Biochemical and Biophysical Research Communications 441 (2013) 988–993

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Disease-associated single amino acid mutation in the calf-1 domain of integrin α3 leads to defects in its processing and cell surface expression

Masashi Yamada, Kiyotoshi Sekiguchi *

Laboratory of Extracellular Matrix Biochemistry, Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan

ARTICLE INFO

Article history:
Received 17 October 2013
Available online 9 November 2013

Keywords:
Glycosylation
Posttranslational modification
Basement membrane
Laminin
Tetraspanin
CD151

ABSTRACT

Integrin α3β1, a receptor for laminins, is involved in the structural and functional organization of epithelial organs, including the lung, kidney, and skin. Recently, a missense mutation that causes substitution of Arg628 with Pro (R628P) in the calf-1 domain of human α3 was shown to be associated with disorders of the lung, kidney, and skin. Here, we found that the R628P mutation leads to aberrations in the posttranslational processing of α3. Specifically, α3 with the R628P mutation showed hardly any cleavage at the calf-2 domain, which usually occurs in the Golgi apparatus during the delivery of de novo-synthesized α3. The mutant α3 retained the ability to associate with integrin β1, but not with the tetraspanin CD151, and the bound β1 was a partially glycosylated immature form, the maturation of which also takes place in the Golgi apparatus. Furthermore, the cell surface expression of the mutant protein was markedly reduced. These results suggest that the R628P mutation leads to a deficit in the transport of α3β1 from the ER to the Golgi apparatus. When Arg628 was mutated to Gln or Glu, instead of Pro, the resulting mutants did not display aberrations in processing or CD151 binding, indicating that the presence of Pro, rather than the absence of Arg, at amino acid residue 628 of α3 is important for the abnormalities in the R628P mutant. In support of this notion, a homology modeling analysis of the calf-1 domain of α3 showed that replacement with Pro, but not with Gln or Glu, caused partial disruption of the β-sheet structures. Furthermore, the ER-associated degradation of the R628P mutant was not enhanced compared with that of the wild-type protein, suggesting that the deficits in the posttranslational processing and cell surface expression of the R628P mutant are independent of the ER-associated degradation, but arise from the defect in its export from the ER. We conclude that the calf-1 domain is required for the transport of α3 from the ER to the Golgi apparatus to maintain the integrity of epithelial tissues, and hence the impairment of the calf-1 domain by the R628P mutation leads to severe diseases of the kidneys, lungs, and skin.

1. Introduction

Integrin α3β1 is a receptor for laminin-511, -521, and -332, the major components of epithelial basement membranes [1]. Integrin α3 is a type I transmembrane protein with a large extracellular region, a single transmembrane helix domain, and a short unstructured cytoplasmic tail [2]. The extracellular region is composed of a seven-bladed β-propeller, a thigh, and two calf domains with flexible linkers, and is posttranslationally cleaved at the calf-2 domain to produce heavy (~115 kDa) and light (~35 kDa) chains that are linked by a disulfide bond [3]. The α3 subunit is expressed in many epithelial organs, including the kidney, lung, and skin, during development, and α3 knockout mice show severe defects in the lungs and kidneys, and aberrations in the epidermis [4,5].

Several mutations of the human *ITGA3* gene have recently been reported to lead to abnormalities of the lungs and kidneys, consistent with the phenotypes of α3 knockout mice [6,7]. Has et al. [6] presented three kinds of mutations: a deletion mutation in exon 8; a point mutation in intron 11 that abolishes the splice acceptor site of exon 12, leading to exon 12 skipping; and a single base substitution in exon 14 that results in a missense mutation, in which Arg628 is changed to Pro. All three mutations produce similar clinical features, such as congenital nephritic syndrome, interstitial

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; ER, endoplasmic reticulum; mAb, monoclonal antibody; pAb, polyclonal antibody; PBS, phosphate-buffered saline.

* Corresponding author. Address: Laboratory of Extracellular Matrix Biochemistry, Division of Protein Chemistry, Institute for Protein Research, Osaka University, Suita, Osaka 565-0871, Japan. Fax: +81 6 6879 8619.

E-mail address: sekiguch@protein.osaka-u.ac.jp (K. Sekiguchi).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.11.003

lung disease, and epidermolysis bullosa. Conversely, it remains unclear how the missense mutation in exon 14 causes the disease-associated dysfunction of α3, although the other two mutations induce premature termination. Nicolaou et al. [7] reported that substitution of Ala349 with Ser in α3 results in a gain of glycosylation, causing kidney and lung abnormalities that largely resemble those caused by the above-mentioned three mutations. This mutation not only abolishes the association of α3 with integrin β1, but also causes disorders in its posttranslational processing and cell surface expression, indicating that the biosynthetic processing of α3 from the endoplasmic reticulum (ER) to the plasma membrane is important for the exertion of its biological functions.

In the present study, we found that the missense mutation (replacement of Arg628 with Pro, R628P) in exon 14 of α3 led to a defect in its posttranslational processing, failure of its association with the mature form of integrin β1, absence of its CD151 binding, and a reduction in its cell surface expression. Our results raise the possibility that the R628P mutation induces conformational perturbations of the calf-1 domain of α3 that impair the transport of α3 from the ER to the Golgi apparatus, but do not affect its ER-associated ubiquitin-dependent degradation.

2. Materials and methods

2.1. Cell culture, antibodies and reagents

A549 human lung adenocarcinoma cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Sigma, St. Louis, MO, USA) supplemented with heat-inactivated 10% (v/v) fetal bovine serum (JRH Biosciences, Lenexa, KS, USA) as described previously [8].

A mouse monoclonal antibody (mAb) against human CD151 (8C3) was produced as described previously [9]. A polyclonal antibody (pAb) against the cytoplasmic tail of human integrin α3A was generated as described previously [10]. An anti-integrin α3 goat pAb and anti-ubiquitin mouse mAb (P4D1) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-integrin β1 and anti-calnexin mouse mAbs were from BD Transduction Laboratories (Lexington, KY, USA). Anti-actin rabbit pAb, anti-FLAG mouse mAb (M2), and anti-FLAG mAb (M2)-conjugated agarose were from Sigma. Anti-calnexin rabbit pAb was from Stressgen (Ann Arbor, MI, USA). Peroxidase-conjugated AffiniPure anti-mouse IgG, anti-rabbit IgG, and anti-goat IgG were from Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Alexa 488-conjugated anti-mouse IgG and Alexa 546-labeled anti-rabbit IgG were from Molecular Probes (Eugene, OR, USA). Lactacystin was obtained from Peptide Institute Inc. (Osaka, Japan).

2.2. Transfection of DNA and siRNA

cDNAs encoding full-length human integrin α3A with a C-terminal FLAG tag and full-length human CD151 were inserted into the pCAGIPuro vector (a kind gift from Dr. Hitoshi Niwa, RIKEN CDB). A cDNA for a siRNA-resistant mutant of CD151 was generated as described previously [11]. The point mutations in α3 were introduced using a QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA). The expression vectors were introduced into the cells using Lipofectamine LTX (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. In transient expression experiments, the cells were subjected to assays at 24–26 h after transfection. Stable transformants expressing wild-type CD151 and its siRNA-resistant mutant were obtained by selection with 2 μg/ml puromycin after transfection.

For RNA interference against CD151, A549 cells were transfected with 50 nM siRNA using Lipofectamine RNAiMAX (Invitrogen) in accordance with the manufacturer's instructions as described previously [11]. At 3 days after transfection, the cells were analyzed by immunoblotting as described below.

2.3. Immunoprecipitation and immunoblotting

Cells were washed with ice-cold phosphate-buffered saline (PBS), and lysed in a lysis buffer containing 1% (w/v) Triton X-100, 5% (v/v) glycerol, 150 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl pH 7.5, and 1 mM phenylmethylsulfonyl fluoride. The lysates were then processed as described previously [11]. For immunoprecipitation, anti-FLAG mAb-conjugated beads (Sigma) were added to the lysates and incubated at 4 °C for 1–3 h. The resulting immune complexes were washed with lysis buffer. The precipitated and lysate proteins were subjected to immunoblotting as described previously [11].

2.4. Cell surface labeling

Cells were washed twice with PBS and surface-labeled with 2 mg/ml sulfo-NHS-LC-biotin (Pierce, Rockford, IL, USA) in PBS at room temperature for 10 min. After washing with cold DMEM and PBS, the cells were lysed in a buffer containing 1% (w/v) NP-40, 0.1% (w/v) SDS, 0.5% (w/v) sodium deoxycholate, 5% (v/v) glycerol, 150 mM NaCl, 1 mM EDTA, 20 mM Tris–HCl pH 7.5, and 1 mM phenylmethylsulfonyl fluoride. Immunoprecipitation with anti-FLAG mAb-conjugated agarose was performed as described above. The immunoprecipitates were blotted with peroxidase-conjugated streptavidin (Pierce) as described previously [10].

2.5. Immunofluorescence staining

Cells were plated on glass coverslips coated with 10 nM laminin-511, which was purified from conditioned medium of human choriocarcinoma JAR cells as described previously [11]. At 12 h after plating, the cells were transfected with expression vectors for wild-type α3 and its R628P mutant as described above. At 24 h after transfection, the cells were immunostained with anti-FLAG mouse mAb and anti-calnexin rabbit pAb combined with Alexa 488-conjugated anti-mouse IgG and Alexa 546-labeled anti-rabbit IgG secondary antibodies, as described previously [8].

2.6. Homology modeling

Modeling of the calf-1 domain of human integrin α3 was carried out based on the crystal structure of human integrin αV (PDB ID: 3IJE chain A). The amino acid sequences were aligned with those of human integrin α3 (NCBI Reference Sequence: NP_002195) using ClustalW (DNA Data Bank of Japan). A three-dimensional model was generated with SWISS-MODEL. Energy minimization and single amino acid substitutions were then performed using Swiss-PDB Viewer, followed by analysis with UCSF Chimera.

3. Results and discussion

To examine the effects of Arg628 substitution with Pro (R628P) on the expression of integrin α3, wild-type α3 or its R628P mutant tagged with a C-terminal FLAG sequence (α3-FLAG) was transiently expressed in human lung adenocarcinoma A549 cells, followed by immunoprecipitation with an anti-FLAG mAb. Immunoblotting analysis of the precipitates with an anti-FLAG mAb showed that a full-length immature form was detected for both wild-type α3 and its R628P mutant, while the light chain containing the C-terminal FLAG tag was detected for wild-type α3, but not for the R628P mutant (Fig. 1A). Consistent with these results, immunoblotting

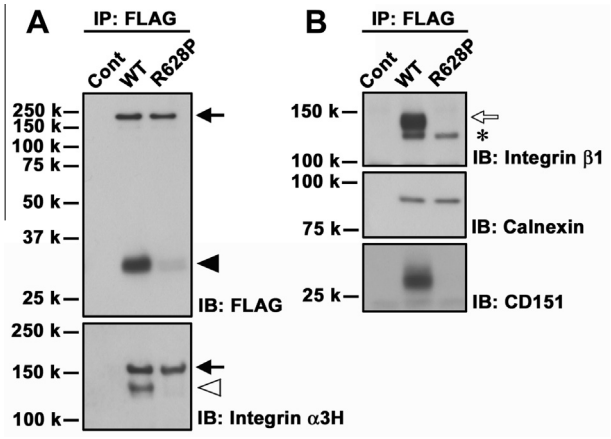

Fig. 1. Effects of the R628P mutation on the posttranslational processing of α3β1 and its binding to CD151. (A and B) A549 cells were transfected with an empty vector (Cont) or expression vector for wild-type (WT) α3-FLAG or its R628P mutant. Immunoprecipitates with an anti-FLAG mAb were subjected to immunoblotting with antibodies against the proteins indicated on the right. Molecular weights are shown on the left. (A) The anti-α3 pAb used recognizes the heavy chain of α3 (integrin α3H). The closed arrows indicate the immature form of α3, the closed arrowhead indicates its light chain, and the open arrowhead indicates its heavy chain. (B)The open arrow indicates the mature form of β1 and the asterisk indicates its immature form.

with an anti-integrin α3 pAb recognizing the N-terminal region resulted in very little detection of the heavy chain in the R628P mutant. These results indicate that mutation of Arg628 to Pro leads to a defect in posttranslational cleavage between the heavy and light chains. Next, we conducted a coimmunoprecipitation assay of integrin β1 with α3-FLAG to examine whether the R628P mutant remained associated with β1 (Fig. 1B). The R628P mutant remained capable of binding to β1, though the mutant failed to coprecipitate with the mature form of β1 but another form that migrated slightly below the mature form was detected on the immunoblot. This faster migrating band has been shown to represent an immature form of β1 arising from incomplete glycosylation [7]. These results indicate that the R628P mutant only stays associated with the partially glycosylated immature form of β1. A heterodimer of α and β subunits is formed in the ER, followed by maturation of β1 with glycosylation in the Golgi apparatus [7]. Since the proteolytic processing of the heavy and light chains of α3 also takes place in the Golgi apparatus [3], it seems likely that the R628P mutant is not completely processed posttranslationally because it is not transported from the ER to the Golgi.

Integrin α3 is known to be strongly associated with the tetraspanin CD151, and this association arises in the ER [7,12]. The association with CD151 is involved in the regulation of cell adhesion and migration mediated by integrin α3β1 [13,14]. As shown in Fig. 1B, the coimmunoprecipitation experiments showed that the R628P mutant was incapable of binding to CD151. This result is in agreement with a report showing that CD151 binding requires the extracellular region comprising amino acids 570–705 of α3 [15]. However, calnexin, a chaperone protein in the ER, was detected in immunoprecipitates from cells expressing wild-type α3 and the R628P mutant to comparable extents (Fig. 1B).

To examine whether the defect in posttranslational processing of the R628P mutant arose from its failure to bind to CD151, we evaluated the possible participation of CD151 in the regulation of integrin α3 processing by cotransfection of α3-FLAG and CD151. The increased expression of CD151 did not alter the amounts of the light chain of wild-type α3, although it augmented the levels of CD151 associated with wild-type α3 (Fig. 2A). In the cells expressing the R628P mutant, CD151 transfection did not influence either the proteolytic processing of the mutant or the binding to CD151, as evidenced by the failure to detect the light chain of α3 or CD151 in the immunoprecipitates. These results indicate that CD151 does not have the ability to promote the posttranslational processing of α3. Next, we examined the effects of siRNA-induced CD151 knockdown on the posttranslational processing of α3. To exclude possible off-target effects of

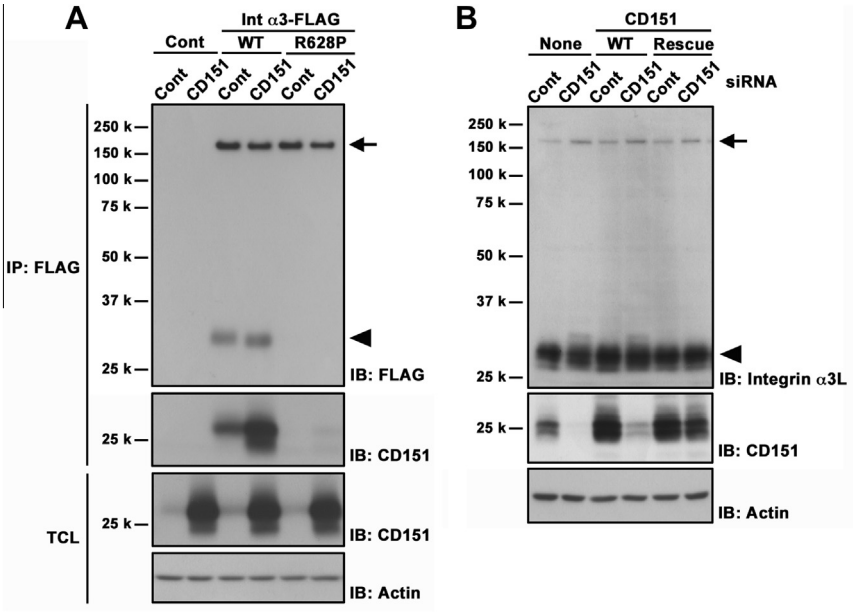

Fig. 2. Effect of CD151 expression on the posttranslational processing of α3. (A) A549 cells were transfected with an empty vector (Cont) or expression vector for wild-type (WT) α3-FLAG or its R628P mutant together with a control (Cont) or CD151 expression vector. Immunoprecipitates with an anti-FLAG mAb (IP: FLAG) and cell lysates (TCL) were blotted with antibodies against the proteins indicated on the right. (B) Control A549 cells (None) or cells stably expressing wild-type (WT) CD151 or its siRNA-resistant mutant (Rescue) were transfected with a control (Cont) or CD151 siRNA. Cell lysates were immunoblotted with antibodies against the proteins indicated on the right. The anti-α3 pAb used recognizes the light chain of α3 (integrin α3L). (A and B) Molecular weights are shown on the left. The closed arrows indicate the immature form of α3 and the closed arrowheads indicate its light chain.

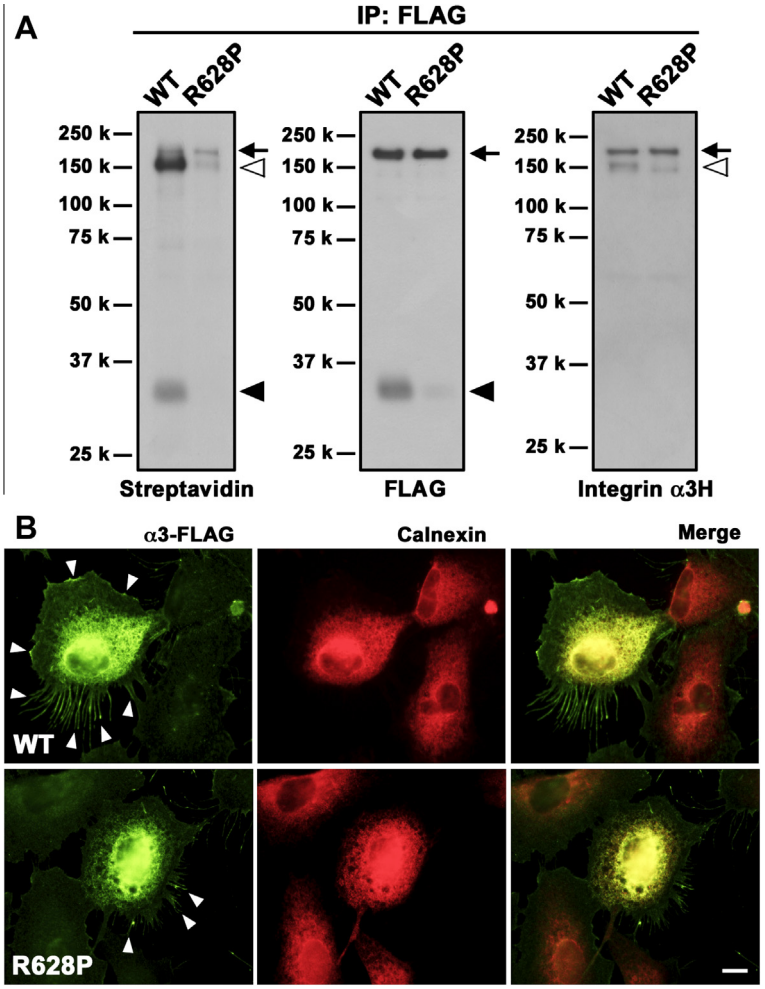

Fig. 3. Loss of cell surface expression of the R628P mutant of α3. (A) A549 cells were transfected with an expression vector for wild-type (WT) α3-FLAG or its R628P mutant, followed by cell surface labeling with biotin. Immunoprecipitates with an anti-FLAG mAb were blotted with peroxidase-conjugated streptavidin, anti-FLAG mAb, and anti-α3 pAb. The anti-α3 pAb used recognizes the heavy chain of α3 (integrin α3H). Molecular weights are shown on the left. The closed arrows indicate the immature form of α3, the closed arrowheads indicate its light chain, and the open arrowheads indicate its heavy chain. (B) A549 cells adhering to laminin-511 were transfected with an expression vector for wild-type (WT) α3-FLAG or its R628P mutant, and then immunostained with anti-FLAG mAb and anti-calnexin pAb. The arrowheads indicate cell peripheries and protrusions positive for α3-FLAG. The bar represents 10 μm.

the CD151 siRNA, we also performed rescue experiments in cells stably expressing siRNA-resistant CD151, in which the sequence targeted by the siRNA was silently mutated. As shown in Fig. 2B, CD151 knockdown did not affect the production of the light chain of α3. The immature form of α3 seemed to be slightly increased by CD151 knockdown, but this increase was not abolished by the expression of the siRNA-resistant CD151, implying that it was caused by an off-target effect of the siRNA. Taken together, these results suggest that the inability of the R628P mutant to bind to CD151 is not responsible for the defects in its posttranslational processing.

Next, we examined whether the R628P mutation has an impact on the cell surface expression of α3. Cells transfected with wild-type α3-FLAG or its R628P mutant were surface-labeled with biotin, followed by immunoprecipitation with an anti-FLAG mAb and blotting with streptavidin. Biotinylated heavy and light chains of α3 were scarcely detected in the R628P mutant, compared with wild-type α3 (Fig. 3A). Only a small amount of biotinylated full-length immature α3 was detected on the blot, but at similar levels for wild-type α3 and the R628P mutant. In agreement with these results, the amounts of the heavy and light chains were both greatly reduced in the R628P mutant in the immunoblot analysis with anti-FLAG mAb and anti-integrin α3 pAb, while the immature form was strongly detected in both wild-type α3 and the R628P mutant to similar extents. These results indicate that the cell surface expression of the R628P mutant is greatly decreased, consistent with its defect in posttranslational processing. In addition, it appears that the α3 detected on the cell surface is almost exclusively a cleaved mature form composed of the heavy and light chains.

We also performed immunocytochemical analyses with anti-FLAG mAb and anti-calnexin pAb in cells transiently transfected with wild-type α3-FLAG or its R628P mutant. The signals of wild-type α3 were detected not only in the calnexin-positive ER, but also at the cell peripheries and protrusions, whereas those of the R628P mutant were almost exclusively detected in the ER (Fig. 3B). These results corroborate our conclusion that the R628P mutant is not expressed on the cell surface and is mostly retained in the ER. Consistent with these results, Glanzmann's thrombasthenia, a bleeding disorder caused by platelet dysfunction, has been shown to arise from several mutations in the calf-1 domain of integrin αIIb, and these mutations also impair the transport from the ER, posttranslational processing, and cell surface expression of αIIbβ3 [16,17]. These lines of evidence suggest that similar mechanisms underlie the aberrations induced by the mutations in the calf-1 domains of α3 and αIIb.

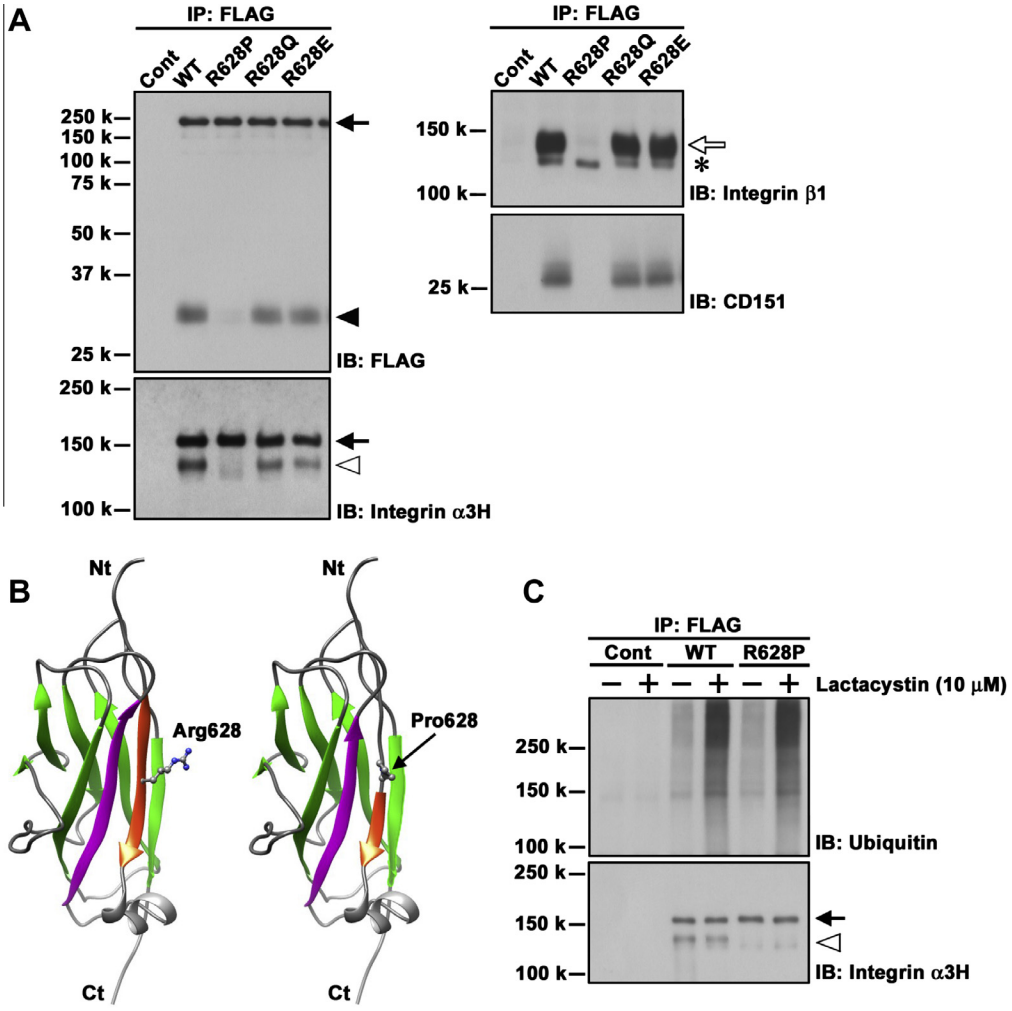

Fig. 4. Pro-dependent defects in the posttranslational processing and protein conformation of α3 after substitution of Arg628. (A) A549 cells were transfected with an empty vector (Cont) or expression vector for wild-type (WT) α3-FLAG or its R628P, R628Q, and R628E mutants. Immunoprecipitates with an anti-FLAG mAb were blotted with antibodies against the proteins indicated on the right. (B) Homology models of the calf-1 domain (amino acids 618–762) of wild-type human α3 (left) and its R628P mutant (right) were generated based on the crystal structure of αV. Note that the first (orange) and second (magenta) β-sheets are partially disrupted. Arg628 is located in the first β-sheet. (C) A549 cells were transfected with an empty vector (Cont) or expression vector for wild-type (WT) α3-FLAG or its R628P mutant. The cells were treated with a proteasome inhibitor, lactacystin, for 6 h before being lysed. Immunoprecipitates with an anti-FLAG mAb were subjected to immunoblotting with antibodies against the proteins indicated on the right. (A and C) The anti-α3 pAb used recognizes the heavy chain of α3 (integrin α3H). Molecular weights are shown on the left. The closed arrows indicate the immature form of α3, the closed arrowhead indicates its light chain, and the open arrowhead indicates its heavy chain. The open arrow indicates the mature form of β1, and the asterisk indicates its immature form. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Pro is a unique amino acid in that its side chain is cyclized back to the backbone amide position, yielding an imido group instead of an amino group. Pro residues are therefore suggested to affect protein secondary structures such as α-helices and β-sheets. However, this is not always the case, because it has been reported that 16% and 11% of the Pro residues in the dataset examined are located in α-helices and β-sheets, respectively [18]. To evaluate the effects of the Pro substitution on the posttranslational processing of integrin α3β1, we employed other mutations in α3, namely replacement of Arg628 by Gln or Glu, producing R628Q and R628E, respectively. As shown in Fig. 4A, the light and heavy chains of the R628Q and R628E mutants were detected to similar extents to those of wild-type α3, whereas those of the R628P mutant were hardly detectable. In addition, the R628Q and R628E mutants, and wild-type α3 were associated with not only the immature form but also the mature form of integrin β1, while the R628P mutant was bound solely to the immature form. Furthermore, CD151 was found to associate with the R628Q and R628E mutants, and wild-type α3. These results indicate that the aberrant posttranslational processing is closely associated with the substitution of Arg628 with Pro, but not with other amino acid residues, thereby raising the possibility that the Pro substitution affects the protein conformation of α3.

To examine this possibility, we performed a homology modeling analysis of the calf-1 domain of α3, in which Arg628 is located, using the data for the crystal structure of integrin αVβ3. As shown in Fig. 4B, replacement of Arg628 with Pro led to partial disruptions of the first and second β-sheet structures. However, substitution with Gln or Glu did not affect these structures (data not shown). These results suggest that the mutation of Arg628 to Pro perturbs the structure of the calf-1 domain, leading to retention of the mutant protein in the ER, and subsequent defects in the posttranslational events and cell surface expression.

Misfolded proteins are known to be removed from the ER by an ER-associated degradation system involving ubiquitin and proteasomes [19]. To examine whether the R628P mutant accelerates the ER-associated degradation of α3, cells transfected with wild-type α3-FLAG or its R628P mutant were treated with a proteasome inhibitor, lactacystin, followed by immunoprecipitation with an anti-FLAG mAb and immunoblotting with an anti-ubiquitin mAb.

As shown in Fig. 4C, the treatment with lactacystin augmented the ubiquitination levels of both the wild-type protein and R628P mutant, and, interestingly, the levels were almost the same. These results indicate that the deficits in the posttranslational processing and cell surface expression of the R628P mutant do not simply result from its increased degradation through the structural aberration. It appears likely that the Pro substitution at Arg628 does not largely perturb the structure of α3, allowing the R628P mutant to avoid the ER-associated degradation. The relatively small structural aberration in the R628P mutant is also supported by our observation that the mutant remains capable of binding to β1. Proteins synthesized de novo in the ER have two possible fates: export to the Golgi apparatus for further maturation and cell surface expression or exclusion by the ER-associated degradation system for protein quality control. The Pro substitution does not appear to affect the commitment of α3 to the ER-associated degradation, but does impair the exit of α3 from the ER.

We conclude that the Pro substitution at Arg628 in the calf-1 domain of α3 perturbs its transport from the ER to the Golgi apparatus, resulting in severe dysfunctions of epithelial cells in the kidneys, lungs, and skin, which in turn lead to congenital nephritic syndrome, interstitial lung disease, and epidermolysis bullosa. Our results provide clear evidence that the calf-1 domain is critically involved in the transport of α3 from the ER.

Acknowledgments

We thank Dr. Hitoshi Niwa (RIKEN CDB) for the generous gift of the pCAGIPuro expression vector. We thank Drs. Yu Takano, Yuya Sato, and Yukimasa Taniguchi (Osaka University) for advice on the homology modeling. This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to M.Y. and K.S.) and for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to K.S.).

References

[1] R. Nishiuchi, J. Takagi, M. Hayashi, H. Ido, Y. Yagi, N. Sanzen, T. Tsuji, M. Yamada, K. Sekiguchi, Ligand-binding specificities of laminin-binding integrins: a comprehensive survey of laminin–integrin interactions using recombinant α3β1, α6β1, α7β1 and α6β4 integrins, Matrix Biol. 25 (2006) 189–197.
[2] I.D. Campbell, M.J. Humphries, Integrin structure, activation, and interactions, Cold Spring Harb. Perspect. Biol. 3 (2011).
[3] J.C. Lissitzky, J. Luis, J.S. Munzer, S. Benjannet, F. Parat, M. Chretien, J. Marvaldi, N.G. Seidah, Endoproteolytic processing of integrin pro-α subunits involves the redundant function of furin and proprotein convertase (PC) 5A, but not paired basic amino acid converting enzyme (PACE) 4, PC5B or PC7, Biochem. J. 346 (2000) 133–138.
[4] J.A. Kreidberg, M.J. Donovan, S.L. Goldstein, H. Rennke, K. Shepherd, R.C. Jones, R. Jaenisch, A3β1 integrin has a crucial role in kidney and lung organogenesis, Development 122 (1996) 3537–3547.
[5] C.M. DiPersio, K.M. Hodivala-Dilke, R. Jaenisch, J.A. Kreidberg, R.O. Hynes, A3β1 Integrin is required for normal development of the epidermal basement membrane, J. Cell Biol. 137 (1997) 729–742.
[6] C. Has, G. Sparta, D. Kiritsi, L. Weibel, A. Moeller, V. Vega-Warner, A. Waters, Y. He, Y. Anikster, P. Esser, B.K. Straub, I. Hausser, D. Bockenhauer, B. Dekel, F. Hildebrandt, L. Bruckner-Tuderman, G.F. Laube, Integrin α3 mutations with kidney, lung, and skin disease, N. Engl. J. Med. 366 (2012) 1508–1514.
[7] N. Nicolaou, C. Margadant, S.H. Kevelam, M.R. Lilien, M.J. Oosterveld, M. Kreft, A.M. van Eerde, R. Pfundt, P.A. Terhal, B. van der Zwaag, P.G. Nikkels, N. Sachs, R. Goldschmeding, N.V. Knoers, K.Y. Renkema, A. Sonnenberg, Gain of glycosylation in integrin α3 causes lung disease and nephrotic syndrome, J. Clin. Invest. 122 (2012) 4375–4387.
[8] M. Yamada, G. Mugnai, S. Serada, Y. Yagi, T. Naka, K. Sekiguchi, Substrate-attached materials are enriched with tetraspanins and are analogous to the structures associated with rear-end retraction in migrating cells, Cell Adhes. Migr. 7 (2013) 304–314.
[9] R. Nishiuchi, N. Sanzen, S. Nada, Y. Sumida, Y. Wada, M. Okada, J. Takagi, H. Hasegawa, K. Sekiguchi, Potentiation of the ligand-binding activity of integrin α3β1 via association with tetraspanin CD151, Proc. Natl. Acad. Sci. USA 102 (2005) 1939–1944.
[10] M. Yamada, Y. Tamura, N. Sanzen, R. Sato-Nishiuchi, H. Hasegawa, L.K. Ashman, E. Rubinstein, M. Yanez-Mo, F. Sanchez-Madrid, K. Sekiguchi, Probing the interaction of tetraspanin CD151 with integrin α3β1 using a panel of monoclonal antibodies with distinct reactivities toward the CD151-integrin α3β1 complex, Biochem. J. 415 (2008) 417–427.
[11] M. Yamada, Y. Sumida, A. Fujibayashi, K. Fukaguchi, N. Sanzen, R. Nishiuchi, K. Sekiguchi, The tetraspanin CD151 regulates cell morphology and intracellular signaling on laminin-511, FEBS J. 275 (2008) 3335–3351.
[12] F. Berditchevski, E. Gilbert, M.R. Griffiths, S. Fitter, L. Ashman, S.J. Jenner, Analysis of the CD151-α3β1 integrin and CD151–tetraspanin interactions by mutagenesis, J. Biol. Chem. 276 (2001) 41165–41174.
[13] M.E. Hemler, Tetraspanin functions and associated microdomains, Nat. Rev. Mol. Cell Biol. 6 (2005) 801–811.
[14] C.S. Stipp, Laminin-binding integrins and their tetraspanin partners as potential antimetastatic targets, Expert Rev. Mol. Med. 12 (2010) e3.
[15] R.L. Yauch, A.R. Kazarov, B. Desai, R.T. Lee, M.E. Hemler, Direct extracellular contact between integrin α3β1 and TM4SF protein CD151, J. Biol. Chem. 275 (2000) 9230–9238.
[16] C. Gonzalez-Manchon, M. Fernandez-Pinel, E.G. Arias-Salgado, M. Ferrer, M.V. Alvarez, S. Garcia-Munoz, M.S. Ayuso, R. Parrilla, Molecular genetic analysis of a compound heterozygote for the glycoprotein (GP) IIb gene associated with Glanzmann's thrombasthenia: disruption of the 674–687 disulfide bridge in GPIIb prevents surface exposure of GPIIb–IIIa complexes, Blood 93 (1999) 866–875.
[17] N. Rosenberg, R. Yatuv, V. Sobolev, H. Peretz, A. Zivelin, U. Seligsohn, Major mutations in calf-1 and calf-2 domains of glycoprotein IIb in patients with Glanzmann thrombasthenia enable GPIIb/IIIa complex formation, but impair its transport from the endoplasmic reticulum to the Golgi apparatus, Blood 101 (2003) 4808–4815.
[18] M.W. MacArthur, J.M. Thornton, Influence of proline residues on protein conformation, J. Mol. Biol. 218 (1991) 397–412.
[19] M.H. Smith, H.L. Ploegh, J.S. Weissman, Road to ruin: targeting proteins for degradation in the endoplasmic reticulum, Science 334 (2011) 1086–1090.