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Insulin-Like Growth Factor-I Increases Laminin, Integrin Subunits and Metalloprotease ADAM12 in Mouse Myoblasts*

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The extracellular matrix (ECM) is considered a part of the myogenesis signaling mechanism. We hypothesized that insulin-like growth factor-I (IGF-I) modifies ECM during differentiation of mouse C2C12 cells. The myogenic effect of IGF-I (30 nmol/l) was manifested by increased myogenin and myosin heavy chain (MyHC) levels as well as fusion index (2.6 times over control) on the 3rd day of differentiation. IGF-I markedly augmented laminin, but not fibronectin. Cellular contents of integrin α3, α5 and β1 during 3-day differentiation increased in the presence of IGF-I. Treatment with IGF-I increased the expression of the long form of metalloprotease ADAM12 (100 kDa) in myocytes. In conclusion: i) IGF-I caused an increase of laminin, integrin α3 and β1 in C2C12 myogenic cells that can be secondary to stimulation of myogenesis; ii) IGF-I augmented integrin α5 and ADAM12 levels, suggesting a role of this growth factor in determination of the pool of reserve cells during myogenesis.

Key words: ADAM12, fibronectin, IGF-I, integrins, laminin, myogenesis.

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Skeletal muscle growth and development is a complex process controlled by interactions between muscle cells and the surrounding microenvironment. Muscle cell differentiation is governed by a network of muscle regulatory factors (MRFs) such as: MyoD, Myf-5, myogenin, and MRF-4 (CHARGE & RUDNICKI 2004), however, the importance of extracellular matrix (ECM) molecules as a part of the myogenesis signaling mechanism has also been demonstrated (KRAUSS *et al*. 2005; LEI *et al*. 2013). In fact, neither the expression of myogenin nor its localization to myoblast nuclei was sufficient to drive skeletal muscle differentiation, if interactions between cell-ECM were inhibited (OSSES & BRANDAN 2002). The extracellular matrix is essential for response to growth factors and some ECM molecules, i.e. decorin, fibronectin and laminin may bind to and suppress the activity of myostatin, a negative regulator of muscle cell proliferation and differentiation (KISHIOKA *et al*. 2008; MIURA *et al*. 2010; YASAKA *et al*. 2013). Integrins, heterodimeric transmembrane receptors, play multiple critical roles in conveying extracellular signals to intracellular responses ("outside-in" signaling) as well as in altering the extracellular matrix interactions based upon intracellular changes ("inside-out" signaling) (ASKARI *et al*. 2009). Despite the large overall number of integrin receptor complexes, skeletal muscle integrin receptors are limited to seven α subunits, all associated with the β1 integrin subunit (SCHWANDER *et al*. 2003). Matrix metalloproteinases are thought to play an important role in skeletal muscle cell growth and differentiation, as these proteins contribute to activation of cytokines and growth factors (WANG *et al*. 2009; MOHAMED *et al*. 2010). Among matrix enzymes synthesized and secreted by myogenic cells, ADAM (a disintegrin and metalloprotease) proteins received much interest, due to their involvement in interactions with integrins and syndecans and in interplay with intracellular signaling molecules (JACOBSEN & WEWER 2009). Taken together, the ECM and its receptors provide an appropriate and permissive environment for

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specific differentiation to occur (OSSES *et al*. 2009).

Insulin-like growth factor-I, an anabolic factor in skeletal muscle, is critical in promoting muscle hypertrophy and muscle regeneration following injury (NYSTROM *et al*. 2009). In our recent study IGF-I increased levels of cyclins promoting cell cycle progression in proliferating C2C12 myoblasts and augmented the expression of cyclin D3 associated with the cell cycle exit in cultures subjected to differentiation (GRABIEC *et al*. 2014). This growth factor increased levels of the early (myogenin), and late (myosin heavy chain) markers of myogenesis (OKSBJERG *et al*. 2004). The IGF-I system was often targeted by proinflammatory cytokines, exerting catalytic effects in skeletal muscle cells (WIETESKA-SKRZECZYŃSKA *et al*. 2011b; GRZELKOWSKA-KOWALCZYK *et al*. 2015). However, whether and how IGF-I affects components of the extracellular matrix during skeletal myogenesis is not well known. The present work therefore focused on alterations of the expression of ECM-related proteins in mouse C2C12 myogenic cells subjected to 3-day differentiation in the presence of IGF-I.

Material and Methods

Cell culture

The mouse C2C12 myoblast cell line purchased from the European Collection of Animal Cell Culture (ECACC) was used for the study. These cells are derived from adult skeletal muscle and mimic skeletal muscle differentiation in cell culture (YAFFE & SAXEL 1977), i.e. they proliferate in the presence of serum and differentiate upon serum withdrawal with upregulation of specific markers such as cell cycle inhibitor p21, myogenin, and myosin heavy chain (KIM *et al*. 2006). Cell cultures free of contamination were maintained in an exponential phase of growth in 10% (v/v) FBS/DMEM (Fetal Bovine Serum/Dulbecco Modified Eagle Medium) with an antibiotic-antimycotic mixture, in controlled humidified air supplemented with 5% CO_2, at 37°C. The growth medium was changed every 48 h. Cells at ~90% confluence were subjected to differentiation (switch to differentiation medium – 2% (v/v) horse serum HS/DMEM) in the presence of IGF-I (30 nmol/l). This concentration of IGF-I was close to its physiological concentration in normal fed mice (IRESJÖ *et al*. 2013). To preserve the characteristics of the C2C12 cell line, the splitting of cells was done up to a maximum of 7 times.

Assessment of DNA content and cell viability

The crystal violet assay was performed to determine the total amount of nuclear DNA corresponding with cell number. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl- tetrazolium bromide (MTT) assay as described previously (GRABIEC *et al*. 2014). In both assays absorbance was measured on a multidetection microplate reader Infinite 200 PRO Tecan™ (TECAN, Mannedorf, Switzerland) at a wavelength of 570 nm.

Myoblast fusion

In order to visualize the morphological changes in C2C12 cultures on the 3rd day of myogenesis, cell monolayers were washed twice with ice-cold PBS, fixed with 75% methanol (v/v) and then Giemsa staining was performed. Nuclei were counted using a phase-contrast microscope (IX 70, Olympus Optical Co, Hamburg, Germany), and the average number of nuclei in ten random fields was recorded for each dish. The results were presented as: fusion index (%) = (number of nuclei in myotubes)/(total number of nuclei in myoblasts and myotubes) × 100, as described previously (WIETESKA-SKRZECZYŃSKA *et al*. 2011a).

Immunofluorescence staining and confocal microscopy

Cell cultures were carried out directly on glass Lab-tec coverslips (Nunc Inc., USA), fixed with 3.7% paraformaldehyde and permeabilized with 0.05% Triton X-100 in PBS, as described previously (GRABIEC *et al*. 2014). The cells were incubated overnight in darkness at 4°C with appropriate primary antibody (all purchased from Santa Cruz Biotechnology): anti-fibronectin (rabbit polyclonal, sc-9068), anti-laminin (rabbit polyclonal, sc-20142), anti-integrin α3 (rabbit polyclonal, sc-28665), anti-integrin α5 (rabbit polyclonal, sc-10729), anti-integrin β1 (rabbit polyclonal, sc-8978), anti-ADAM12 (rabbit polyclonal, sc-25579). The slides were rinsed with PBS and incubated for 1 h with Alexa Fluor 488 secondary antibody (Eugene, USA). For nuclear visualization cells were stained with 7-aminoactinomycin D (7-AAD, 5 μg/ml). The cells were visualized by the confocal laser scanning microscope FV-500 system (Olympus Optical Co, Hamburg, Germany). The combination of excitation/emission were: Argon 488 nm laser with 505-525 nm filter for Alexa Fluor 488 and HeNe 543 nm laser with 610 nm filter for 7AAD nucleus staining. Stacks of cross-sections were gathered separately for each fluorescence channel. Ten independent fields from each repetition of control and experimentally-treated cell cul-

tures were photographed and the IOD values were measured using the MicroImage analysis system (Olympus Optical Poland). To visualize the cellular profile of fluorescence, distribution lines were drawn on representative cells and the fluorescence intensity was estimated along with these lines.

Immunoblotting

Cell lysates comprised of cytosolic and membrane-enriched fractions were obtained using RIPA buffer supplemented with a protease and phosphatase inhibitor cocktail and octyl-glucopiranoside (Sigma-Aldrich, St. Louis, MO, USA). Aliquots of cell lysates corresponding to 100 μg of total protein (determined using the BCA kit according to the manufacturer's instructions) were subjected to SDS-PAGE. The membranes were probed with appropriate primary antibody (listed above, Santa Cruz Biotechnology), followed by 1h-incubation with secondary antibody. The secondary antibody, conjugated with appropriate IR fluorophores: IRDye® 680 or IRDye® 800 CW (IR-longer-wavelength near-infrared), enables detection of specific proteins directly on the PVDF membrane using the Odyssey Infrared Imaging System (LI-COR Biosciences). Scan resolution of the instrument was set at 169 μm, and the intensity at 4, in standard protocol. Quantification of the integrated optical density (IOD) was performed and presented in arbitrary units. The membranes were also reprobed with anti-actin antibody (goat polyclonal, sc-1616, Santa Cruz Biotechnology), to ensure that all lanes contain equal amounts of total protein.

Statistics

The results of MTT and CV tests are representative of four separate experiments performed in triplicate (n = 12). The data obtained from immunoblotting analysis represent three separate experiments performed in triplicate (n = 9). The experiments visualizing the morphological changes or the cellular localization of examined proteins were performed three times with 3 wells/treatment. Ten randomly selected non-overlapping fields with similar cell density were photographed. The individual data (n = 10) used for the statistical analysis represents average numbers of nuclei observed in myotubes and the total fluorescence, respectively. All results were presented as means ± SE. For each assay the Student *t*-test was used for the comparison of two means (control vs. experimental treatment) and the criterion for statistical significance was $P<0.05$. The analyses were performed using GraphPad Prism 5 (GraphPad Software, USA).

Results and Discussion

The extracellular matrix is important in creating the niche for muscle precursor cells which controls proliferation and differentiation essential for skeletal muscle tissue maintenance (WITSCHUT *et al*. 2010). In our study exogenous IGF-I at concentration 30 nmol/l augmented cell viability (by 37%, $P<0.0001$), increased DNA content (by 37%, $P<0.0001$) and fusion index (over 2.6 times in comparison to control, $P<0.0001$, Table 1) on the 3rd day of myogenesis. Levels of the early (myogenin) and late (myosin heavy chain, Fig. 1) markers of myogenesis in IGF-I-treated cells were also elevated, in agreement with the well-documented myogenic effect of this growth factor (DUAN *et al*. 2010; WIETESKA-SKRZECZYŃSKA *et al*. 2011a). Myogenesis is accompanied by remodeling of ECM as well as by changes in integrin receptor expression pattern (CACHAÇO *et al*. 2005). During differentiation the fibronectin amount decreased, being present mainly in nonfused cells but not in

Table 1

The effect of insulin-like growth factor-I (IGF-I, 30 nmol/l) on DNA content, cell viability and fusion of mouse C2C12 myoblasts on the 3rd day of differentiation. Data represent means of absorbances (± SE), with n=12/treatment condition. * – significantly different vs. control value (Ctrl).

	Ctrl	IGF-I
DNA content	1.87 ± 0.06	2.56 ± 0.12 *
Cell viability	1.00 ± 0.05	1.37 ± 0.05 *
Fusion index	4.75 ± 0.32	12.5 ± 0.95 *

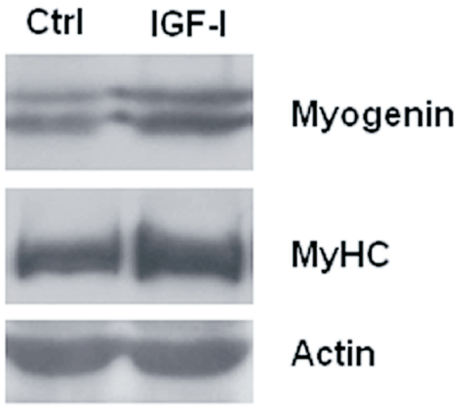

Fig. 1. The effect of insulin-like growth factor-I (IGF-I, 30 nmol/l) on myogenin and myosin heavy chain (MyHC) in mouse C2C12 myoblasts on the 3rd day of differentiation. Blots are representative for three separate experiments.

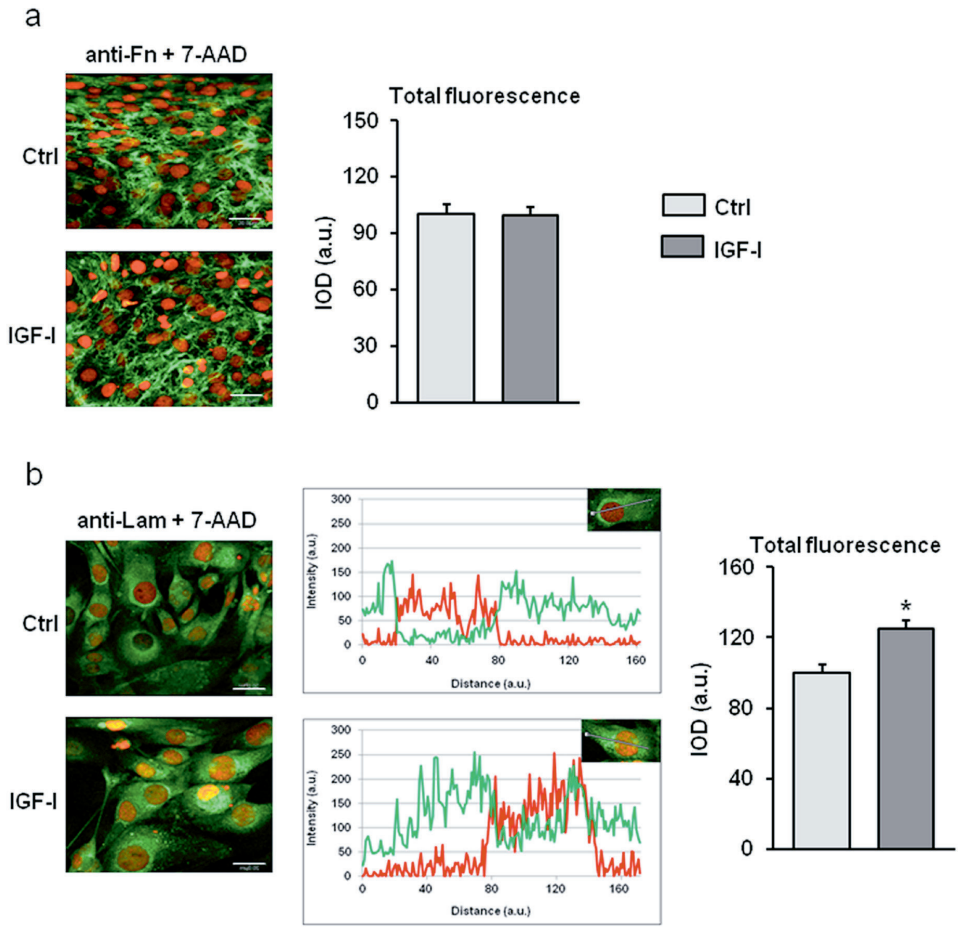

Fig. 2. The effect of IGF-I (30 nmol/l) on fibronectin (Fn, A) and laminin (Lam, B) in C2C12 myogenic cells on the 3rd day of differentiation. The content and localization of proteins was assessed by confocal microscopy. The images are representative for three separate experiments. Bar, 20 μm. The integrated optical density (IOD) was presented in arbitrary units (a.u.) and the value obtained in control (Ctrl) cells was set as 100%. Representative profiles of fluorescence distribution are presented and the estimated cells are indicated on profile diagrams. * – significantly different vs. control value.

myotube cultures, and was replaced by laminins. Consequently, proliferating and migrating myoblasts express high amounts of the fibronectin-binding α5β1 integrin, and they switch to the laminin-binding integrins during myotube formation (BRAKEBUSCH & FARSLER 2005).

Fibronectin was expressed in C2C12 myogenic cells differentiating in control conditions and in the presence of IGF-I, and appeared mainly in the extracellular environment (Fig. 2A). The level of fibronectin was not affected by IGF-I. KNOBLAUCH *et al*. (2007) detected laminin on the surface of newly formed myotubes during C2C12 myogenesis. In our study immunofluorescence staining of differentiating cultures on the 3rd day after induction of myogenesis did not reveal an extracellular assembly of laminin (Fig. 2B). Laminin appeared ultimately in the cytosolic fraction, confirming delayed synthesis of this protein during myogenesis, in comparison to fibronectin. The cellular level of laminin in IGF-I-treated cells was markedly higher than the control value (P=0.001), and the strong laminin-specific immunofluorescence was visible in the perinuclear area of cells. The lack of the appearance of laminin in the extracellular space in our study could result from different myogenesis dynamics and the very early stage of differentiation.

Integrins α3 and β1 were present predominantly in cell membranes and were abundant in myotubes (Fig. 3). Exposure to IGF-I markedly augmented levels of α3 and β1 integrin subunits, proved by strong immunofluorescence and immunoblotting analyses. These changes in α3 and β1 integrins under IGF-I treatment could be associated with the stimulation of myogenesis and increased myotube formation. High levels of α3 and β1 integrins noticed on the 3rd day correspond with the onset of fusion, suggesting that these integrin subunits are important at this stage of myogenesis. Our results are in agreement with an earlier study using rat primary myoblasts, showing that overexpression of the full-length integrin α3 subunit induced myoblast fusion, whereas the inhibition of integrin α3

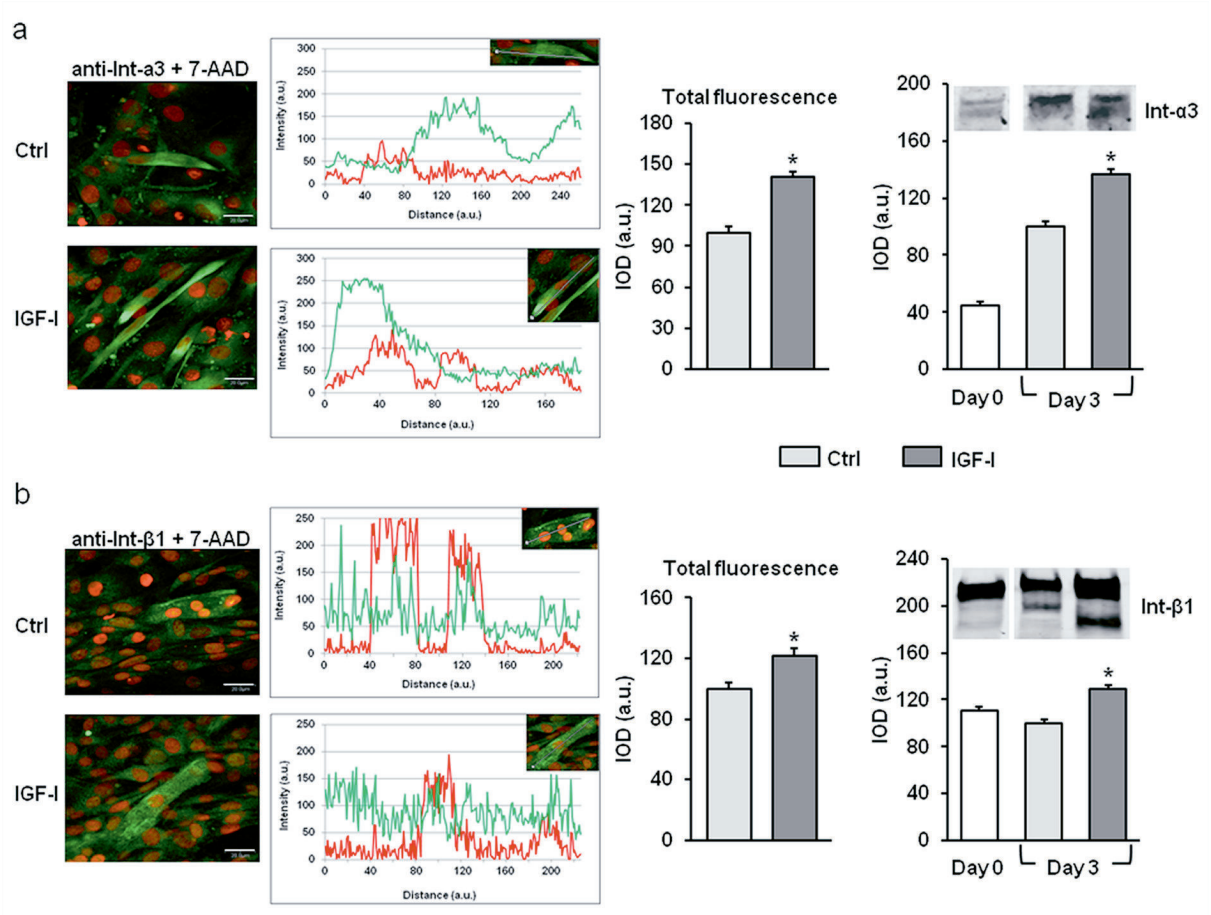

Fig. 3. The effect of IGF-I (30 nmol/l) on integrin-α3 (Int-α3, A) and integrin-β1 (Int-β1, B) in C2C12 myogenic cells on the 3rd day of differentiation. The content and localization of proteins was assessed by confocal microscopy. The images are representative for three separate experiments. Bar, 20 μm. The integrated optical density (IOD) was presented in arbitrary units (a.u.) and the value obtained in control (Ctrl) cells was set as 100%. Representative profiles of fluorescence distribution are presented and the estimated cells are indicated on profile diagrams. * – significantly different vs. control value.

extracellular domain impaired this process (BRZÓSKA *et al*. 2006). Similarly, disruption of integrin β1 *in vivo* and *in vitro* has profound effects on myogenesis. Lack of integrin β1 had no apparent effect on the migration and proliferation of myoblasts, however clear alterations occurred at the later stages of myogenesis and were manifested by impaired fusion (SCHWANDER *et al*. 2003). Indeed, according to a previous study, muscle-specific integrin β1, appearing in a doublet form, was used as a marker of differentiation (GALLIANO *et al*. 2000). The high level of integrin β1 in cell cultures exposed to IGF-I (Fig. 3B) could be therefore associated with increased myotube number due to myogenesis stimulation (Table 1), but also could result from the rise in integrin β1 content in a single myotube, indicating the activation of integrin signaling accompanying fusion.

Interestingly, in our study integrin α5, a subunit of fibronectin surface receptor, was significantly augmented by IGF-I ($P<0.0001$, Fig. 4A), which was rather unexpected in view of the myogenic effect of the growth factor. This observation corresponds with the lack of a decrease in fibronectin (Fig. 2A), the ECM protein attributed primarily to proliferating myoblasts. A potential explanation for this phenomenon is the unique, among growth factors, ability of IGF-I to stimulate both proliferation and differentiation of myogenic cells, processes usually considered as mutually exclusive (PAUL & ROSENTHAL 2002).

Skeletal myoblasts induced *in vitro* to differentiate form either multinucleated myotubes or give rise to quiescent undifferentiated "reserve cells" that share several characteristics with muscle satellite cells. CAO *et al*. (2003) reported that the expression level of metalloprotease ADAM12 is much higher in proliferating C2C12 myoblasts and in reserve cells than in mature myotubes. Remarkably, overexpression of ADAM12 induced a quiescent-like phenotype and did not stimulate differentiation. In the present study, treatment with IGF-I increased the expression of ADAM12 in myocytes ($P<0.0001$, Fig. 4B). The immunoblot-

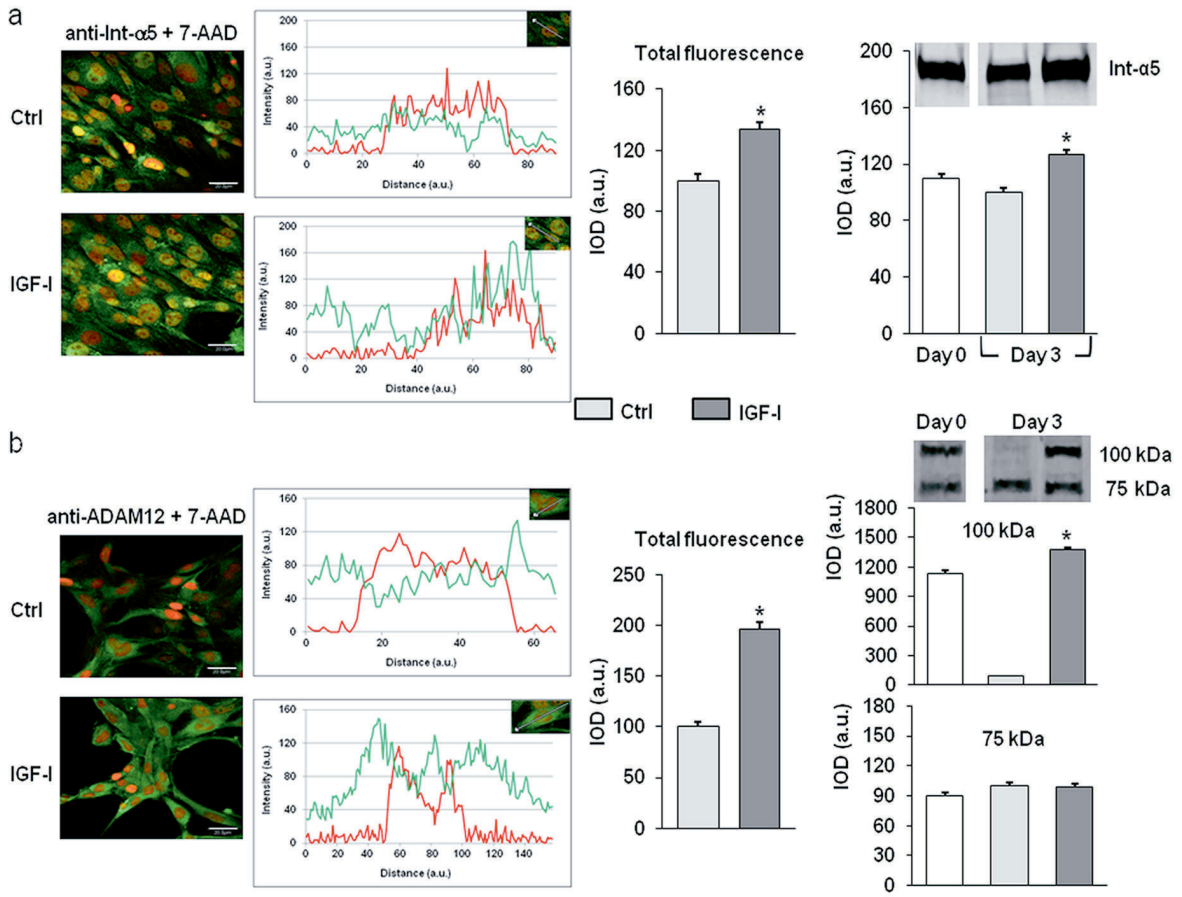

Fig. 4. Effect of IGF-I (30 nmol/l) on integrin-α5 (Int-α5, A) and metalloprotease ADAM12 (B) in C2C12 myogenic cells on the 3rd day of differentiation. The content and localization of proteins was assessed by confocal microscopy. The images are representative for three separate experiments. Bar, 20 μm. The integrated optical density (IOD) was presented in arbitrary units (a.u.) and the value obtained in control (Ctrl) cells was set as 100%. Representative profiles of fluorescence distribution are presented and the estimated cells are indicated on profile diagrams. * – significantly different vs. control value.

ting analysis revealed the presence of two main isoforms of ADAM12. The 100 kDa uncleaved proform of ADAM12 increased dramatically in myogenic cells treated with IGF-I ($P<0.0001$) and its level was similar to that found in proliferating myoblasts. This observation suggest that IGF-I could play a role in determining the pool of reserve cells during myogenic differentiation acting via stimulation of ADAM12 expression in nonfused cells. The effect of IGF-I in maintaining cell proliferation under conditions that favour myogenesis can be supported by increased DNA content (Table 1) as well as by an increase in cyclin A, an established marker of regenerative tissue potential (CHENG *et al*. 2007), reported in our recent study in C2C12 myogenic cells differentiating in the presence of this growth factor (GRABIEC *et al*. 2014). The cellular distribution of ADAM12 was in agreement in several studies showing that it is mainly located inside cells, indicating that translocation of this protein from intracellular storage to the cell surface might be restricted and regulated (WEWER *et al*. 2005). A shorter (75 kDa) form of ADAM12 probably represents the mature form of the protein or ADAM12 without the metalloproteinase domain, able to bind with integrins (ZHAO *et al*. 2004). Interestingly, some studies have implicated the involvement of ADAM12 in fusion of muscle precursor cells. LAFUSTE *et al*. (2005) reported that expression of ADAM12 and integrin α9 subunit parallel and culminate at the time of myoblast fusion and inhibition of the ADAM12/α9β1 integrin interaction dramatically impaired this process.

In conclusion, IGF-I caused increases in extracellular matrix-related proteins in C2C12 myogenic cells. Some of these alterations, i.e. enhanced expression of laminin, integrin α3 and β1 can be secondary to IGF-I-dependent stimulation of myogenesis. However, other IGF-I-exerted ECM modifications, i.e. an increased level of integrin α5 and ADAM12, suggest that this growth factor also plays a role in determination of the pool of reserve cells during myogenesis.

References

ASKARI J.A., BUCKLEY P.A., MOULD P.A., HUMPHRIES J.M. 2009. Linking integrin conformation to function. J. Cell Sci. **122**: 165-170.

BRAKEBUSCH C., FARSLER R. 2005. Beta1 integrin function *in vivo*: adhesion, migration and more. Cancer Metastasis Rev. **24**: 403-411.

BRZÓSKA E., BELLO V., DARRIBERE T., MORACZEWSKI J. 2006. Integrin α3 subunit participates in myoblast adhesion and fusion *in vitro*. Differentiation **74**: 105-118.

CACHAÇO A.S., PEREIRA C.S., PARDAL R.G., BAJANCA F., THORSTEINSDÓTTIR S. 2005. Integrin repertoire on myogenic cells changes during the course of primary myogenesis in the mouse. Dev. Dyn. **232**: 1069-1078.

CAO Y., ZHAO Z., GRUSZCZYNSKA-BIEGALA J., ZOLKIEWSKA A. 2003. Role of metalloprotease disintegrin ADAM12 in determination of quiescent reserve cells during myogenic differentiation *in vitro*. Mol. Cell. Biol. **23**: 6725-6738.

CHARGE S.B., RUDNICKI M.A. 2004. Cellular and molecular regulation of muscle regeneration. Physiol. Rev. **84**: 209-238.

CHENG R.K., ASAI T., TANG H., DASHOUSH N.H., KARA R.J., COSTA K.D., NAKA Y., WU E.X., WOLGEMUTH D.J., CHAUDHRY H.W. 2007. Cyclin A2 induces cardiac regeneration after myocardial infarction and prevents heart failure. Circ. Res. **100**: 1741-1748.

DUAN C., REN H., GAO S. 2010. Insulin-like growth factors (IGFs), IGF receptors, and IGF-binding proteins: roles in skeletal muscle growth and differentiation. Gen. Comp. Endocrinol. **167**: 344-351.

GALLIANO M.F., HUET C., FRYGELIUS J., POLGREN A., WEWER U.M., ENGVALL E. 2000. Binding of ADAM12, a marker of skeletal muscle regeneration, to the muscle-specific actin-binding protein, alpha -actinin-2, is required for myoblast fusion. J. Biol. Chem. **275**: 13933-13939.

GRABIEC K., GAJEWSKA M., MILEWSKA M., BŁASZCZYK M., GRZELKOWSKA-KOWALCZYK K. 2014. The influence of high glucose and high insulin on mechanisms controlling cell cycle progression and arrest in mouse C2C12 myoblasts: the comparison with IGF-I effect. J. Endocrinol. Invest. **37**: 233-245.

GRZELKOWSKA-KOWALCZYK K., WICIK Z., MAJEWSKA A., TOKARSKA J., GRABIEC K., KOZŁOWSKI M., MILEWSKA M., BŁASZCZYK M. 2015. Transcriptional Regulation of Important Cellular Processes in Skeletal Myogenesis Through Interferon-γ. J. Interferon Cytokine Res. **35**: 89-99.

IRESJÖ B-M., SVENSSON J., OHLSSON C., LUNDHOLM K. 2013. Liver-deriver endocrine IGF-I is not sufficient for activation of skeletal muscle protein synthesis following oral feeding. BMC Physiol. **13**: 7.

JACOBSEN J., WEWER U.M. 2009. Targeting ADAM12 in human disease: head, body or tail? Curr. Pharm. Des. **15**: 2300-2310.

KIM H.K., LEE Y.S., SIVAPRASAD U., MALHOTRA A., DUTTA A. 2006. Muscle-specific microRNA miR-206 promotes muscle differentiation. J. Cell Biol. **174**: 677-687.

KISHIOKA Y., THOMAS M., WAKAMATSU J., HATTORI A., SHARMA M., KAMBADUR R., NISHIMURA T. 2008. Decorin enhances the proliferation and differentiation of myogenic cells through suppressing myostatin activity. J. Cell Physiol. **215**: 856-867.

KNOBLAUCH A., WILL C., GONCHARENKO G., LUDWIG S., WIXLER V. 2007. The binding of Mss4 to alpha-integrin subunits regulates matrix metalloproteinase activation and fibronectin remodeling. FASEB J. **21**: 497-510.

KRAUSS R.S., COLE F., GAIO U., TAKAESU G., ZHANG W., KANG J.S. 2005. Close encounters: regulation of vertebrate skeletal myogenesis by cell-cell contact. J. Cell Sci. **118**: 2355-2362.

LAFUSTE P., SONNET C., CHAZAUD B., DREYFUS P.A., GHERARDI R.K., WEWER U.M., AUTHIER F.J. 2005. ADAM12 and α9β1 integrin are instrumental in human myogenic cell differentiation. Mol. Biol. Cell. **16**: 861-870.

LEI H., LEONG D., SMITH L.R., BARTON E.R. 2013. Matrix metalloproteinase 13 is a new contributor to skeletal muscle regeneration and critical for myoblast migration. Am. J. Physiol. Cell. Physiol. **305**: C529-C538.

MIURA T., KISHIOKA Y., WAKAMATSU J., HATTORI A., NISHIMURA T. 2010. Interaction between myostatin and extracellular matrix components. Anim. Sci. **81**: 102-107.

MOHAMED A., HUET E., DELBE J., LEDOUX D., MENASHI S., MARTELLY I. 2010. Extracellular matrix metalloproteinase inducer (emmprin/cd147) as a novel regulator of myogenic cell differentiation. J. Cell Physiol. **226**: 141-149.

NYSTROM G., PRUZNAK A., HUBER D., FROST R.A., LANG C.H. 2009. Local insulin-like growth factor I prevents sepsis-induced muscle atrophy. Metabolism **58**: 787-797.

OKSBJERG N., GONDRET F., VESTERGAARD M. 2004. Basic principles of muscle development and growth in meat-producing mammals as affected by the insulin-like growth factor (IGF) system. Domest. Anim. Endocrinol. **27**: 219-240.

OSSES N., BRANDAN E. 2002. ECM is required for skeletal muscle differentiation independently of muscle regulatory factor expression. Am. J. Physiol. Cell. Physiol. **282**: C383-C394.

OSSES N., CASAR J.C., BRANDAN E. 2009. Inhibition of extracellular matrix assembly induces the expression of osteogenic markers in skeletal muscle cells by a BMP-2 independent mechanism. BMC Cell Biol. **10**: 73.

PAUL A.C., ROSENTHAL N. 2002. Different modes of hypertrophy in skeletal muscle fibers. J. Cell Biol. **156**: 751-760.

SCHWANDER M., LEU M., STUMM M., DORCHIES O.M., RUEGG U.T., SCHITTNY J., MÜLLER U. 2003. β1 integrins regulate myoblast fusion and sarcomere assembly. Dev. Cell. **4**: 673-685.

WANG W., PAN H., MURRAY K., JEFFERSON B.S., LI Y. 2009. Matrix metalloproteinase-1 promotes muscle cell migration and differentiation. Am. J. Pathol. **174**: 541-549.

WEWER U.M., ALBRECHTSEN R., ENGVALL E. 2005. ADAM12. The long and the short of it. (In: The ADAM Family of Proteases. N. M. Hooper and U. Lendeckel eds., Springer, Netherlands): 123-146.

WIETESKA-SKRZECZYŃSKA W., GRZELKOWSKA-KOWALCZYK K., TOKARSKA J., GRABIEC K. 2011a. Growth factor and cytokine interactions in myogenesis. Part I. The effect of TNF-alpha and IFN-gamma on IGF-I-dependent differentiation in mouse C2C12 myogenic cells. Pol. J. Vet. Sci. **14**: 417-424.

WIETESKA-SKRZECZYŃSKA W., GRZELKOWSKA-KOWALCZYK K., REJMAK E. 2011b. Growth factor and cytokine interactions in myogenesis. Part II. Expression of IGF binding proteins and protein kinases essential for myogenesis in mouse C2C12 myogenic cells exposed to TNF-alpha and IFN-gamma. Pol. J. Vet. Sci. **14**: 425-431.

WITSCHUT K.J., HAAGSMAN H.P., ROELEN B.A. 2010. Extracellular matrix components direct porcine muscle stem cell behavior. Exp. Cell Res. **316**: 341-352.

YAFFE D., SAXEL O. 1977. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature **270**: 725-727.

YASAKA N., SUZUKI K., KISHIOKA Y., WAKAMATSU J., NISHIMURA T. 2013. Laminin binds to myostatin and attenuates its signaling. Anim. Sci. **84**: 663-668.

ZHAO Z., GRUSZCZYNSKA-BIEGALA J., CHEUVRONT T., YI H., MARK H., MARK K., KAUFMAN S.J. ZOLKIEWSKA A. 2004. Interaction of the disintegrin and cysteine-rich domains of ADAM12 with integrin alpha7beta1. Exp. Cell Res. **298**: 28-37.