Expression of integrin β1 by fibroblasts is required for tissue repair in vivo

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Summary
In tissue repair, fibroblasts migrate into the wound to produce and remodel extracellular matrix (ECM). Integrins are believed to be crucial for tissue repair, but their tissue-specific role in this process is poorly understood. Here, we show that mice containing a fibroblast-specific deletion of integrin β1 exhibit delayed cutaneous wound closure and less granulation tissue formation, including reduced production of new ECM and reduced expression of α-smooth muscle actin (α-SMA). Integrin-β1-deficient fibroblasts showed reduced expression of type I collagen and connective tissue growth factor, and failed to differentiate into myofibroblasts as a result of reduced α-SMA stress fiber formation. Loss of integrin β1 in adult fibroblasts reduced their ability to adhere to, to spread on and to contract ECM. Within stressed collagen matrices, integrin-β1-deficient fibroblasts showed reduced activation of latent TGFβ. Addition of active TGFβ alleviated the phenotype of integrin-β1-deficient mice. Thus integrin β1 is essential for normal wound healing, where it acts, at least in part, through a TGFβ-dependent mechanism in vivo.

Key words: α-SMA, Myofibroblast, Matrix contraction, Integrins

Introduction
During normal healing of skin, connective tissue is repaired exclusively through the action of fibroblasts, which migrate into the wound site where they synthesize and remodel a new extracellular matrix (ECM) (Martin, 1997; Eckes et al., 1999; Gurtner et al., 2004). The ability of cells to migrate, attach to and remodel ECM is mediated by specialized cell surface structures termed focal adhesions (FAs), which mediate adhesion between the ECM and the actin cytoskeleton through integrin cell surface receptors (Burridge and Chrzanowska-Wodnicka, 1996; Chrnansowska-Wodnicka, 1996; Zamir and Geiger, 2001) and from the ECM (Burridge and Chrzanowska-Wodnicka, 1996; Zamir and Geiger, 2001) and from the ECM (Burridge and Chrzanowska-Wodnicka, 1996; Zamir and Geiger, 2001). Given their role in cell adhesion and adhesive signaling, integrin expression by fibroblasts must be important for tissue repair in vitro, although the contribution of individual integrins to this process is still being elucidated (Hynes, 2002; Schultz and Wysocki, 2009). As an example, fibroblasts lacking focal adhesion kinase (FAK) spread poorly, cannot migrate, and cannot properly respond to TGFβ (Sie et al., 1995; Liu et al., 2007). Recently, we have shown that fibroblast expression of Rac1, a small GTPase that is expressed ubiquitously and is recruited to FAs by paxillin (Burridge and Wennemmer, 2004; Ishibe et al., 2004), is required for fibrogenesis and cutaneous tissue repair in vivo (Liu et al., 2008; Liu et al., 2009a) and for induction of myofibroblast formation by endothelin-1 (Shi-wen et al., 2004). Moreover, we have shown that activation of cell adhesion is sufficient to cause increased expression of mRNAs encoding fibrogenic proteins (Kennedy et al., 2008). Collectively, these results suggest that adhesion and adhesive signaling has a key role in tissue repair and fibrogenic responses in vivo.

Given their role in cell adhesion and adhesive signaling, integrin expression by fibroblasts must be important for tissue repair in vitro, although the contribution of individual integrins to this process is still being elucidated (Hynes, 2002; Schultz and Wysocki, 2009). Integrin β1 is a common mediator of fibroblast attachment to collagen type I and fibronectin (Lafrenie and Yamada, 1996; Gabbiani, 2003), suggesting that expression of integrin β1 by fibroblasts has a key role in cutaneous tissue repair. However, this hypothesis has not yet been tested. As integrin-β1-knockout mice die in utero (Stephens et al., 1995), conditional-knockout mouse models are necessary to address this question. Mice harboring the integrin β1 allele flanked
by loxP sites have been generated, and these mice have been used successfully to delete integrin β1 in vivo using mice expressing Cre recombinase under the control of a tissue-specific promoter (Raghavan et al., 2000; Piwko-Czuchra et al., 2009; Bauer et al., 2009). Recently, we have shown that mice containing a fibroblast-specific deletion of integrin β1 show resistance to bleomycin-induced skin fibrosis (Liu et al., 2009b). In this report, we study the influence of deletion of integrin β1 in adult fibroblasts on cutaneous wound healing and the underlying cell and molecular processes involved. Our data provide new and valuable insights into the contribution of integrin β1 to fibroblast biology.

**Results**

**Deletion of integrin β1 causes delayed cutaneous wound repair**

To test whether integrin-β1-deficient mice showed impaired cutaneous wound closure, we subjected 8-week-old mice homozygous for deletions in the integrin β1 gene (Itgb1), or wild-type littermate counterparts, to dermal punch wounding. Loss of β1 integrin was shown by real-time PCR and western blot analysis of fibroblasts cultured from control (C/C) and integrin-β1-deficient (K/K) animals (Fig. 1A, B). Compared with wounded control animals, integrin-β1-deficient animals showed a significantly reduced rate of wound closure (Fig. 1C). Studies using wild-type mice treated with or without tamoxifen revealed that tamoxifen alone did not affect wound closure (not shown). Integrin-β1-deficient animals were examined 7 days after wounding. They displayed reduced collagen production and less granulation tissue (Fig. 2), as well as myofibroblasts with decreased α-SMA and PCNA expression at the site of injury (Fig. 3) compared with control animals.

**Deletion of integrin β1 causes impaired migration, adhesion, proliferation and spreading**

To study the molecular mechanism(s) of failed wound repair, we cultured primary dermal fibroblasts from explants of animals

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**Fig. 1. Conditional deletion of integrin β1 in fibroblasts results in impaired cutaneous wound healing.** Mice deleted for integrin β1 (K/K) and control mice (C/C) that were otherwise genetically identical were generated and genotyped. (A) Fibroblasts from integrin β1 and control mice were cultured. RNA was extracted from cells and subjected to real-time PCR analysis to detect mRNA encoding integrin β1 and GAPDH, and relative expression was calculated by the ΔΔCt method. Six mice per group were analyzed. Data represent means + s.d. (*P<0.05; Student’s t-test). (B) Fibroblasts from integrin β1 and control mice were cultured. Cell extracts were subjected to SDS-PAGE and western blot analysis with anti-integrin-β1 antibody. Before hybridization with antibody, blots were stained using Ponceau Red to verify equal loading. (C) Mice were wounded and wound closure was measured on day 0, day 3, day 7 and day 10. Six mice per group (four wounds per mouse) were analyzed. Data represent means + s.d. (*P<0.05; Student’s t-test).

**Fig. 2. Conditional deletion of integrin β1 in fibroblasts results in impaired cutaneous wound healing.** Mice were wounded and quality of wound closure was measured. Histological sections of mouse wounds 7 days after wounding were investigated using hematoxylin and eosin (H&E, top) and trichrome (bottom) stain to detect collagen. Area of granulation tissue and hydroxyproline levels were measured and results are shown in graphs on right. For all assays, six mice per group were analyzed. Data represent means + s.d. (*P<0.05, **P<0.01; Student’s t-test). Note that integrin-β1-deficient wounds had defects in wound closure as revealed by reduced granulation tissue in the wound. epi, epidermis; der, dermis.
deleted for integrin β1 or not. Using a scratch wound assay of in vitro wound repair, we showed that integrin-β1-deficient cells migrated more slowly (Fig. 4A). Single-cell tracking confirmed that persistence of migration in integrin-β1-deficient fibroblasts on collagen I and on tissue culture plastic was significantly diminished (Fig. 4B). Integrin-β1-deficient cells also showed reduced proliferation (Fig. 5A) and an impaired ability to adhere to a fibronectin substrate (Fig. 5B) than observed with control cells. Cell spreading was also monitored microscopically after adhesion, using antibodies against Rhodamine-phalloidin and vinculin. Loss of integrin β1 also resulted in abnormal spreading on fibronectin, as revealed with anti-vinculin antibody (green) and Rhodamine-phalloidin (red) staining to detect actin (Fig. 5C).

Deletion of integrin β1 causes impaired myofibroblast differentiation and ECM contraction

Myofibroblasts are the major cell type responsible for ECM remodeling during wound healing (Dugina et al., 2001; Hinz et al., 2007). We showed that integrin-β1-deficient fibroblasts contained reduced numbers of α-SMA-containing stress fibers (Fig. 6A) compared with control cells, suggesting that integrin-β1-deficient cells had impaired myofibroblast differentiation. Consistent with this observation, integrin-β1-deficient fibroblasts also had reduced α-SMA protein expression (Fig. 6B). Moreover, loss of integrin β1 resulted in cells that displayed reduced expression of mRNAs encoding profibrotic proteins, including α-SMA and type I collagen (Fig. 6C). Finally, integrin-β1-deficient fibroblasts were less able to migrate and thus to generate contractile forces to contract free-floating and tethered collagen gel matrices (Fig. 7A,B). These data suggest that integrin β1 deficiency results in a dramatic failure of fibroblast attachment to collagen and differentiation into myofibroblasts.

Loss of integrin β1 results in reduced TGFβ activation

It has been proposed that integrins (in particular integrin αv) mediate the activation of latent TGFβ during myofibroblast
integrin-β1-deficient fibroblasts were isolated by explant culture from mice containing the integrin β1 gene (C/C) or not (K/K). For all assays, fibroblasts from six mice per group were analyzed. Cells were subjected to (A) a proliferation assay and (B) a fibronectin adhesion assay (means ± s.d. are shown; *P<0.05; Student’s t-test). (C) Cell spreading on fibronectin was monitored for 2 hours. Cells were fixed and stained with anti-vinculin antibody to detect focal adhesions and Rhodamine-phalloidin to detect actin (red). Nuclei are stained with DAPI (blue). Representative images are shown.

Fig. 5. Loss of integrin β1 results in a reduced ability of fibroblasts to adhere to ECM, proliferate and spread on ECM. Fibroblasts were isolated by explant culture from mice containing the integrin β1 gene (C/C) or not (K/K). For all assays, fibroblasts from six mice per group were analyzed. Cells were subjected to (A) a proliferation assay and (B) a fibronectin adhesion assay (means ± s.d. are shown; *P<0.05; Student’s t-test). Cells were fixed and stained with anti-vinculin antibody (green) to detect focal adhesions and Rhodamine-phalloidin to detect actin and α-SMA (red). Colocalization of vinculin and actin is seen as yellow staining. Intensity of actin and α-SMA was assessed by linear scans across fibroblasts (white lines) using image analysis software (Northern Eclipse, Empix); representative traces are shown below. Integrin-β1-deficient cells have a reduced number of actin stress fibers, indicating reduced myofibroblast formation. Nuclei are stained with DAPI (blue). (B) Cells were subjected to western blot analysis with an anti-α-SMA antibody. (C) Real-time PCR analysis. mRNA harvested from cells (ten mice total per group, each assay performed in triplicate, means ± s.d. are shown) was subjected to real-time PCR analysis to detect the mRNAs indicated (*P<0.05; Student’s t-test).

Western blot analyses of wild-type (C/C) and integrin-β1-deficient (K/K) cells showed no difference in levels of TGFβ type I or TGFβ type II; however, integrin-β1-deficient cells showed elevated integrin β3 expression (Fig. 9), an integrin that has previously been associated with decreased rates of tissue repair (Reynolds et al., 2005).

Based on these results, we investigated whether phenotype of integrin-β1-deficient mice could be rescued by addition of exogenous active TGFβ1 to mice subjected to the dermal punch model of tissue repair. By day 3 after wounding, addition of TGFβ1 resulted in increased wound closure in both wild-type and integrin-β1-deficient mice (Fig. 10A). By day 5 and day 7 after wounding, TGFβ1 increased wound closure in integrin-β1-deficient mice to the level observed in wild-type mice (Fig. 10A). In addition, application of TGFβ1 to day 7 wounds of integrin-β1-deficient
mice resulted in levels of granulation tissue, collagen, and $\alpha$-SMA production that were similar to those observed with the control-injected wild-type mice (Fig. 10B–D). Taken together, these data indicate that the addition of exogenous active TGF$\beta$ effectively rescued the wound-healing defects of integrin-$\beta_1$-deficient mice. These data strongly suggest that expression of integrin $\beta_1$ by fibroblasts is required for efficient wound closure, specifically proper granulation tissue formation and dermal repair, via a TGF$\beta$-dependent mechanism.

Discussion
Integrin $\beta_1$ is essential for embryonic development and the maintenance of several tissues (Brakebusch and Fässler, 2005; Fässler and Meyer, 1995). Integrin-$\beta_1$-knockout mice die in utero (Stephens et al., 1995), indicating an essential role for this integrin in development. Cell-type-specific deletions of the integrin $\beta_1$ gene has revealed a key role for integrin $\beta_1$ in supporting stem and progenitor cell properties, such as adhesion to their niche and the appropriate orientation of the mitotic spindle to control the symmetry of cell division (Lechler and Fuchs, 2005; Kuang et al., 2007). Mutant mice lacking integrin $\beta_1$ in skin epidermis possessed severe blistering as a result of impaired attachment of basal keratinocytes to the basement membrane, impaired keratinocyte proliferation, dermal fibrosis and severely delayed wound healing (Raghavan et al., 2000; Brakebush et al., 2000; Grose et al., 2002). However, the role of integrin $\beta_1$ in fibroblasts and myofibroblast-
like cells in vivo is largely unknown, as is the precise cell and molecular mechanism underlying integrin β1 action via fibroblasts in tissue repair.

Integrin β1 is a key mediator of fibroblast adhesion to type I collagen and fibronectin (Lafrenie and Yamada, 1996; Gabbiani, 2003). In this study, we show that loss of integrin β1, specifically in adult fibroblasts, results in impaired cutaneous tissue repair in vivo. Integrin-β1-deficient mice showed reduced rates of wound closure, and decreased myofibroblast production and collagen deposition. Consistent with these results, cultured fibroblasts derived from integrin-β1-deficient mice possessed decreased levels of α-SMA mRNA and protein expression. Consistent with these data, integrin-β1-deficient fibroblasts exhibited reduced α-SMA stress fiber formation and reduced expression of mRNA encoding type I collagen and connective tissue growth factor (CTGF). Moreover, integrin β1 was required for optimal cell migration, adhesion and ECM contraction. These results are in sharp contrast to the conventional knock-out mice lacking integrin β3, which show enhanced wound healing that is complete several days earlier than in wild-type mice, and enhanced dermal fibroblast infiltration into wound sites (Reynolds et al., 2005). Interestingly, we found that loss of integrin β1 resulted in an increase in expression of integrin β3, providing a possible mechanistic basis for our study. It is also interesting to note that wound healing in the integrin-β2-knockout mouse was similar to that in our fibroblast-specific knockout of β1 integrin (Peters et al., 2005). Here, the mechanisms underlying the severely impaired wound healing were associated with a severe reduction of neutrophils and macrophages into the wounds, causing a lack of secreted TGFβ1. As our mice are only deficient in integrin β1 in fibroblasts, the molecular mechanism underlying impaired healing is likely to be different. Indeed, it was previously suggested that mechanical contraction of ECM by fibroblasts activates TGFβ in a fashion requiring integrin αV and an intact cytoskeleton (Wipff et al., 2007). To determine whether such a mechanism might be responsible for the phenotype of integrin-β1-knockout fibroblasts, we showed that wild-type...
fibroblasts, but not integrin-β1-deficient fibroblasts, embedded within a collagen gel were able to support high levels of activity of a TGFβ-responsive promoter in a fashion that depended on myosin-mediated contraction. TGFβ rescued the phenotype of integrin-β1-deficient mice. These results collectively suggest that expression of integrin-β1 by fibroblasts is essential for wound healing, and that the function of integrin β1 occurs partly through a TGFβ-dependent mechanism probably through a defect in activation of latent TGFβ. Our results demonstrate for the first time that integrin β1 expression by fibroblasts is involved with this process and is required for formation of granulation tissue in skin.

Fibrosis is characterized by the persistence of myofibroblasts within scars (Dugina et al., 2001; Chen et al., 2005; Gabbiani, 2003). Although myofibroblasts can form through the presence of extracellular signaling molecules including TGFβ, ET-1 and CCL2, it is now increasingly appreciated that adhesional signaling also contributes to their formation (Werner and Grose, 2003; Leask, 2006; Shi-wen et al., 2006a,b; Shi-wen et al., 2007). Our data showing that integrin β1, a protein that mediates cell attachment of ECM, was required for tissue repair in vivo is consistent with this latter notion. Taken together with observations that the persistent fibrotic phenotype of lung scleroderma fibroblasts is at least partially mediated by integrin β1 (Shi-wen et al., 2007), which is upregulated in scleroderma fibroblasts (Chen et al., 2005; Chen et al., 2008), our results strongly suggest that the targeting of adhesional signaling is a novel, appropriate strategy for anti-fibrotic drug intervention. In summary, the observations presented here indicate that integrin β1 is essential for proper granulation tissue formation and thus for wound closure and might have future implications for the treatment of non-healing ulcers and fibrosis.

Materials and Methods

**Generation of integrin β1 conditional-knockout mice**

Integrin β1 conditional-knockout mice were generated as described (Liu et al., 2008; Liu et al., 2009a; Reddy and Enwemeka, 1996). Wound tissues were homogenized in saline, hydrolyzed with 2N NaOH for 30 minutes at 120°C, followed by the determination of hydroxyproline by modification of the Neumann and Logan’s reaction using Chloramidine T and Ehrlich’s reagent using a hydroxyproline standard curve and measuring at 550 nm. Values were expressed as mg hydroxyproline per mg protein.

**Cell culture, immunofluorescence and western analysis**

Primary dermal fibroblasts were isolated from explants (4- to 6-week-old animals) as described (Chen et al., 2005). Cells were subjected to anti-tumor, immunofluorescence analysis as described (Chen et al., 2005; Kennedy et al., 2007) using anti-rabbit-tissue, Rhodamine-phallloidin (Sigma) and anti-vinculin (Sigma) antibodies, followed by an appropriate secondary antibody (Jackson Immunoresearch). Cells were photographed (Zeiss Axiphot B-100, Empix). Alternatively, cells were lysed in 2% SDS, proteins quantified (Pierce) and subjected to western blot analysis as described (Shi-wen et al., 2004; Chen et al., 2005; Kennedy et al., 2007). Anti-integrin β1 antibody was from R&D Systems; anti-integrin-β3, anti-TGFβ type I receptor, anti-TGFβ type II receptor and anti-GAPDH antibodies were from Santa Cruz Biotechnology. Fluorescence intensity of α-SM, fibronectin was quantified by line scan measurement using Northern Eclipse (Empix) software (Liu et al., 2007). MLEC-PAI-Luc, a nuc lung epithelial cell line stably transfected with an expression plasmid containing a TGFβ-responsive region (~799 to +71) of human plasminogen activator inhibitor-1 (PAI1) gene fused to the firefly luciferase reporter gene. Cells were maintained in DMEM supplemented with 5% fetal calf serum.

**Real-time polymerase chain reaction (RT-PCR)**

RT-PCR was performed essentially as described (Kennedy et al., 2007; Kennedy et al., 2008; Pala et al., 2008). A total of ten control and conditional-knockout mice were analyzed independently for each data point (mean ± s.d. shown from these ten independent animals; Student’s paired t-test). Cells were cultured until 80% confluent, serum starved for 24 hours and total RNA was isolated (Qiagen). Integrity of the RNA was verified by gel electrophoresis. Total RNA (25 ng) was reverse transcribed and amplified (TaqMan Assays on Demand; Applied Biosystems) as described (Chen et al., 2005). Cells were subjected to real-time reverse transcription PCR using the ABI Prism 7900 HT sequence detector (Perkin-Elmer-Cetus, Vaudreuil, QC). Triplicates of each samples were run, and expression values were standardized to values obtained with control 18S RNA primers using the ΔΔCt method.

**Adhesion assay**

 Fibroblasts were isolated and cultured as described above. Adhesion assays were performed essentially as previously described (Chen et al., 2004; Chen et al., 2005). Wells of 96-well plates were incubated overnight at 4°C with 10 µg/ml fibrinectin (Sigma) in 0.5% bovine serum albumin (BSA), 1× PBS. Subsequently, cells were blocked for 1 hour in 10% BSA in PBS, room temperature. Fibroblasts were harvested with 2 mM EDTA in PBS (20 minutes, room temperature), washed twice with DMEM serum-free medium containing 1% BSA (Sigma), resuspended in the same medium at 2.5×10^5 cells/ml and 100 µl suspension was incubated in each well for the times indicated. Non-adherent cells were removed by washing with PBS. To detect cell adhesions, the acid phosphatase assay was used; adherent cells were quantified by incubation with 100 µl substrate solution (0.1 M sodium acetate, pH 5.5; 10 mM p-nitrophenolphosphate and 0.1% Triton X-100) for 2 hours at 37°C. The reaction was stopped by the addition of 15 µl of 1 N NaOH per well and A405 measured.
was measured. Comparison of adhesive abilities was performed by using Student’s unpaired t-test. P<0.05 was considered statistically significant.

Collagen gel contraction

Experiments were performed essentially as described (Shi-wen et al., 2004). For a floating gel assay, 24-well tissue culture plates were pre-coated with BSA. Cells were used at passage three. Trypsinized fibroblasts were suspended in MCD2B medium (Sigma) and mixed with collagen solution [one part 0.2 M HEPES, pH 8.0, four parts 3 mg/ml collagen (Nutragen, Inamed) and five parts of 2× MCD2B] for a final concentration of 80,000 cells per ml in 1.2 mg/ml collagen. Collagen cell suspension (1 ml) was then added to each well to polymerize. Gels were then detached from wells by adding 1 ml MCD2B medium. Gel contraction was quantified by measuring changes in weight. When indicated, contraction assays were performed in the presence of DMSO and 100 µM blebbistatin (myosin ATPase/contraction inhibitor, Calbiochem).

Fibroblast-populated collagen lattices

Measurement of contractile forces generated within a three-dimensional FPCL was performed as described (Shi-wen et al., 2004). Briefly, using 1×10⁴ cells/ml of collagen gel (First Link, UK), we measured the force generated across a collagen lattice using a culture force monitor which measures forces exerted over 24 hours. A rectangular FPCL was cast, floated in medium in 2% FCS and attached to a ground point at one end and a force transducer at the other. Cell-generated tensile forces on the collagen gel are measured by the force transducer and logged into a personal computer (t-CFM) (Denton et al., 2009). Graphical readings are produced every 15 seconds providing a continuous output of force (Dynes: 1×10⁻⁶ N). Experiments were run in parallel and three independent times, and a representative trace is shown.

Migration and proliferation assays

For in vitro wounding (migration) experiments, fibroblasts obtained from integrin β1 conditional (C/C) and knockout (K/K) mice were cultured in 12-well plates (2×10⁴ cells/well). The next day, the cells were confluent. Medium was removed, and cells rinsed with serum-free medium with 0.1% BSA and cultured for an additional 24 hours in serum-free medium plus 0.1% BSA. The monolayer was artificially injured by scratching across the plate with a blue pipette tip (approximately 100 µm wide; 1×10⁴ cells/ml) or tissue culture plastic. Cells were observed at 24 hours (Barrett et al., 2003). For a measurement of adhesive abilities, fibroblasts were plated at low density (2000 cells/cm²) on type I collagen (30 µg/ml in PBS, acid-extracted from newborn calf skin) or tissue culture plastic. Cells were observed for 5 hours in an incubator chamber attached to an IX81 microscope (Olympus). Interactions of fibroblasts with the extracellular matrix: implications for the understanding of fibrosis. Springer Semin. Immunopathol. 21, 415-450. Fässler, R. and Meyer, M. (1995). Consequences of lack of β1 integrin gene expression in mice. Genes Dev. 9, 1896-1908. Gabbiani, G. (2003). The myofibroblast in wound healing and fibrocontractive diseases. Exp. Cell Res. 290, 500-503. Geiger, B., Spatz, J. P. and Bershadsky, A. D. (2009). Environmental sensing through focal adhesions. Nat. Rev. Mol. Cell Biol. 10, 21-33. Gros, R., Hutter, C., Bloch, W., Thorey, I., Watt, F. M., Fässler, R., Brakebusch, C. and Werner, S. (2002). A crucial role of β1 integrins for keratinocyte migration in vitro and during cutaneous wound repair. Development 129, 2303-2315. Gurtner, G. C., Werner, S., Barrandon, Y. and Longaker, M. T. (2008). Wound repair and regeneration. Nature 453, 314-321. Hinz, B., Pham, S. H., Thannickal, V. J., Galli, A., Bochaton-Pierrat, M. L. and Gabbiani, G. (2007). The myofibroblast: one function, multiple origins. Am. J. Pathol. 170, 1807-1816. Hynes, R. O. (2002). Integrins: bidirectional, allosteric signaling machines. Cell 110, 673-687. Ilic, D., Furuta, Y., Kanazawa, S., Takeda, N., Sobue, K., Nakatsuji, N., Nomura, S., Fujimoto, J., Okada, M., Yamamoto, T. et al. (1995). Reduced cell motility and enhanced focal adhesion contact formation in cells from FAK-deficient mice. J. Biol. Chem. 270, 23184-23188. Ishibe, S., Joly, D., Ishibe, S., Joly, D., Liu, Z. X. and Cantley, L. G. (2002). Paxillin serves as an ERK- regulated scaffold for coordinating FAK and Rac activation in epithelial morphogenesis. Mol. Cell, 6, 257-267. Kennedy, L., Liu, S., Shi-wen, X., Carter, D., Lyons, K., Black, C. M., Abraham, D. J. and Leask, A. (2007). CCN2 is essential for fibroblast function. Exp. Cell Res. 313, 952-964. Kennedy, L., Shi-wen, X., Carter, D. R., Breitkreutz, A., Logue, P., Black, C. M., Abraham, D. J. and Leask, A. (2008). Adhesion of cells to fibronectin induces a tissue repair gene expression program. Matrix Biol. 27, 274-281. Kuan, S., Kuroda, K., Le Grand, F. and Rudnick, M. A. (2007). Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129, 999-1010. Lafrenie, R. M. and Yamada, K. M. (1996). Integrin-dependent signal transduction. J. Cell Biochem. 61, 543-553. Leask, A. (2006). Scar wars: Is TGFβ the phantom menace in scleroderma? Arthritis Res. Ther. 8, 213. 3681
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