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Cross-talk between integrin receptor and insulin-like growth factor receptor in regulation of collagen biosynthesis in cultured fibroblasts

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ABSTRACT

Purpose: Cellular processes are regulated by signals generated by adhesion receptors and growth factor receptors. IGF-binding protein 1 (IGFBP-1) is a molecule which may affect the both signaling pathways through inactivation of IGF-I (ligand for IGF-IR) and binding to RGD region of integrin receptors. Whether this phenomenon is important in communication between insulin-like growth factor receptor (IGF-IR) and β1-integrin receptor in regulation of prolidase activity and collagen biosynthesis is the aim of this study.

Material and Method: We studied the effects of IGFBP-1, IGF-I, thrombin (integrin activator), echistatin (disintegrin), phosphatidylinositol 3-kinase inhibitor (LY-294002) and ERK 1/2 inhibitors (PD98059 and UO126) on prolidase activity, collagen biosynthesis and expression of proteins participating in pathways generated by these receptors.

Results: Stimulation of β1-integrin and IGF-I receptors by standard ligands was proved to up-regulate collagen synthesis in cultured fibroblasts. IGFBP-1, similarly as echistatin and studied inhibitors, contributed to down-regulation of ERK1/2, Akt, mTOR expression and up-regulation of NFκB. It was accompanied by parallel decrease in prolidase activity and collagen biosynthesis.

Conclusion: The data suggest that "cross talk" between IGF-I receptor and integrin receptor may play important role in regulation of prolidase activity and collagen biosynthesis.

Key words: Prolidase, β1-integrins, Collagen, IGFBP-1, IGF-IR

INTRODUCTION

Cellular processes are regulated by signals generated by adhesion receptors and growth factors receptors. Several lines of evidence indicate that the intensity of metabolism and cell differentiation is contingent upon the communication between these receptors to modulate their signalling pathways. This phenomenon is known in literature as "cross talk". One of the examples is "cross talk" between insulin-like growth factor receptor (IGF-IR) and the β_1-integrin receptor. It has been demonstrated that in fibroblasts and some cancer cell lines these two receptors generate in cooperation signalling pathways that stimulate adhesion, migration and cell differentiation [1,2].

The best known IGF-IR signaling is phosphatidylinositol pathway leading to activation of Akt and transcription factors which stimulate expression of genes involved in the metabolism and transport of carbohydrates, protein biosynthesis and apoptosis [3,4,5].

Another IGF-IR-dependent signal transduction is the Ras-Raf-dependent pathway of MAP-kinase activation through the proteins Shc, GRB-2 and Sos [6]. Activation of this pathway leads to induction of transcription factors that stimulate gene expression of proteins regulating cell growth

and differentiation. It was shown that through this pathway IGF-I stimulates collagen synthesis, cell proliferation, prolidase activity and inhibits apoptosis [6,7,8].

The amount of free IGF in circulation is regulated by family of IGF-I binding proteins (IGF BPs). They play a role as a transport proteins, regulate the half-life of IGF-I, protect IGF-I against proteolytic enzymes and may form reservoir of IGF-I. So far has been detected and mapped the sequence of fifteen IGFBPs. They differ between each other in size and strength of binding of IGF. Particularly noteworthy are IGFBP-1 and IGFBP-2, which in their structure contain the RGD sequence, which indicates the possibility of interaction with the integrin receptors [9,10].

Integrin receptors belong to a group of membrane receptors involved in cell-cell and cell-extracellular matrix protein interactions. They are heterodimers formed from two subunits (α and β). So far it has been identified 25 different α subunits and 9 β subunits [11].

In addition to participation in the processes of adhesion and migration, integrin receptors mediate the processes of reorganization of the cellular skeleton, intracellular ion transport, metabolism, kinase activation, gene expression and regulation of the cell cycle. The result of integrin receptor stimulation by a ligand is activation of focal adhesion kinase (FAK), which through interaction with the Src kinase activates pathway leading to activation of MAP-kinase via Ras-Raf-dependent way. The similar pathway (except FAK participation) is generated by IGF-I receptor. The response is an increased expression of numerous proteins [2,11,12,13,14].

Recent data support the important role of pathways generated by IGF-I receptor and integrin receptor in the regulation of many cellular functions. Akt is involved in regulation of cellular processes at the level of cytoplasm (cell growth, glucose metabolism regulation, activation of mTOR complex 1), mitochondria (apoptosis and glucose metabolism) and nucleus (regulation of transcriptional factors and apoptosis) [15]. Both the Akt and ERK signaling pathways take part in the regulation of mTOR, which is known as a regulator of various cellular processes, including proliferation, growth, differentiation and survival [16].

IGFBP-1 inhibits collagen biosynthesis by inactivation of IGF-I, the major inducer of biosynthesis of this protein in fibroblasts. The ability of IGFBP-1 to bind to β_1-integrin receptor suggests that this protein may also inhibit the signaling pathway generated by this receptor leading to the inhibition of collagen biosynthesis. Whether collagen biosynthesis is regulated by cross-talk between β_1-integrin and IGF-I receptors is the aim of this study.

MATERIALS AND METHODS

Reagents

Dulbecco's phosphate buffered saline (DPBS), 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent (BCIP/NBT), thrombin, mouse anti-phospho-mitogen activated protein kinase (MAPK) antibody, rabbit anti-phospho-AKT, rabbit anti-AKT antibody, anti-mouse IgG, anti-rabbit IgG, and anti-goat IgG, were provided by Sigma Corp., USA, as were most other chemicals and buffers used. Dulbecco's minimal essential medium (DMEM) and fetal bovine serum (FBS) used in cell culture were products of Gibco, USA. Glutamine, penicillin and streptomycin were obtained from Quality Biologicals Inc., USA. Rabbit NFκB, rabbit anti-phospho-mTOR and rabbit anti-mTOR antibodies were provided by Cell Signaling Technology, Inc., USA. IGF-BP1 was provided by R&D Systems, USA. IGF-I and L-5[^{3}H] proline (28 Ci/mmol) were purchased from Amersham, UK. Nitrocellulose membrane (0.2 μm), sodium dodecylsulphate (SDS), polyacrylamide, molecular weight standards and Coomassie Briliant Blue R-250 were received from Bio-Rad Laboratories.

Cell culture

Human skin fibroblasts were purchased from the American Type Culture Collection, (Manassas, Virginia, USA). The cells were maintained in DMEM supplemented with 10% FBS (HyClone Laboratories Logan, UT, USA), 2 mM glutamine, 50 U/ml penicillin, and 50 μg/ml streptomycin at 37°C in a 5% CO_2 incubator.

Determination of prolidase activity

The activity of prolidase was determined according to the method of Myara et al. [17], which is based on colorimetric determination of proline using Chinard's reagent. Cells were scraped off and centrifuged at 200×g for 15 min and the supernatant was discarded. The cell pellet was suspended in 1 ml of 50 mM HEPES, pH 7.8, and sonicated for 3×10 s at 0°C. Samples were then centrifuged (12,000 ×g, 30 min) at 4°C and the supernatant was used for protein determination [18] and prolidase activity assays. Activation of prolidase requires incubation with Mn(II): 100 μl of cell extract supernatant was mixed with 100 μl of 50 mM HEPES, pH 7.8 containing $MnCl_2$ at a final concentration of 1 mM in the mixture. After incubation for 24 h at 37°C, the prolidase reaction was initiated by adding 100 μl of the activated mixture to 100 μl of 94 mM glycyl-proline (Gly-Pro) for a final concentration of 47 mM. After additional incubation for 1 h at 37°C, the reaction was terminated with the addition of 1 ml of 0.45M trichloroacetic acid. To parallel blank tubes, trichloroacetic

acid was added at time "zero". Samples were centrifuged at 10,000 ×g for 15 min. The released proline was determined by adding 0.5 ml of the trichloroacetic acid supernatant to 2 ml of a 1:1 mixture of glacial acetic acid: Chinard's reagent (25 g of ninhydrin dissolved at 70°C in 600 ml of glacial acetic acid and 400 ml of 6 M orthophosphoric acid) and incubated for 10 min at 90°C. The amount of proline released was determined colorimetrically by monitoring absorbance at 515 nm and calculated using proline standards. Enzyme activity was reported as nanomoles of proline released per minute per milligram of protein.

Collagen biosynthesis

Cells were labeled for 24 h with the 5-[^{3}H]proline (5 µCi/ml, 28 Ci/mmol). Its incorporation into proteins was measured as described previously [19]. Incorporation of the tracer into collagen was determined by digesting proteins with purified *Clostridium histolyticum* collagenase, according to the method of Peterkofsky et al. [20]. The results are shown as combined values for the cell plus medium fractions.

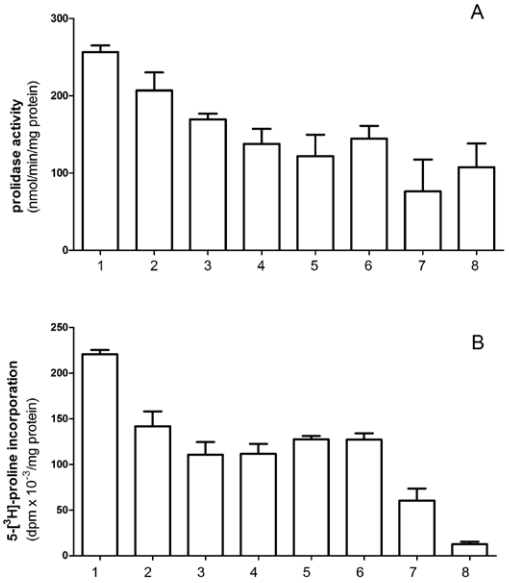
Western blot analysis

Slab SDS/PAGE was used, according to the method of Laemmli [21]. Equal amounts of total cellular protein (25 µg) were electrophoresed on SDS-PAGE gels. After SDS-PAGE, the gels were allowed to equilibrate for 5 min. in 25mM Tris, 0.2M glycine in 20% (v/v) methanol. The proteins were transferred to 0.2 µm pore-sized nitrocellulose at 100 mA for 1 h by using a LKB 2117 Multiphor II electrophoresis unit. The nitrocellulose was incubated with: monoclonal anti-phospho-MAPK antibody (ERK1/ERK/2) at concentration of 1:1,000, monoclonal anti-phospho-AKT antibody at concentration of 1:1,000, polyclonal anti-phospho-mTOR antibody at concentration of 1:1,000, polyclonal NFκB antibody at concentration 1:1,000 in 5% dried milk in Tris buffered saline with Tween 20 (TBS-T) (20 mmol/l Tris–HCl buffer, pH 7.4, containing 150 mmol/l NaCl and 0.05% Tween 20) for 1 h. In order to analyze phospho-mTOR, mTOR, phospho AKT and NFκB, a second antibody, alkaline phosphatase conjugated, anti-rabbit immunoglobulin (whole molecule) was added at a dilution 1:5000; in order to analyze phosphorylated MAPK (ERK1/ERK2), a second antibody, alkaline phosphatase conjugated, anti-mouse immunoglobulin (whole molecule) was added at a dilution 1:5000 in Tris-buffered saline containing Tween 20 and incubated for 30 min under gentle shaking. Then nitrocellulose was washed with TBS-T (5×5 min) and submitted to 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium liquid substrate reagent (BCIP/NBT).

Statistical analysis

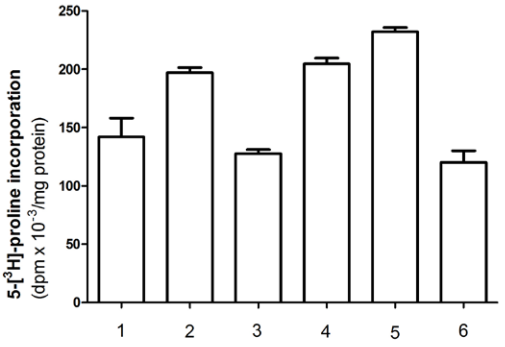
In experiments shown on *Fig. 1* and *Fig. 2* the mean values for 3 assays ± S.D. were calculated. The results were submitted to statistical analysis using the unpaired, one-sided student's t test, accepting $P < 0.05$ versus control as significant.

Figure 1. **Prolidase activity (A) and collagen biosynthesis measured by 5-[^{3}H]proline incorporation into proteins susceptible to the action of bacterial collagenase (B) in confluent human skin fibroblasts incubated for 24 h in medium with 10% FBS (1) and without FBS (2), containing Echistatin at concentration 100 nM (3), IGFBP-1 at concentration 200 ng/ml (4) and 400 ng/ml (5), PD98059 at concentration 20 µM (6), UO126 at concentration 20 µM (7) and LY-294002 at concentration 50 µM (8).**



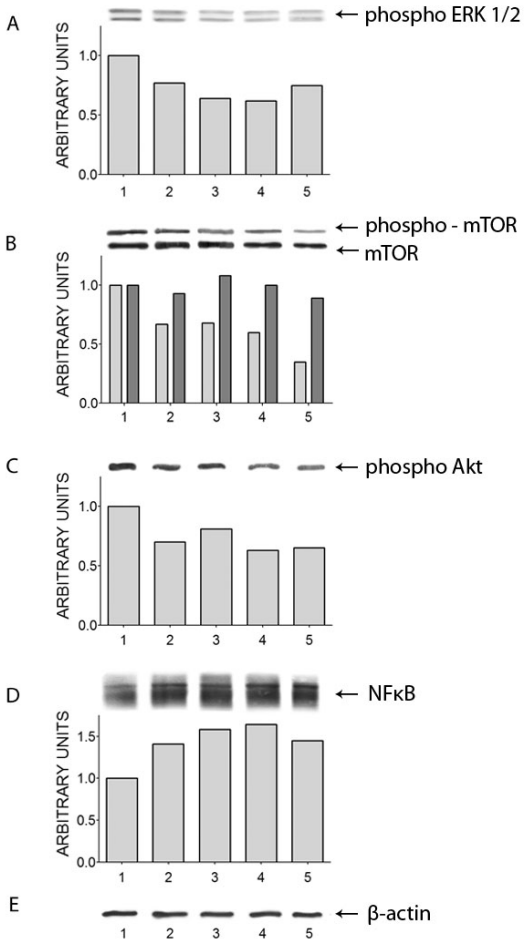
Mean values from three independent experiments done in duplicates ± S.D. are presented. P<0.05 versus control cells cultured without FBS (2).

Figure 2. **Collagen biosynthesis measured by 5-[^{3}H]proline incorporation into proteins susceptible to the action of bacterial collagenase in confluent human skin fibroblasts incubated for 24 h in medium without FBS (1), containing thrombin at concentration 0.1 U/ml (2), IGFBP-1 at concentration 400 ng/ml (3), IGF-I at concentration 40 ng/ml (4), IGF-I at concentration 40 ng/ml with thrombin at concentration 0.1 U/ml (5) and IGF-I at concentration 40 ng/ml with IGFBP-1 at concentration 400 ng/ml (6).**



Mean values from three independent experiments done in duplicates ± S.D. are presented. P<0.05 versus control cells (1).

Figure 3. **Western immunoblot analysis for phospho-ERK 1/2 (A), phospho-mTOR and total mTOR (B), phospho-Akt (C), NFκB (D) and β-actin (E) in confluent human skin fibroblast incubated for 24h in medium with 10% FBS (1) or without FBS (2) containing Echistatin at concentration 100 nM/ml (3), IGFBP-1 at concentration 200 ng/ml (4) and 400 ng/ml (5).**



Mean values from three independent experiments done in duplicates ± S.D. are presented. P<0.05 versus control cells (1).

Statistical analysis was performed using extended version of MS Excel 2007 software. For Western blot analysis the mean values of 6 pooled cell homogenate extracts are presented. The same amount of supernatant protein (25 μg) was run in each lane. The expression of β-actin served as a control for protein loading.

RESULTS

Prolidase activity and collagen biosynthesis were measured in confluent human dermal fibroblasts treated with Echistatin, a well-known disintegrin, at concentration 100 nM, insulin-like growth factor binding protein 1 (IGFBP-1) at concentration 200 ng/ml and 400 ng/ml, LY-294002 (2-(4-Morpholinyl)-8phenyl-4H-1benzopyran-4-one), a specific inhibitor of phosphatidylinositol 3-kinase, in concentration 50 μM and two ERK 1/2 inhibitors PD98059 and UO126 (1,4-Diamino-2,3-dicyano-1,4-bis(2-aminophenylthio)butadiene) at concentration 20 μM. Fibroblasts were incubated for 24h in medium with or without 10% FBS.

The cells cultured in FBS-deprived medium evoked decrease in prolidase activity (*Fig. 1A*, lane 2) and collagen biosynthesis (*Fig. 1B*, lane 2), compared to control cells, cultured in 10% FBS (Fig.1A and B, lane 1). Further inhibition of both processes were observed in the cells cultured in the presence of Echistatin in FBS-deprived medium (*Fig. 1A* and *Fig. 1B*, lane 3). This is consistent with our previous studies on the role of β_1-integrin receptor in regulation of prolidase activity and collagen biosynthesis [22]. Significant reduction of prolidase activity and collagen biosynthesis were found in the cells cultured in the presence of IGFBP-1 (*Fig.1A* and *Fig. 1B*, lanes 4 and 5). Inhibition of proteins involved in signaling pathways generated by IGF-I receptor and β_1-integrin receptor also resulted in reduction of prolidase activity and collagen biosynthesis. We found that blocking of phosphorylation of Erk 1/2 (Fig.1 A and B, lanes 6 and 7) and PI3K (*Fig. 1A* and Fig. 1B, lane 8) by specific inhibitors (PD98059, UO126 and LY-294002 respectively) contributed to down regulation of both processes. In contrast, activation of β_1-integrin receptor by thrombin (*Fig. 2*, line 2) but not by IGF-BP1 (*Fig. 2*, line 3) stimulated collagen biosynthesis in fibroblasts cultured in FBS-deprived medium. The similar effect was achieved by IGF-I, ligand of IGF-IR (*Fig. 2*, line 4). Supplementation of medium with both IGF-I and thrombin further increased collagen biosynthesis (*Fig. 2*, line 5). The effect was not achieved when IGF-I was added to the cultured medium with IGF-BP1.

In our previous studies we provided evidence that changes in prolidase activity are associated with changes in phosphorylation of several kinases involved in regulation of expression of transcriptions factors [7,23]. We found that the studied substances do not change the expression of integrin receptor and IGF-IR (data not shown) but modify the expression of proteins involved in cellular pathways generated by stimulation of these receptors. The addition of Echistatin and IGFBP-1 to the medium without FBS induces decrease in phosphorylation of ERK 1/2 (*Fig. 2 A*, line 3,4,5), mTOR (*Fig. 2B*, lanes 3,4,5) and Akt (*Fig. 2C*, lanes 3,4,5) and increase in expression of NFκB (*Fig. 2D*, lanes 3,4,5).

DISCUSSION

Collagen is the most abundant extracellular protein in mammals. It constitutes about one-third of total body proteins and is a polypeptide containing the highest amount of iminobonds compared to all known proteins. It plays a significant role in maintaining tissue architecture and

integrity. It has been also recognized as a ligand for β_1-integrin receptors through which it may participate in regulation of some physiological and pathological processes [24,25,26].

The most potent stimulator of collagen biosynthesis is insulin-like growth factor (IGF-I). The changes of circulating IGF-I level lead to changes of the collagen content in tissues [27,28]. Important regulators of IGF-I activity are insulin-like growth factor binding proteins (IGFBPs) [29]. Until now it has been identified and sequenced six of IGFBP's (IGFBP-1 to IGFBP-6) with high affinity to IGF-I and nine of IGFBPs (IGFBP-rP 1-9) with low affinity to IGF-I. The object of our interest is IGFBP-1. This is protein of approximately 25 kDa that is expressed in greatest amounts during fetal development. It is known as a potent inhibitor of IGF-mediated proteins synthesis, both in phosphorylated and non-phosphorylated form. Some data also suggest its participation in the processes that are not related to IGF-I regulation. The IGF-independent effect of IGFBP-1 is the result of its ability to interact with the cell surface integrin receptors [30]. This protein contains an Arg-Gly-Asp (RGD) integrin recognition sequence. Therefore, IGFBP-1 may affect the signaling pathways generated by integrin receptors. It has been proved, that IGFBP-1 has affinity for β_1-integrin receptor, and through this receptor may induce changes in expression, differentiation and cell growth [2,10,31]. Our previous studies have shown that β_1-integrin receptor is involved in signaling, that regulates collagen biosynthesis [14,22]. In this report we suggest that IGFBP-1 may affect collagen biosynthesis through down-regulation of signal generated by the integrin receptor. An addition of IGF-BP1 to the medium of cultured fibroblasts contributed to decrease in collagen biosynthesis as demonstrated on *Fig. 1*. The same effect was achieved by echistatin – β_1-integrin blocker. When thrombin was used as a β_1-integrin stimulatory ligand together with IGF-I as a IGF-IR stimulatory ligand, distinct increase in collagen biosynthesis was found (*Fig. 2*). It suggest cross-talk between both receptors in the process of collagen biosynthesis.

In collagen degradation and biosynthesis an important role plays prolidase. Prolidase is an enzyme which catalyzes hydrolytic reactions of releasing proline or hydroxyproline from C-terminus of iminodi- and iminotripeptides derived from collagen and other proline-containing proteins. Released proline is reused for collagen biosynthesis. This enzyme catalyzes turn-over of proline-containing proteins [17,22,32]. It seems that activity of this enzyme may be a step-limiting factor in post-transcriptional regulation of collagen biosynthesis. Several data suggests that prolidase-dependent regulation of collagen biosynthesis may take place at the transcriptional level [23,33,34]. Of special interest is β_1 subunit of integrin receptor. It plays an important role in biosynthesis of collagen. Stimulation of β_1-integrin, for example, by thrombin (plasma coagulation factor, serine protease able to activate $\alpha_2\beta_1$-integrin receptor) activates the signal pathway leading to increased production of collagen and an increase in prolidase activity and expression. Decrease in prolidase activity corresponds to increase in expression of NFκB. This transcription factor inhibits the expression of subunits of type I collagen [35]. It cannot be excluded that NFκB activation undergo indirectly through Sp factor involved in ERK/p38 MAPK signaling [36]. In this study we suggest that prolidase activity and collagen biosynthesis are regulated by both receptors: IGF-IR and β_1-integrin receptor through proteins participating in pathways generated by these receptors, such as PI3K, Erk 1/2, Akt and mTOR. It suggests that "cross talk" between IGF-I receptor and integrin receptor play important role in regulation of prolidase activity and collagen biosynthesis.

REFERENCES

1. Ross RS. Molecular and mechanical synergy: cross-talk between integrins and growth factor receptors. Cardiovasc Res. 2004 Aug 15;63(3):381-90.

2. Shakibaei M, John T, Souza P De, Rahmanzadeh R, Merker HJ. Signal transduction by beta1 integrin receptors in human chondrocytes in vitro: collaboration with the insulin-like growth factor-I receptor. Biochem J. 1999 Sep 15;342 Pt 3:615-23.

3. Butler AA, Yakar S, Gewolb IH, Karas M, Okubo Y, LeRoith D. Insulin-like growth factor-I receptor signal transduction: at the interface between physiology and cell biology. Comp Biochem Physiol B Biochem Mol Biol. 1998 Sep;121(1):19-26.

4. del Peso L, González-García M, Page C, Herrera R, Nuñez G. Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science. 1997 Oct 24;278(5338):687-9.

5. Marte BM, Downward J. PKB/Akt: connecting phosphoinositide 3-kinase to cell survival and beyond. Trends Biochem Sci. 1997 Sep;22(9):355-8.

6. Bonfini L, Migliaccio E, Pelicci G, Lanfrancone L, Pelicci PG. Not all Shc's roads lead to Ras. Trends Biochem Sci. 1996 Jul;21(7):257-61.

7. Miltyk W, Karna E, Wołczyński S, Pałka J. Insulin-like growth factor I-dependent regulation of prolidase activity in cultured human skin fibroblasts. Mol Cell Biochem. 1998 Dec;189(1-2):177-83.

8. Tanaka H, Wakisaka A, Ogasa H, Kawai S, Liang CT. Effect of IGF-I and PDGF administered in vivo on the expression of osteoblast-related genes in old rats. J Endocrinol. 2002 Jul;174(1):63-70.

9. Galiano RD, Zhao LL, Clemmons DR, Roth SI, Lin X, Mustoe TA. Interaction between the insulin-like growth factor family and the integrin receptor family in tissue repair processes. Evidence in a rabbit ear dermal ulcer model. J Clin Invest. 1996 Dec 1;98(11):2462-8.

10. Jones JI, Gockerman A, Busby WH Jr, Wright G, Clemmons DR. Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its Arg-Gly-Asp sequence. Proc Natl Acad Sci U S A. 1993 Nov 15;90(22):10553-7.

11. Ginsberg MH, Partridge A, Shattil SJ. Integrin regulation. Curr Opin Cell Biol. 2005 Oct;17(5):509-16.

12. LaFlamme SE, Auer KL. Integrin signaling. Semin Cancer Biol. 1996 Jun;7(3):111-8.

13. Tucker GC. Inhibitors of integrins. Curr Opin Pharmacol. 2002 Aug;2(4):394-402.

14. Karna E, Miltyk W, Surazyński A, Pałka JA. Protective effect of hyaluronic acid on interleukin-1-induced deregulation of beta1-integrin and insulin-like growth factor-I receptor signaling and collagen biosynthesis in cultured human chondrocytes. Mol Cell Biochem. 2008 Jan;308(1-2):57-64.

15. Bononi A, Agnoletto C, De Marchi E, Marchi S, Patergnani S, Bonora M, Giorgi C, Missiroli S, Poletti F, Rimessi A, Pinton P. Protein kinases and phosphatases in the control of cell fate. Enzyme Res. 2011;2011:329098.

16. Winter JN, Jefferson LS, Kimball SR. ERK and Akt signaling pathways function through parallel mechanisms to promote mTORC1 signaling. Am J Physiol Cell Physiol. 2011 May;300(5):C1172-80.

17. Myara I, Charpentier C, Lemonnier A. Optimal conditions for prolidase assay by proline colorimetric determination: application to imidodipeptiduria. Clin Chim Acta. 1982 Oct 27;125(2):193-205.

18. Lowry OH, Rosenbregh N, Far AL, Randall IR. Protein measurement with the Folin reagent. J Biol Chem. 1951 Nov;193(1):265-75.

19. Oyamada I, Pałka JA, Schalk EM, Takeda K, Peterkofsky B. Scorbutic and fasted guinea pig sera contain an insulin-like growth factor I reversible inhibitor of proteoglycan and collagen synthesis in chick embryo chondrocytes and adult human skin fibroblasts. Arch Biochem Biophys. 1990 Jan;276(1):85-93.

20. Peterkofsky B, Palka J, Wilson S, Takeda K, Shah V. Elevated activity of low molecular weight insulin-like growth factor-binding proteins in sera of vitamin C-deficient and fasted guinea pigs. Endocrinology. 1991 Apr;128(4):1769- 79.

21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970 Aug 15;227(5259):680-5.

22. Surazyński A, Sienkiewicz P, Wołczyński S, Pałka J. Differential effect of echistatin and thrombin on collagen production and prolidase activity in human dermal fibroblasts and their possible implication in beta1-integrin-mediated signaling. Pharmacol Res. 2005 Mar;51(3):217-21.

23. Surazynski A, Miltyk W, Prokop I, Palka J. Prolidase-dependent regulation of TGF β and TGF β receptor expressions in human skin fibroblasts. Eur J Pharmacol. 2010 Dec 15;649(1-3):115-9.

24. Ricard-Blum S, Ruggiero F. The collagen superfamily: from the extracellular matrix to the cell membrane. Pathol Biol (Paris). 2005 Sep;53(7):430-42.

25. Arbet-Engels C, Janknecht R, Eckhart W. Role of focal adhesion kinase in MAP kinase activation by insulin-like growth factor-I or insulin. FEBS Lett. 1999 Jul 9;454(3):252-6.

26. Juliano RL, Haskill S. Signal transduction from the extracellular matrix. J Cell Biol. 1993 Feb;120(3):577-85.

27. Gillery P, Leperre A, Maquart FX, Borel JP. Insulin-like growth factor-I (IGF-1) stimulates protein synthesis and collagen gene expression in monolayer and lattice cultures of fibroblasts. J Cell Physiol. 1992 Aug;152(2):389-96.

28. Feld SM, Hirschberg R, Artishevsky A, Nast C, Adler SG. Insulin-like growth factor I induces mesengial proliferation and increases mRNA and secretion of collagen. Kidney Int. 1995 Jul;48(1):45-51.

29. Clemmons DR. Role of insulin-like growth factor binding proteins in controlling IGF actions. Mol Cell Endocrinol. 1998 May 25;140(1-2):19-24.

30. Frost RA, Lang CH. Differential effects of insulin-like growth factor I (IGF-I) and IGF-binding protein-1 on protein metabolism in human skeletal muscle cells. Endocrinology. 1999 Sep;140(9):3962-70.

31. Ivaska J, Reunanen H, Westermarck J, Koivisto L, Kahari VM, Heino J. Integrin a2b1 mediates isoform-specific activation of p38 and up-regulation of collagen gene transcription by a mechanism involving the alpha2 cytoplasmic tail. J Cell Biol. 1999 Oct 18;147(2):401-16.

32. Surazynski A, Miltyk W, Palka J, Phang JM. Prolidase-dependent regulation of collagen biosynthesis. Amino Acids. 2008 Nov;35(4):731-8.

33. Palka JA, Phang JM. Prolidase activity in fibroblasts is regulated by interaction of extracellular matrix with cell surface integrin receptor. J Cell Biochem. 1997 Nov 1;67(2):166-75.

34. Phang JM, Hu CA, Valle D. Disorders of proline and hydroxyproline metabolism, In: Scriver, CR, Beaudet AL, Sly WS, Valle D, editors. The Metabolic and Molecular Basis of Inherited Disease. 8th ed. New York: McGraw-Hill; 2001. p. 1821–38.

35. Rippe RA, Schrum LW, Stefanovic B, Solis-Herruzo JA, Brenner DA. NF-kappaB inhibits expression of alpha1(I) collagen gene. DNA Cell Biol. 1999 Oct;18(10):751 61.

36. Sun J, Ramnath RD, Zhi L, Tamizhselvi R, Bhatia M. Substance P enhances NF-kappaB transactivation and chemokine response in murine macrophages via ERK1/2 and p38 MAPK signaling pathways. Am J Physiol Cell Physiol. 2008 Jun;294(6):C1586-96.