govern cell migration speed through cell-substratum adhesiveness, Nature 385 (1997) 537–540.
[25] D. Ilic, Y. Furuta, S. Kanazawa, N. Takeda, K. Sobue, N. Nakatsuji, S. Nomura, J. Fujimoto, M. Okada, T. Yamamoto, Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice, Nature 377 (1995) 539–544.
[26] A.T. Ho, E.B. Voura, P.D. Soloway, K.L. Watson, R. Khokha, MMP inhibitors augment fibroblast adhesion through stabilization of focal adhesion contacts and up-regulation of cadherin function, J. Biol. Chem. 276 (2001) 40215–40224.
[27] B.K. Pilcher, J.A. Dumin, B.D. Sudbeck, S.M. Krane, H.G. Welgus, W.C. Parks, The activity of collagenase-1 is required for keratinocyte migration on a type I collagen matrix, J. Cell Biol. 137 (1997) 1445–1457.
[28] P. Tremble, C.H. Damsky, Z. Werb, Components of the nuclear signaling cascade that regulate collagenase gene expression in response to integrin-derived signals, J. Cell Biol. 129 (1995) 1707–1720.
[29] G. Giannelli, J. Falk-Marzillier, O. Schiraldi, W.G. Stetler-Stevenson, V. Quaranta, Induction of cell migration by matrix metalloprotease-2 cleavage of laminin-5, Science 277 (1997) 225–228.
[30] N. Koshikawa, G. Giannelli, V. Cirulli, K. Miyazaki, V. Quaranta, Role of cell surface metalloprotease MT1-MMP in epithelial cell migration over laminin-5, J. Cell Biol. 148 (2000) 615–624.
[31] G. Giannelli, C. Bergamini, E. Fransvea, F. Marinosci, V. Quaranta, S. Antonaci, Human hepatocellular carcinoma (HCC) cells require both α3β1 integrin and matrix metalloproteinases activity for migration and invasion, Lab. Invest. 81 (2001) 613–627.