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ORIGINAL PAPER

Modulation of cutaneous extracellular collagen contraction by phosphorylation status of p130Cas

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Abstract Skin can respond to various types of internal and/or external mechanostimuli, such as excessive tension caused by body growth or decompression due to weight loss, which significantly affect skin morphology. Mechanosensors, including p130Cas, are reported to play a role in deformation and subsequent recovery of various tissues including skeletal muscles and blood vessels. However, the role of mechanotransduction via p130Cas in the regulation of skin size remains unclear. In this report, p130Cas activation was manipulated using a fibroblast-embedded collagen gel model or mouse skin contraction model. Inhibition or activation of Src family kinase-mediated phosphorylation of p130Cas significantly depressed and accelerated collagen gel contraction, respectively. The results also demonstrated age-dependent depression of cutaneous p130Cas activation in vivo. Inhibition of p130Cas signaling in our mouse model significantly suppressed recovery from cutaneous deformation. Taken together, our study highlighted the important role of p130Cas in cutaneous mechanotransduction for skin homeostasis.

Keywords p130Cas · Mechanotransduction · Skin morphology · Aging · Extracellular matrix

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Abbreviations

CSK	C-terminal Srk kinase
ECM	Extracellular matrix
FAK	Focal adhesion kinase
FBS	Fetal bovine serum
HEK	Human embryonic kidney
MMP	Matrix metalloproteinase
p130Cas	p130 Crk-associated substrate
PP2	4-amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo [3,4-*d*] pyrimidine

Introduction

During daily living activity, various internal and/or external mechanical stimuli affect human skin tension [1]. The response of the dermal tissue to mechanical stimuli depends mainly on the structural integrity of the skin, especially on the quality and/or quantity of the cutaneous extracellular matrix (ECM) [2]. In aged skin, the contact between fibroblasts and collagen fibrils is reported to be reduced by 20 %, with a 25 % decrease in the cross-sectional surface area of the dermis, compared with that of younger skin [3]. What are the mechanisms responsible for the fall in ECM-related interaction in the aged skin? While the exact reasons are yet to be identified, previous studies indicated that delayed wound healing process, dermatochalasis and blepharoptosis are age-related morphological disorders associated with the age-related dysregulated ECM homeostasis [4, 5]. These quantitative changes seem to lead to insensitive mechanoreception of dermatocytes. Consistent with the aforementioned findings, the collagen gel contractility ratio, a cell-related mechanotransduction process, was reported to be lower in dermal fibroblasts of the elderly than young individuals, suggesting impairment

of skin fibroblasts during the aging process [6–8]. In addition, both the expression of collagen and number of elastic fibrils were reported to be increased in human and rodent skin subjected to expansion forces both in vitro and in vivo [9, 10]. These findings suggest that the interactions between dermatocytes and between the cells and ECMs play important roles in the skin response to mechanical stimuli as well as the wound healing and/or repair processes [11, 12].

Mechanotransduction is the process by which cells convert a mechanical stimulus into chemical reaction [13, 14]. There are a variety of mechanotransduction systems in various tissues, including skeletal muscle, bone, cartilage, blood vessels, heart, and skin. In blood vessels, high blood pressure stimulates focal adhesion kinase (FAK)-mediated signaling and nitric oxide production [15]. Furthermore, muscle atrophy caused by unloading stress upregulates myostatin-mediated signaling [16] and downregulates insulin-like growth factor-I (IGF-I)-mediated signaling [17]. Several cell adhesion molecules and/or their related factors (e.g., integrin complex and platelet endothelial cell adhesion molecule-1) are also reported to be involved in mechanotransduction. However, the exact mechanism involved in mechanical stimuli-induced cutaneous signal transduction remains elusive.

To elucidate such mechanism(s), we focused in the present study on p130 Crk-associated substrate (p130Cas) among focal adhesion molecules. This particular signaling pathway was selected because p130Cas is known as a potent mediator of cell motility, invasion and proliferation [18–20]. The results showed expression of p130Cas in human and mouse skin and that such expression was markedly suppressed in the aged skin. In addition, p130Cas is located close to collagens via integrin β1 and formed a component of the focal adhesion complex. We also analyzed the physiological role of p130Cas in skin mechanotransduction using cutaneous cell cultures and collagen gel and mouse skin flabbiness models. These results suggested the possibility that the focal adhesion system focusing on p130Cas plays a pivotal role in skin morphology regulation by mediating mechanotransduction signals to drive the focal adhesion machinery-based cell contraction as well as ECM reorganization.

Materials and methods

Tissue samples

Skin tissues of 4- and 30-week-old female mice were purchased from Primary Cell (Hokkaido, Japan). Normal human dermal fibroblasts (NHDFs; Kurabo Corporation, Osaka, Japan) were maintained in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Tokyo, Japan) containing 5 % (v/v) fetal bovine serum (FBS). Normal human epidermal keratinocytes (NHEKs; Life Technologies) were cultured in Epilife medium (Life Technologies) containing HuMedia-KG additional agent kit (Kurabo). Both cell lines were cultured at 37 °C in a humidified atmosphere containing 5 % CO_2. Before the construction of 3D-collagen gels, NHDFs and NHEKs seeded on cell culture plates were transfected with small inhibitory RNAs (siRNAs) specific for the sequences of p130Cas, CSK or with a non-specific siRNA together with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA) in OptiMEM I medium (Invitrogen), using the instructions provided by the manufacturer. Another batch of cells was treated with 5 or 25 μM 4-amino-5-(4-chlorophenyl)-7-(*t*-butyl) pyrazolo [3,4-*d*] pyrimidine (PP2) (Calbiochem, San Diego, CA), an inhibitor of C-terminal Srk kinase (CSK). Assessment of collagen gel contraction with siRNA-treated cells was initiated 48 h after the transfection.

Western blotting analysis

Cells and mouse skins were lysed in phosphatase extraction buffer (Novagen, Darmstadt, Germany). Briefly, 20 μg of each cell extract (NHDFs or NHEKs) and of mouse skin extract, were separated on 7.5 % or 12 % SDS gels (Bio-Rad Laboratories, Hercules, CA). Following the separation, the samples were transferred to PVDF membranes (Bio-Rad Laboratories) and then incubated with antibodies specific for human phospho-p130Cas Y410 or Y165 (Cell Signaling, Danvers, MA), human p130Cas (BD Biosciences San Jose, CA), human CSK (Santa Cruz Biotechnology, Santa Cruz, CA), human collagen 1α chain 1 (Santa Cruz Biotechnology), human MMP-1 (Santa Cruz Biotechnology), and human β actin (Sigma Aldrich, St. Louis, MO). Subsequently, antibody recognition was visualized using Enhanced Chemiluminescence Plus (GE Healthcare, UK) following the instructions recommended by the manufacturers.

Construction of 3D collagen gels and evaluation of contraction

Collagen gel solution (type I-A, 3.0 mg/ml, pH 3.0; Nitta gelatin, Osaka, Japan) was mixed with 250 mM HEPES buffer (pH 7.4), fivefold-concentrated DMEM (low glucose; Life Technologies), 5 % FBS, distilled water and NHDFs treated with siRNAs specific for p130Cas or CSK, or with PP2, followed by dispensation of a 600-μl portion into each well of a 24-well plate. This mixture was then incubated at 37 °C for collagen polymerization. Following the construction of NHDF-embedded collagen gels, NHEKs were seeded on the top of the gels for evaluation of

the role of p130Cas in keratinocytes. To assess the effect of p130Cas on gel contraction, gels were detached from the well walls immediately after the addition of 100 µl medium. Following culture for 48–96 h at 37 °C, photos of the gels were taken to calculate their areas using image analysis software (Image J). The ratio of the contracted gel area to the area of the gel at start-up represented the percentage of gel contraction.

Immunochemical staining of normal human dermal fibroblasts and skin tissues

NHDFs were cultured on collagen [type I-C (3.0 mg/ml, pH 3.0), Nitta gelatin]-coated chamber slides (Thermo-Scientific, MA) for 24 h with DMSO or PP2, then fixed in 4 % paraformaldehyde and stained as described previously [3]. Briefly, after 20 min fixation, the cells were treated with 0.1 % Triton-X for permeabilization. The cells were also concomitantly stained for actin expression and nuclear counterstaining. Stained cells were examined by confocal laser scanning microscopy (Carl Zeiss, Jena, DE). Alexa Fluor 546 phalloidin (Life Technologies) was used to visualize actin filaments, and nuclei were counterstained with the nuclear dye DAPI (Chemicon International, Inc., Temecula, CA).

Human skin punch biopsies taken from the thick arm of normal Hispanic women volunteers (age: 30s and 60s) were obtained from Stephens and Associates, Carrollton, TX. The collections of skin tissues were approved by the Institutional Review Board of IntegReview Ltd. (Austin, TX). This study was conducted according to the Declaration of Helsinki protocols and informed consent was obtained from each volunteer prior to the procedure. Skin punch biopsies were fixed in 10 % buffered formalin and then embedded in paraffin. The immunoreactivities of phospho-p130Cas and total p130Cas were estimated with anti-BCAR1 antibody (phospho Y410; Abcam, Cambridge, UK) and anti-p130Cas antibody (BD Biosciences), respectively. Immunoreactivity was visualized using a Histofine kit (Nichirei, Tokyo).

Establishment of in vivo model for skin recovery from slack

Five week-old naked mice (HR-1) obtained from Japan SLC (Shizuoka, Japan) were handled according to the guidelines of the Institutional Animal Care and Use Committee of Tokushima University. The mice were kept under specific pathogen-free conditions throughout the experiments. Under anesthesia, chambers (Renner, Dannstadt, Germany) were placed under the dorsal skin of mice for continuous stretch stimulation of the skin for 1 week, followed by assessment of recovery from the stretch at 3 weeks after the removal of the embedded chamber. To examine the role of p130Cas in the recovery process, the dorsal skins of the mice were injected with 200 µl of PBS or 250 µM PP2/200 µl PBS every day from day 1 before chamber removal to 3 weeks after the removal. Skin excess was measured every week after chamber removal.

Statistical analysis

Differences in mean or raw values of various parameters between two groups were analyzed by the Student *t*-test. A p value < 0.05 denoted the presence of a statistically significant difference.

Results

Mechanical stress-mediated phosphorylation of p130Cas in human skin fibroblasts

The status of p130Cas phosphorylation was examined when NHDFs were subjected to mechanical stimulated conditions, such as stretching on the culture dish. Western blotting analysis using anti-phospho-p130Cas (Tyr410) antibody showed phosphorylation of p130Cas by constant biaxial stretching for 1 min (Fig. 1).

Effects of depletion of p130Cas signaling on collagen gel contraction

Next, p130Cas expression in NHDFs was knocked down using siRNA and the cells were cultured on collagen gels. p130Cas protein expression was significantly knocked down by its specific siRNA and the expression level remained low at least for 4 days after the treatment (Fig. 2a). Culture of NHDFs transfected with control siRNA on the collagen gel also resulted in time-dependent gradual reduction in the area of collagen gel, relative to that on day 0 (just after culture) (Fig. 2b). In contrast, the area

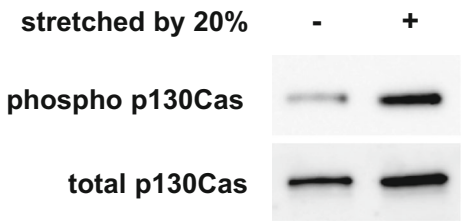

Fig. 1 Stretch-dependent tyrosine phosphorylation of p130Cas. NHDFs at 100,000 cells cultured on collagen (Type I)-coated stretchable silicone dishes (STREX, Japan) were biaxially extended by 20 % or left unstretched for 1 min. Western blot analysis using cell lysates shows stretch-dependent tyrosine phosphorylation of p130Cas in NHDFs

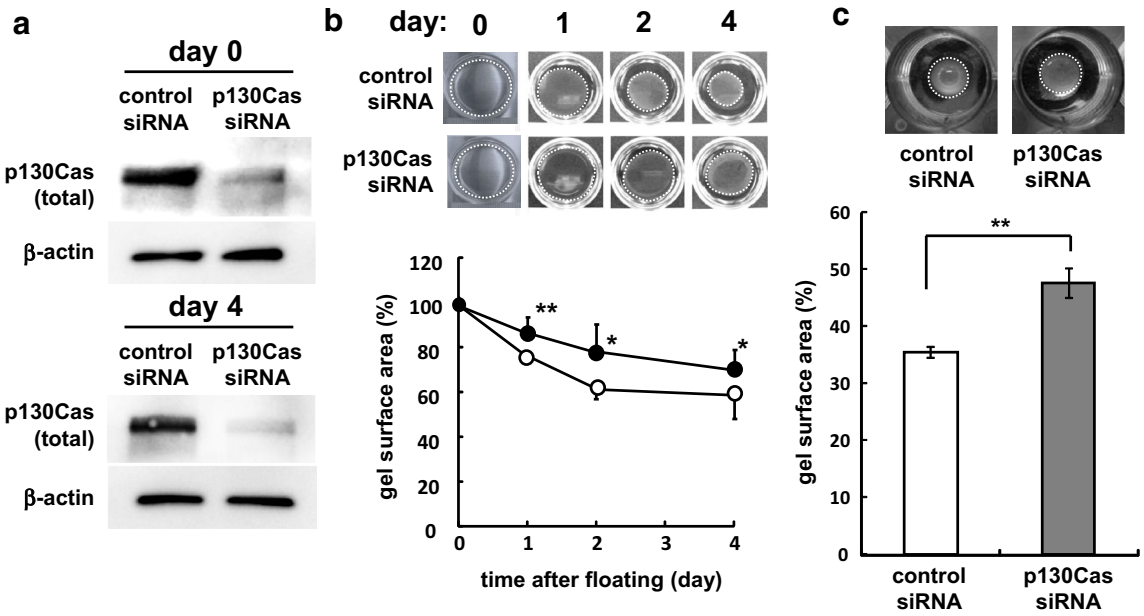

Fig. 2 Inhibitory effect of siRNA-driven p130Cas knockdown on collagen gel contraction. **a** Western blot analysis with NHDFs transfected with a siRNA specific for p130Cas (p130Cas siRNA) or with a scrambled non-specific siRNA (control siRNA) before and 4 days after gel contraction. **b** Inhibition of contraction of NHDF-embedded collagen gel by knockdown of p130Cas expression. The surface areas of the collagen gels were measured and the relative contraction ratios were assessed. Data are mean ± SD ($n = 3$ per group). **c** Significant inhibition of contraction of skin models of siRNA-treated NHEK- and NHDF-embedded collagen gels. Two days after release from the well walls, photographs of the skin models were taken, followed by measurement of their sizes. Data are mean ± SD ($n = 6$ per group). $^{*}p < 0.05$, $^{**}p < 0.01$

of collagen gel was significantly larger in cultures of p130Cas-knocked-down NHDFs compared with that of control siRNA-treated NHDFs at all indicated time points. Since the proliferation rate of NHDFs transfected with control siRNA was similar to that of cells transfected with p130Cas siRNAs (data not shown), these findings indicated that the delay in contraction was caused, at least in part, by knockdown of p130Cas expression in NHDFs. A similar phenomenon was also observed in NHEKs (Fig. 2c). Knockdown of p130Cas also significantly abrogated the time-dependent reduction in collagen gel area, suggesting that p130Cas plays an important role in the contraction ability of keratinocytes as well as that of fibroblasts.

Effects of p130Cas phosphorylation status on collagen gel contraction

To determine whether the dephosphorylation (inactivation) or phosphorylation (activation) of p130Cas mediates collagen gel contraction, NHDFs were cultured on collagen gel in the presence of PP2, an inhibitor of Src family kinase-mediated phosphorylation of p130Cas. Treatment with 5 and 25 μM PP2 prevented the adhesion-mediated p130Cas phosphorylation in a dose-dependent manner (Fig. 3a). Culture of NHDFs with PP2 also significantly inhibited collagen gel contraction in a dose-dependent manner, compared with vehicle (DMSO) treatment (Fig. 3b). The estimated 50 % inhibitory concentration (IC_{50}) of PP2 was 5 μM. In other experiments, we knocked down CSK, an inhibitor of Src family kinase, to activate phosphorylation of p130Cas. Following confirmation of enhanced adhesion-mediated p130Cas phosphorylation by CSK knockdown (Fig. 3c), increased phosphorylation of p130Cas resulted in acceleration of collagen gel contraction by about 20 %, compared with control siRNA-transfected NHDFs cultured with control collagen gel (Fig. 3d).

Morphological changes in human dermal fibroblasts associated with inactivation of p130Cas

To elucidate the mechanism(s) underlying the reduction of collagen gel contraction induced by dephosphorylation of p130Cas, we performed immunocytochemical analysis of NHDFs treated with vehicle or 25 μM PP2 for 24 h. Vehicle-treated NHDFs with intact p130Cas phosphorylation (Fig. 3a) appeared polygonal in shape and contained many actin fibers (Fig. 4a). In contrast, NHDFs with significantly inactivated p130Cas by PP2 (Fig. 3a) appeared filamentous and shrunken, containing only a few actin fibers and damaged actin fiber network (Fig. 4b).

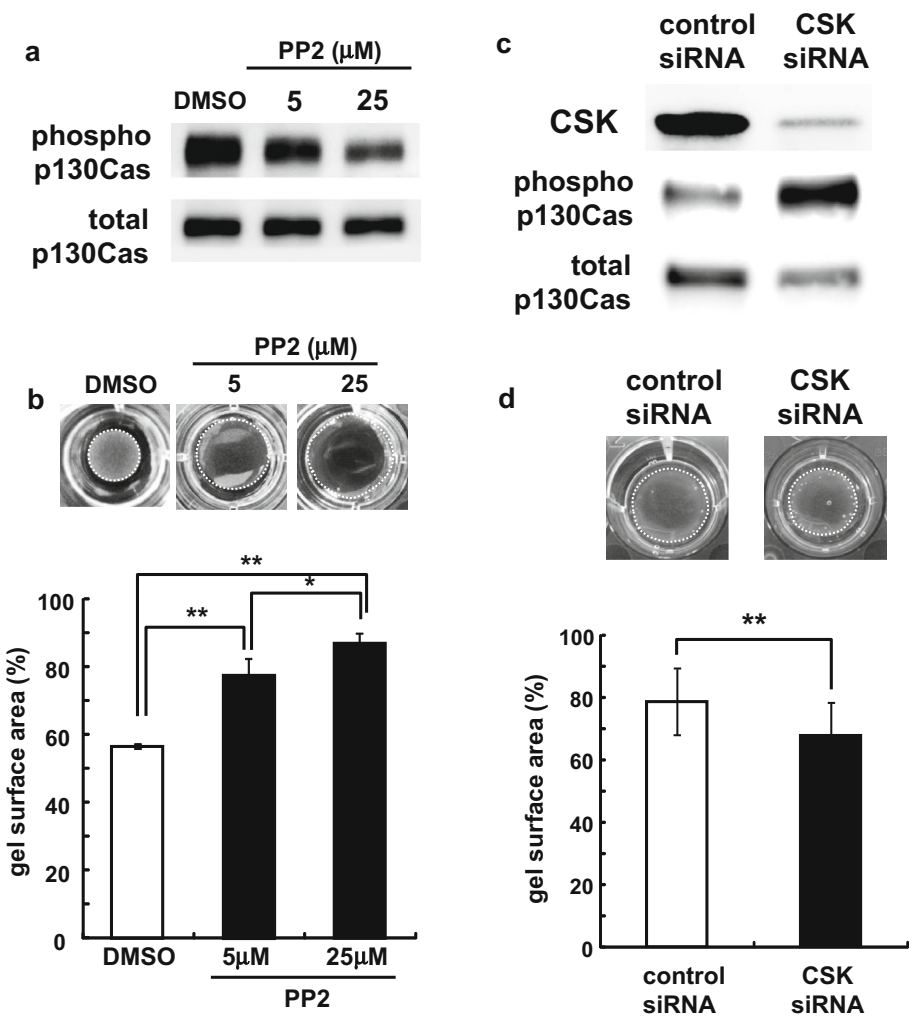

Fig. 3 Effect of depression or acceleration of p130Cas signaling on the contraction of collagen gels containing NHDFs. **a** and **c** Phosphorylation of p130Cas is suppressed or accelerated by the addition of PP2 or by treatment with siRNA specific for CSK, respectively. **b** Contraction of NHDFs-embedded collagen gels is significantly inhibited by PP2, an inhibitor of the Src signaling pathway, which stimulates p130Cas in a dose-dependent manner. **d** Collagen gel contraction is enhanced by a siRNA specific for a negative regulator of Src family kinases (CSK). Data are mean ± SD ($n = 3$ per group). $^{**}p < 0.01$. *Images* are representative of three independent samples

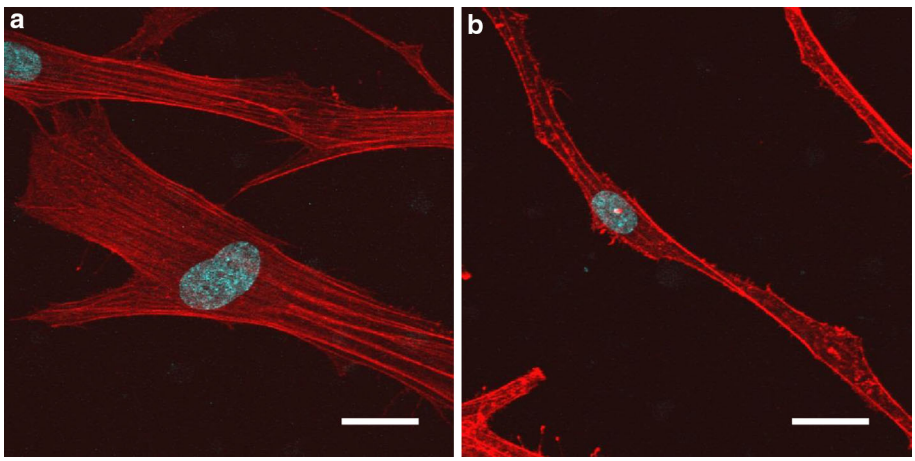

Fig. 4 Morphological changes caused by the addition of PP2. Fibroblasts were cultured on glass chamber slides with a collagen I coating followed by immunostaining to visualize actin filaments. Actin filament (*red*) and nuclei (*blue*) were stained after 24-h incubation in the presence of DMSO (**a**) or 5 μM PP2 (**b**). The cell image was captured by confocal fluorescence microscopy. *Scale bar* 50 μm. Fibroblasts treated with DMSO are spread and show alignment with actin filaments in the major vertex, whereas fibroblasts incubated with 5 μM PP2 for 24 h show cell shrinkage with diffused actin filaments at the periphery and cytoplasm of the cells (color figure online)

Disturbed recovery of p130Cas-inactivated mouse skin following stretching load

To verify the contribution of mechanotransduction via p130Cas signaling to the morphological adjustment in the skin, a mouse skin slack model was established. Mouse skin surplus was measured every week after the release of stretching load (i.e., chamber removal) (Fig. 5a, b). The slack of mouse skin injected with PBS (vehicle) gradually improved, whereas the surplus of mouse skin injected with PP2 gradually increased. The extension of mouse skin injected with PP2 for 3 weeks was significantly longer than that of control skin injected with PBS.

Dephosphorylation of p130Cas in aged mouse and human skin

Since fibroblasts in aged or photo-damaged skin [3, 21] show morphological changes similar to those described

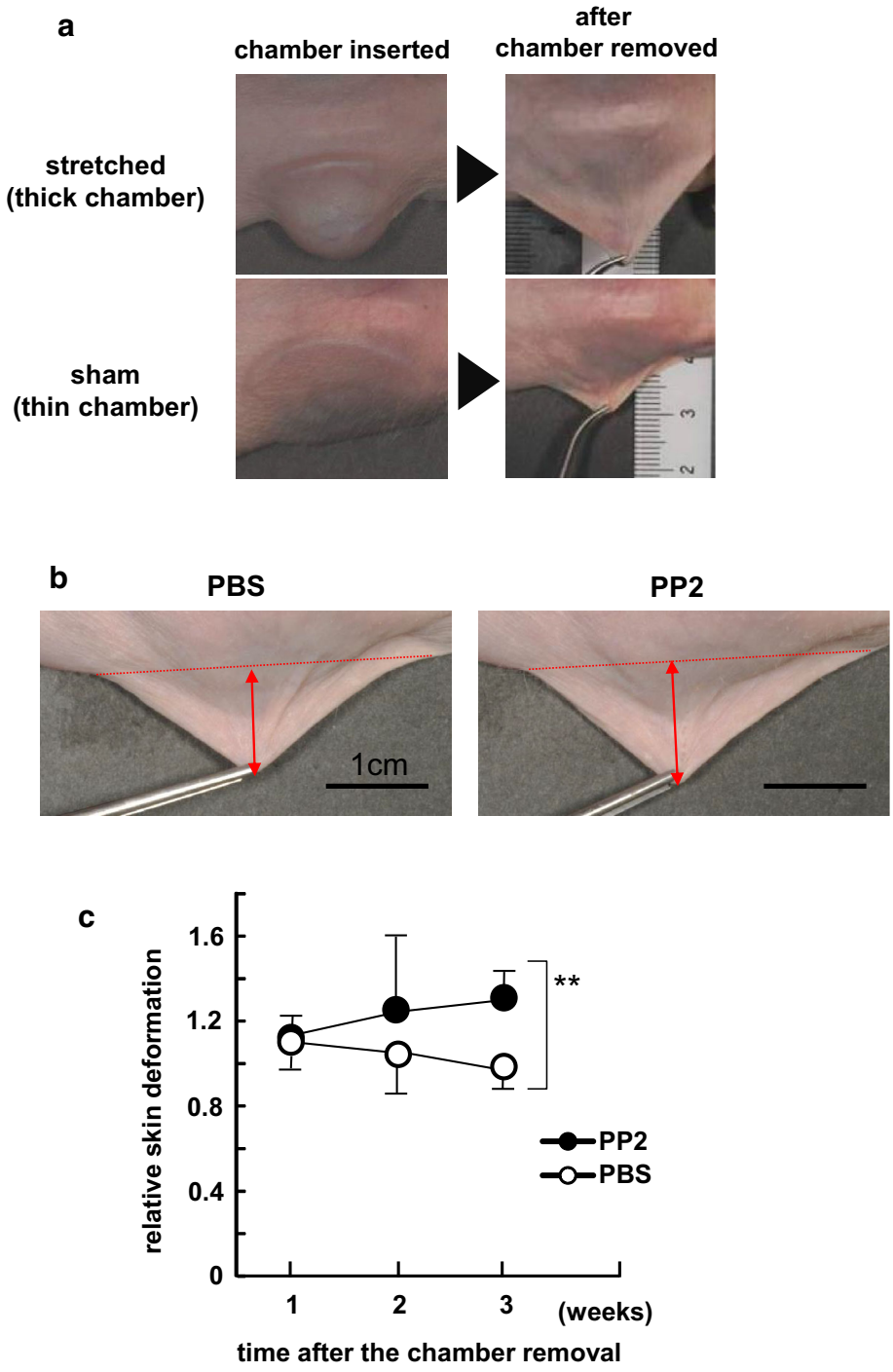

Fig. 5 Contribution of p130Cas to recovery after skin stretching. **a** The stretched skin by thick chamber insertion and the sham-treated skin by thin chamber insertion are shown. Skin recovery after stretching load was assessed immediately after removal of the chamber during the course of 3 weeks of treatment with PP2, an inhibitor of p130Cas. PP2 or PBS was administered 1 day before chamber removal. **b** The stretched skin 3 weeks after chamber removal followed by PBS injection (*left panel*) or PP2 injection (*right*). **c** Serial changes in relative skin deformation; the ratio of extent of skin extension under thick chamber insertion was expressed relative to the that under thin chamber insertion. Data are mean ± SD ($n = 3$–5) at 3 weeks after chamber removal. $*p < 0.05$

above and the decline in their ability to show gel contraction was similar to that of PP2-treated NHDFs (Fig. 3b), we examined the phosphorylation status of p130Cas in fibroblasts of aged mouse skin. Although the expression of total p130Cas in aged mouse skin was similar to that in younger mouse skin, significant dephosphorylation of p130Cas was noted in aged mouse skin (Fig. 6a, b). In addition, immunohistochemical staining of skin samples obtained from young and elderly Hispanic women showed similar numbers of total p130Cas-positive cells per high power field in both young and elderly human skins. However, the number of phosphorylated p130Cas-positive dermal cells per high power field in young human skin was greater than in elderly human skins (Fig. 6c). The immunohistochemical staining also showed that phospho-p130Cas positive keratinocytes had decreased in elderly epidermis. Stretch dependent phosphorylation of p130Cas was also observed in keratinocytes (data not shown). These two results suggest that keratinocytes might also

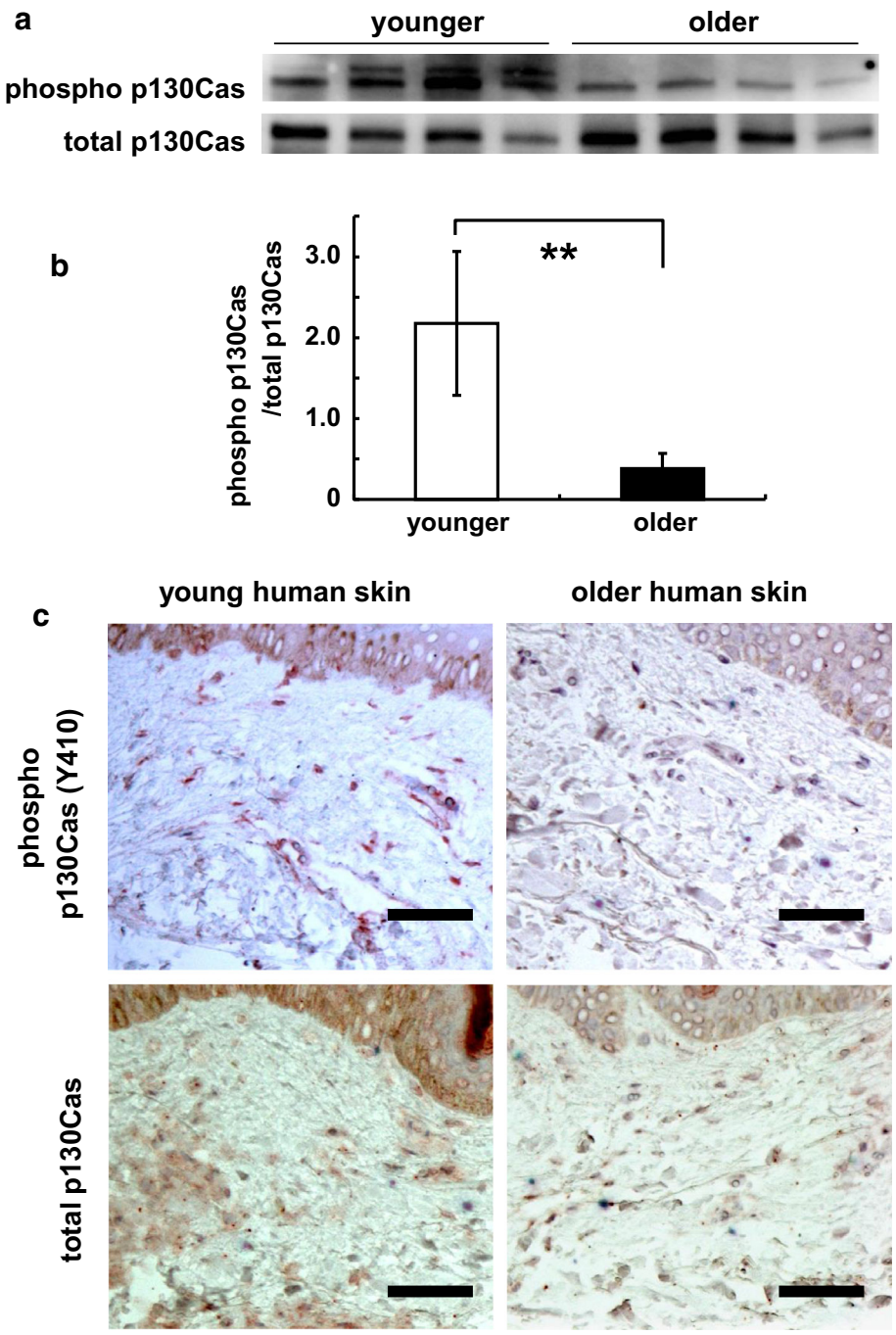

Fig. 6 Age-related suppression of p130Cas phosphorylation in the dermis. **a** Phosphorylation of p130Cas was analyzed in young and old mouse skin samples by western blot using anti-phospho-p130Cas (phospho Y165) and total p130Cas antibodies. **b** Mean ± SD expression level of phosphorylated p130Cas ($n = 4$ per group). $^{**}p < 0.01$. **c** Immunohistological staining using anti-human phospho-p130Cas (Y410) and total p130Cas antibodies using paraffin-embedded sections of skin specimens taken from the ventral upper arm of donors aged in their 30s (young) and 60s (older). *Scale bars* 50 μm

potentially be involved in the regulation of morphological skin changes.

Discussion

Since human dermal fibroblasts interact with the ECM via the integrin complex, we focused on one of the sensor proteins, p130Cas, which is located subjacent to the integrin complex following integrin-ECM interaction [22]. In certain cells, p130Cas acts as a primary force-sensor through the extension of its substrate domain, which primes it for phosphorylation [20, 23]. However, the pathological relevance of p130Cas inactivation (dephosphorylation), especially in skin in vivo, is poorly understood. We reported in the present study that inactivation of p130Cas signaling in fibroblasts was associated with a reduced number of actin fibers and extracellular collagen contraction. In addition, inactivation of p130Cas negatively modulated restoration of mouse skin from deformation. Furthermore, we also examined the dephosphorylated (inactivated) p130Cas in aged mouse and human skin samples. Based on our findings, we suggest that the age-related inactivation of p130Cas signaling plays, at least in part, a role in the reduced skin contraction power, probably through damage of the actin network, and that the reduced contraction of extracellular collagen seems to be the mechanism of the aging-dependent cutaneous slack (Fig. 7). In general, cell stretching activates (phosphorylates) p130Cas [22, 24], which in turn enhances intracellular signaling to augment the expression of extracellular proteins. Our results indicate, however, that manipulation of cellular p130Cas signaling can regulate extracellular contraction, resulting in cellular and/or tissue morphological coordination.

Two relevant questions need to be considered; the first is what type of intrinsic factors mediate the inactivation of p130Cas during aging? Since the extent of oxidative stress in skin varies according to age and exposure to ultraviolet [25–27], oxidative stress is probably a potential factor responsible for inhibition of p130Cas signaling. However, several studies reported that oxidative stress induced phosphorylation (activation) of p130Cas, although in almost all previous studies the cells were treated with high levels of oxidative stress for a short duration [28–30]. Further studies are needed to examine the effects of long-term and low-dose oxidative stress on p130Cas phosphorylation. With regard to oxidative stress, it has been reported that exposure of the skin to sun generates oxidative stress and that the anti-oxidative properties of the skin degrades with aging [26, 31]. Thus, p130Cas activity in photoaged skin is important for our understanding of the effects of oxidative stress on p130Cas function in vivo. The second question is why inactivation of p130Cas was associated with degradation of the actin network. Previous studies demonstrated the involvement of p130Cas phosphorylation in adhesion-induced actin organization [32]. Based on this finding, we hypothesized that dephosphorylated p130Cas cannot interact with actin, resulting in instability of the actin network and consequently its degradation. The aberration of cell attachment would appear to be a loss of mechanical tension leading to decreased collagen synthesis, and disorder of ECM turnover [3]. In our investigation, NHDF whose p130Cas was knocked down by siRNA showed dysregulation of collagen expression (data not shown). The reason that our mouse model showed long-term morphological change might include changes of ECM turnover resulting in lack of dermal fibroblast mechanical stimulation.

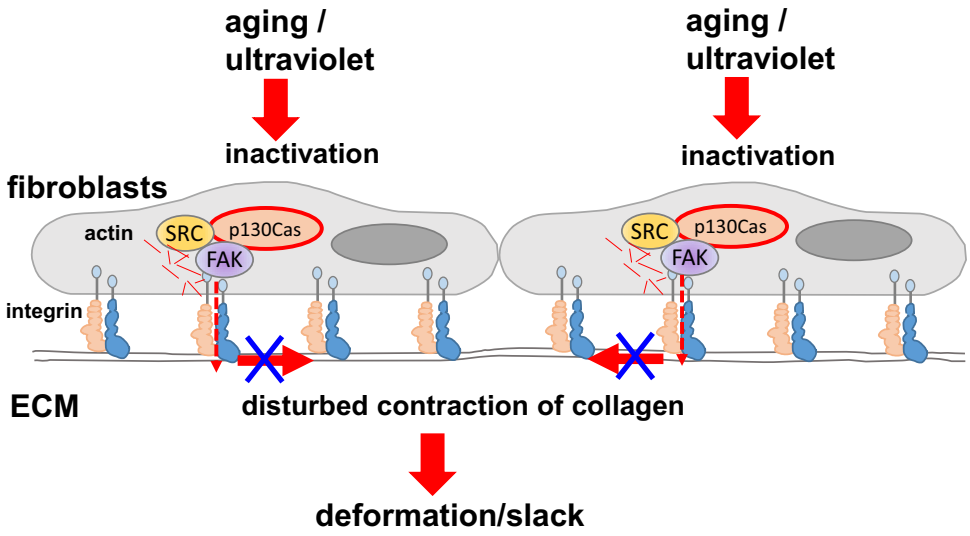

Fig. 7 Age-dependent change in p130Cas signaling. Age-related factors or signaling lead to p130Cas inactivation and induction of actin deformation, resulting in deterioration of collagen gel contraction and morphological regulation

In addition to the roles of components of focal adhesion complex in external network formation with collagens, p130Cas is also thought to play a role in the internal network establishment.

Although further investigations using a phosphodefective mutant of p130Cas are needed to prove the specific function of p130Cas activity, we showed that phosphorylation (activation) of p130Cas can potentially prevent the age-dependent deformation of the skin. This process could be a potential therapeutic target for aging-dependent slackness of the skin, and potential treatment of dermatochalasis and blepharoptosis. At present, anti-oxidative reagents and/or various nutrients are used as anti-aging formulas for the skin. Although oxidative stress can induce phosphorylation of p130Cas [29–31], the anti-aging effect of these reagents on the skin remains questionable. Activation of p130Cas phosphorylation is a promising novel concept in the prevention of skin aging and treatment of dermatochalasis and blepharoptosis.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

1. Eckes B, Krieg T (2004) Regulation of connective tissue homeostasis in the skin by mechanical forces. Clin Exp Rheumatol 22:73–76
2. Naylor EC, Watson RE, Sherratt MJ (2006) Molecular aspects of skin ageing. Maturitas 69:249–256
3. Varani J, Dame MK, Rittie L, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ (2006) Decreased collagen production in chronologically aged skin: role of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 168:1861–1868
4. Balzani A, Chilgar RM, Nicoli M, Sapountzis S, Lazzeri D, Cervelli V, Nicoli F (2013) Novel approach with fractional ultrapulse CO_2 laser for the treatment of upper eyelid dermatochalasis and periorbital rejuvenation. Lasers Med Sci 28:1483–1487
5. Gerstein AD, Phillips TJ, Rogers GS, Gilchrest BA (1993) Wound healing and aging. Dermatol Clin 11:749–757
6. Kono T, Tanii T, Furukawa M, Mizuno N, Kitajima J, Ishii M, Hamada T (1990) Correlation between ageing and collagen gel contractility of human fibroblasts. Acta Derm Venereol 70:241–244
7. Yamato M, Yamamoto K, Hayashi T (1993) Age-related changes in collagen gel contraction by cultured human lung fibroblasts resulting in cross-over contraction curves between young and aged cells. Mech Ageing Dev 67:149–158
8. Fujimura T, Hotta M, Kitahara T, Takema Y (2011) Loss of contraction force in dermal fibroblasts with aging due to decreases in myosin light chain phosphorylation enzymes. Arch Pharm Res 34:1015–1022
9. Stabellini G, Calastrini C, Marcucci A, De Mattei M, Luna-Carbonel MI, Pellati A, Mariani G, Franchella A (2000) Tissue expander: histological and histochemical study 6 months after transplant—our experience. J Long Term Eff Med Implants 10:279–290
10. Plenz G, Löffler A, Siegert R, Weerda H, Müller PK (1998) The effect of tissue expansion on the expression of collagen type I and type III mRNA in distinct areas of skin in the dog as an animal model. Eur Arch Otorhinolaryngol 255:473–477
11. Parsons M, Kessler E, Laurent GJ, Brown RA, Bishop JE (2001) Mechanical load enhances procollagen processing in dermal fibroblasts by regulating levels of procollagen C-proteinase. Exp Cell Res 252:319–331
12. Kessler D, Dethlefsen S, Haase I, Plomann M, Hirche F, Krieg T, Eckes B (2001) Fibroblasts in mechanically stressed collagen lattices assume a synthetic phenotype. J Biol Chem 276:36575–36585
13. Katsumi A, Orr AW, Tzima E, Schwartz MA (2004) Integrins in mechanotransduction. J Biol Chem 279:12001–12004
14. Liu M, Qin Y, Liu J, Tanswell AK, Post M (1996) Mechanical strain induces pp60src activation and translocation to cytoskeleton in fetal rat lung cells. J Biol Chem 271:7066–70671
15. Peter F (1995) Flow-mediated endothelial mechanotransduction. Davies Physiol Rev 75:519–560
16. Solomon AM, Bouloux PM (2006) Modifying muscle mass-the endocrine perspective. J Endocrinol 191:349–360
17. Hu BS, Landeen LK, Aroonsakool N, Giles WR (2007) An analysis of the effects of stretch on IGF-I secretion from rat ventricular fibroblasts. Am J Physiol Heart Circ Physiol 293:677–683
18. Bouton AH, Riggins RB, Bruce-Staskal PJ (2001) Functions of the adapter protein Cas: signal convergence and the determination of cellular responses. Oncogene 20:6448–6458
19. Defilippi P, Di Stefano P, Cabodi S (2006) p130Cas: a versatile scaffold in signaling networks. Trends Cell Biol 16:257–263
20. Sawada Y, Tamada M, Dubin-Thaler BJ, Cherniavskaya O, Sakai R, Tanaka S, Sheetz MP (2006) Force sensing by mechanical extension of the Src family kinase substrate p130Cas. Cell 127:1015–1026
21. Varani J, Schuger L, Dame MK, Leonard C, Fligiel SE, Kang S, Fisher GJ, Voorhees JJ (2004) Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. J Invest Dermatol 122:1471–1479
22. Vuori K, Hirai H, Aizawa S, Ruoslahti E (1996) Introduction of p130cas signaling complex formation upon integrin-mediated cell adhesion: a role for Src family kinases. Mol Cell Biol 16:2606–2613
23. Sawada Y, Sheetz MP (2002) Force transduction by Triton cytoskeletons. J Cell Biol 156:609–615
24. Petch LA, Bockholt SM, Bouton A, Parsons JT, Burridge K (1995) Adhesion-induced tyrosine phosphorylation of the p130 src substrate. J Cell Sci 108:1371–1379
25. Sander CS, Chang H, Salzmann S, Müller CS, Ekanayake-Mudiyanselage S, Elsner P, Thiele JJ (2002) Photoaging is associated with protein oxidation in human skin in vivo. J Invest Dermatol 118:618–625
26. Masaki H, Atsumi T, Sakurai H (1995) Detection of hydrogen peroxide and hydroxyl radicals in murine skin fibroblasts under UVB irradiation. Biochem Biophys Res Commun 206:474–479
27. Jurkiewicz BA, Buettner GR (1996) EPR detection of free radicals in UV-irradiated skin: mouse versus human. Photochem Photobiol 64:918–922
28. Chan HL, Chou HC, Duran M, Gruenewald J, Waterfield MD, Ridley A, Timms JF (2010) Major role of epidermal growth factor receptor and Src kinases in promoting oxidative stress-dependent loss of adhesion and apoptosis in epithelial cells. J Biol Chem 285:4307–4318
29. Basuroy S, Dunagan M, Sheth P, Seth A, Rao RK (2010) Hydrogen peroxide activates focal adhesion kinase and c-Src by a

phosphatidylinositol 3 kinase-dependent mechanism and promotes cell migration in Caco-2 cell monolayers. Am J Physiol Gastrointest Liver Physiol 299:186–195
30. Gozin A, Franzini E, Andrieu V, Da Costa L, Rollet-Labelle E, Pasquier C (1998) Reactive oxygen species activate focal adhesion kinase, paxillin and p130cas tyrosine phosphorylation in endothelial cells. Free Radic Biol Med 25:1021–1032
31. Masaki H (2010) Role of antioxidants in the skin: anti-aging effects. J Dermatol Sci 58:85–90
32. Nakamura I, Jimi E, Duong LT, Sasaki T, Takahashi N, Rodan GA, Suda T (1998) Tyrosine phosphorylation of p130Cas is involved in actin organization in osteoclasts. J Biol Chem 273:11144–11149