Novel insights into cadherin processing by subtilisin-like convertases

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Abstract Proprotein convertases (PCs) are known to activate many important molecules and their overexpression plays a significant role in tumor progression. Only little is known about the involvement of PCs in the processing of cadherin adhesion molecules, which are potent tumor suppressors. Here we show in a baculovirus overexpression system that the desmosomal cadherins Dsg1 and Dsg3 are substrates for the PC furin. Accordingly, inhibition of PCs in differentiating mouse keratinocytes by α1-anti-trypsin Portland (α1-PDX) negatively interfered with pro-epithelial (proE)-cadherin processing, but unexpectedly also resulted in a dramatic reduction of E-cadherin, Dsg1 and Dsg3 protein and Dsg1 mRNA. Because loss of intercellular adhesion is a rate-limiting step in the transition from benign to malignant tumors, these results have significant implications for the use of PC inhibitors as possible therapeutic tools.

Key words: Desmoglein; E-cadherin; Furin; Proprotein cleavage; α1-PDX

1. Introduction

Intercellular adhesion plays an important role in embryonic development, differentiation and maintenance of pluricellular organisms. It is achieved by two types of structurally related junctions known as adherens junctions and desmosomes. Both are composed of transmembrane glycoproteins of the cadherin family that link to the cytoskeleton through multiple cytoplasmic adapter proteins [1,2]. While epithelial cadherin (E-cadherin) is the major cadherin present in adherens junctions, desmosomal cadherins are desmogleins and desmocollins. The importance of cadherin-mediated intercellular adhesion is highlighted by the tumor suppressor function of E-cadherin and desmosomal cadherins [3]. Given [4,5] these important roles, it is crucial to know all events leading to the correct assembly of intercellular adhesion junctions. Like many other proteins, cadherins are initially synthesized as inactive propeptide precursors that are post-translationally processed to become biologically active mature proteins. This processing includes removal of the propeptide fragment at the NH_2-terminal end of immature proteins. A family of calcium-dependent endoproteases named subtilisin-like proprotein convertases (PCs) is largely responsible for the activation of precursor proteins by cleavage at a specific recognition sequence [Arg/Lys-$(X)_n$-Lys/Arg-Arg] ($n = 0$, 2, 4 or 6) [6,7]. To date seven mammalian PCs have been described: furin, PC1/3, PC2, PC4, PC5/6, PC7/8, and PACE4. Of these, furin, PC5/6, PC7/8, and PACE4 have a wide tissue distribution and function in the proteolytic processing of precursors in the constitutive secretory pathway. In a previous study we have demonstrated that proE-cadherin is processed by members of the PC family [8].

Recently, work has focused on the possible involvement of convertases in tumorigenesis [9–14]. Furin and PACE4, for instance, were found to be overexpressed in several tumor cell lines and primary human malignancies [15]. Accordingly, ectopic overexpression of PACE4 was shown to result in a more invasive phenotype of squamous cell carcinoma cells [11] and even promote malignant conversion of non-tumorigenic keratinocytes [14]. PCs are known to process and thereby activate several substrates relevant to cancer development, invasion and metastasis such as growth factors and membrane-type metalloproteinases [8,15,16]. In contrast to this adhesion molecules like E-cadherin become functional upon cleavage of their precursors [17] and thereby act as tumor suppressors. Nevertheless, specific PC inhibitors are discussed as useful therapeutic agents to treat malignancies which overexpress PCs [13,18–20]. Among the most promising candidates so far is the protein-based inhibitor α1-anti-trypsin Portland (α1-PDX) [18,21,22]. Several studies have recently shown that α1-PDX-mediated convertase inhibition reverts the malignant phenotype induced by furin or PACE4 overexpression in different tumor cell lines [13,19,20]. In most of these cases, this effect was likely due to decreased activation of different metalloproteinases. However, effects on adhesion molecules, like cadherins, were not studied in detail.

Due to the presence of a dibasic convertase recognition motif [Arg-X-Lys-Arg], preceding the defined proprotein cleavage site of not only most classical cadherins, but also the desmosomal cadherins Dsg1 and Dsg3, we hypothesized that subtilisin-like convertases are responsible for the maturation of Dsg1 and Dsg3. We first tested whether proDsg1 and proDsg3 are substrates for furin, the prototype member of this convertase family in a baculovirus co-expression system. Furthermore we investigated the impact of PC inhibition by α1-PDX on the expression and processing of E-cadherin, Dsg1 and Dsg3 in mammalian cells.

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Abbreviations: Dsg, desmoglein; PC, proprotein convertase; K14, cytokeratin 14; α1-PDX, α1-anti-trypsin Portland

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2. Materials and methods

2.1. Recombinant baculo- and adenoviruses

The baculovirus constructs encoding the signal peptide, propeptide and entire extracellular part of human Dsg1 (Bac:Dsg1) or human Dsg3 (Bac:Dsg3) fused to the constant region of human IgG1 were a kind gift of Dr. M. Amagai (Keio University, Tokyo, Japan) [23,24]. The baculovirus construct encoding full-length human furin (Bac:fur) was described earlier [25]. Wild-type baculovirus (Bac:WT) was provided by Dr. Hertig (University of Bern, Switzerland). Recombinant adenoviruses encoding α1-PDX together with green fluorescent protein (GFP) (Adtr5PDX), the transactivator tTa (AdCMVtTa) and GFP alone (AdGFP) (kind gift of Dr. B. Vogelstein, Johns Hopkins University, Baltimore, MD, USA) were used in this study. The gene encoding full-length α1-PDX (kindly provided by J. Lipps, Hedral Therapeutics, Portland, USA) was inserted into the multiple cloning site of the transfer vector pAd-TR5F-DC-GFP. It was placed under the control of a modified CMV promoter containing a tetracycline (tet)-repressable, dicistronic expression cassette [26,27] encoding α1-PDX and the GFP tracer. The production of adenoviral vectors was performed as described [27] and titrated by flow cytometry using GFP fluorescence as marker of infection. The adenovirus expressing the transactivator tTa under control of a constitutive CMV promoter was obtained from Dr. B. Massie (Biotechnology Research Institute of Montreal, Montreal, QC, Canada).

2.2. Cell cultures and virus infection

HighFive insect cells (Invitrogen, Basel, Switzerland) were cultured and infected with viruses at increasing MOI (multiplicity of infection) as described [8]. Long-term mouse keratinocytes were cultured as described [28]. Cells were seeded into 8.8 cm^2 culture dishes (NUNCLON™ Surface, NUNC, Denmark) and grown to approximately 80% confluence prior to co-infection with Adtr5PDX (MOI 200) and AdCMVtTa (MOI 50) in 2 ml CnT02 medium (0.07 mM calcium; CELLnTEC, Bern, Switzerland) for 48 h. As a control, cells were infected with AdGFP (MOI 200) and AdCMVtTa (MOI 50). Transfection efficiency was monitored by visualizing green fluorescence. 48 h post-infection, cells were washed with phosphate-buffered saline (PBS) and partially trypsinized under light microscopic control. After cells lost their contact to neighboring cells, the trypsin was inhibited with 10% fetal calf serum (FCS), followed by washing of cell cultures with PBS and incubation with CnT02 containing 1.2 mM calcium.

2.3. Western blot analysis

Insect cell culture media were harvested at different time-points post-infection (as indicated) and equal volumes were analyzed by immunoblot. The antibodies used were: anti-furin (Alexis Corp., Läufelfingen, Switzerland) and anti-human horseradish peroxidase labeled antibodies for baculovirus-encoded Dsg1 and Dsg3. Further antibodies used were: anti-E-cadherin (DECMA), anti-Dsg1/2 (DG3.10, Progen, Heidelberg, Germany), anti-Dsg3 (kind gift of Dr. J. Stanley, Philadelphia, PA, USA), anti-involucrin (kind gift of F. Watt, Cancer Research Fund, London, UK), anti-loricrin (Covance, Richmond, USA), anti-tubulin (Sigma, St. Louis, MO, USA), anti-cytokeratin 14 (K14) (Biogenex, San Ramon, USA) and horseradish peroxidase-conjugated secondary antibodies.

2.4. Amino acid sequence analysis and sequence comparison

To obtain complete processing, HighFive cells were co-infected with Bac:fur (MOI 5) and Bac:Dsg1 (MOI 10) or Bac:Dsg3 (MOI 5) and incubated for 72 h. Media were concentrated with Centricon 50 (Amicon, Beverly, MA, USA) and subjected to precipitation using protein A-Sepharose (Pharmacia, Dübendorf, Switzerland). Sequence analysis was then performed as described earlier [8].

2.5. Immunofluorescence analysis

Cells grown on coverslips (Lab-Tek™ II, Nunc, Denmark) were co-infected and treated as described in Section 2.2 with the exception that Adtr5PDX and AdGFP were used at a MOI of 100. Cells were fixed with 1% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 for 5 min prior to incubation with primary and Texas red-conjugated secondary antibodies.

2.6. mRNA analysis

Total RNA was isolated from mouse keratinocyte cultures using RNeasy (Qiagen, Basel, Switzerland). Random primed cDNA was prepared using standard techniques. Specific primers for subtilisin-like convertases were adapted from those published for the human sequences [29]: furin (amplification product: 399 bp): tatggctacacg-ggctgttgg (sense), ttcactggtattttcaatctct (antisense); PACE4 (437 bp): ggatgcagaagctctggtc (sense), ggctccattctttcagcttt (antisense); PC5/6 (402 bp): ctgctggttttaaggtgagcc (sense), ccccagcagcccgttct (antisense); PC7/8 (639 bp): gaaagggaggcagaaagtc (sense), gggatctgggtcattagag (antisense). Specificity of polymerase chain reaction (PCR) amplification was confirmed by sequencing. For real-time PCR cells were grown in 24 wells, infected with recombinant adenoviruses and treated as described in Section 2.2. 12 h after addition of 1.2 mM calcium total RNA was extracted and reverse transcribed. Real-time PCR was performed with a 7700 sequence detection system (Applied Biosystems). Each PCR reaction contained 5 μl of cDNA, primers at a concentration of 1 μM and 12.5 μl of 2× SYBER Green Reaction Mix in a total volume of 25 μl. For internal control 18S rRNA was quantified using the TaqMan® ribosomal RNA control reagents kit (Applied Biosystems). Dsg1 primers used were: tcctgctgatctcctgtgatt (sense) and tgtgaattgctccatcagaacat (antisense).

3. Results and discussion

3.1. Expression and processing of Dsg1 and Dsg3 in baculovirus-infected insect cells

We previously demonstrated that ectopically expressed proE-cadherin was inefficiently processed into the mature E-cadherin form in HighFive insect cells [8]. The same phenomenon has been described when Dsg1 and Dsg3 were expressed in Sf9 insect cells [23,24]. To investigate whether Dsg1 and Dsg3 can be processed by furin, we first determined the optimal conditions for expression of baculovirus-encoded Dsg1 and Dsg3 in HighFive insect cells. Cells were infected with different amounts of virus and the culture media were

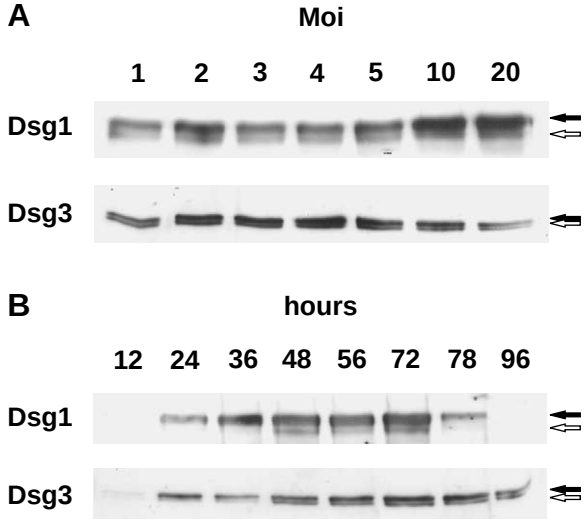

Fig. 1. A: Dose dependence of recombinant proDsg1 and proDsg3 expression in HighFive cells. HighFive cells were infected with increasing MOI of Bac:Dsg1 or Bac:Dsg3 as indicated. Cell culture media were harvested 72 h post-infection and 20 μl medium analyzed by Western blotting. A double band was detected for both proteins with the more abundant higher molecular weight bands corresponding to the unprocessed proforms (black arrow), the lower bands corresponding to the mature desmogleins (white arrow). Note that the size difference between both forms is only 3 kDa. B: Time course of baculovirus-encoded Dsg1 and Dsg3 expression in HighFive cells. HighFive cells were infected with Bac:Dsg1 (MOI 10) or Bac:Dsg3 (MOI 5). Cell culture media were harvested at indicated time-points and analyzed as in A.

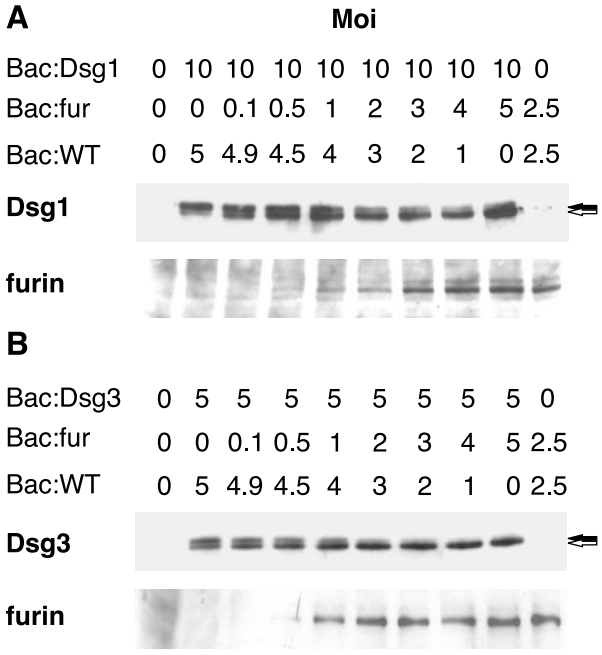

Fig. 2. Enhanced processing of proDsg1 and proDsg3 in HighFive cells by co-expressed mammalian furin. A: HighFive cells were co-infected with Bac:Dsg1 at a MOI of 10 and increasing MOI of baculovirus-expressing mammalian furin (Bac:fur). Wild-type virus (Bac:WT) was added to achieve equal amounts of virus per cell. Non-infected cells (first lane) and cells infected with Bac:fur and Bac:WT only (last lane) served as controls. Cell culture media were harvested 72 h post-infection and analyzed by Western blotting. B: Same experiment as in A using Bac:Dsg3 at a MOI of 5. Black arrow: unprocessed Dsg, white arrow: mature Dsg.

analyzed 72 h post-infection. Maximum levels of secreted proteins were detected using a MOI of 10 for Bac:Dsg1 and a MOI of 2–5 for Bac:Dsg3 (Fig. 1A). To optimize Dsg processing, HighFive cells were subsequently infected with MOI 10 (Bac:Dsg1) or 5 (Bac: Dsg3) and incubated between 12 and 96 h. Western blot analyses of culture media demonstrated that the processing of these 2 mol was overall very inefficient (Fig. 1B). Despite prolonged incubation, the ratio of unprocessed to mature forms of Dsg1 and Dsg3 remained unchanged, regardless of the amount of ectopically expressed protein or incubation time (Fig. 1A). After 72 h amounts of the proteins dropped, due to continuous lysis of the infected cells and proteolytic degradation of the proteins. Together these results indicated that the efficacy of proDsg1 and proDsg3 cleavage in the HighFive-based baculovirus expression system is independent of the expression level of the exogenous protein. This observation is consistent with previous results obtained from the expression of proE-cadherin in HighFive cells [8], indicating that insect-encoded proteases are insufficient to drive maturation of ectopically overexpressed mammalian cadherins.

3.2. Effect of co-expressed furin on Dsg1 and Dsg3 processing

Similar to classical cadherins, the desmosomal cadherins Dsg1 and Dsg3 contain the tetrapeptididyl sequence motif [Arg-X-Lys-Arg] at their predicted proprotein cleavage site [30,31]. We therefore speculated that PCs are involved in prodesmoglein processing. To investigate this possibility, we used a baculovirus-based co-expression system that had allowed efficient and specific cleavage of proE-cadherin by furin [8]. HighFive cells were infected with Bac:Dsg1 or Bac:Dsg3 in parallel with increasing amounts of Bac:fur (Fig. 2). With increasing MOI of Bac:fur, the amounts of furin expressed by HighFive cells increased (Fig. 2). This resulted in a dose-dependent decrease of secreted proDsg1 and proDsg3, an increase of the mature form (Fig. 2) and finally, in virtually complete processing of secreted Dsg1 and Dsg3.

3.3. Determination of the cleavage site of processed Dsg1 and Dsg3

To ensure the specificity of furin-mediated Dsg cleavage in the co-expression system, we determined the N-terminal amino acids of mature Dsg1 and Dsg3. Secreted and processed Dsg fusion proteins from HighFive cells co-infected with recombinant Dsg1- or Dsg3-expressing baculovirus and Bac:fur were purified and subjected to N-terminal proteolytic cleavage. The first N-terminal amino acids were found to be [$^{1P'}$Glu-Trp-Ile-Lys$^{4P'}$] for Dsg1 and [$^{1P'}$Glu-Trp-Val-Lys-Phe-Ala-Lys$^{7P'}$] for Dsg3. These sequences were identical with the published N-terminal sequences of mature human Dsg1 and Dsg3, respectively [30,31]. Both sequence motifs are unique within the entire protein of Dsg1 or Dsg3 and are localized C-terminal to the furin recognition site [Arg-X-Lys-Arg]. Given the sequence identity, the cleavage had occurred in a furin-specific manner and at the predicted cleavage site. These results clearly demonstrate that furin is a Dsg1- and Dsg3-competent processing convertase.

3.4. Inhibition of PCs in cultured mouse keratinocytes

Functional overlap between different PCs active in the regulated secretory pathway is known to occur [32,33]. Using the furin-deficient colon carcinoma cell line Lovo, we have already shown that other subtilisin-like convertases are responsible for processing E-cadherin in vivo or might compensate for the lack of furin [8]. Consistently, a recent study using Lovo cells transfected with different variants of the convertase inhibitor α1-PDX indicated that mainly PC7/8 and to a lesser extent PACE4 are responsible for proE-cadherin cleavage in these cells [18].

To investigate E-cadherin, Dsg1 and Dsg3 processing by subtilisin-like convertases in non-neoplastic cells, we used long-term mouse keratinocyte cultures. These cells represent an ideal tool to investigate cadherin processing because they express large amounts of adhesion molecules upon induction of differentiation with medium containing physiological levels of calcium (0.1–1.5 mM) [28,34]. Furthermore, a recent study using transformed keratinocytes demonstrated that α1-PDX

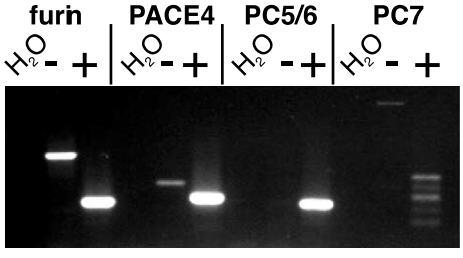

Fig. 3. Convertase expression in long-term mouse keratinocyte cultures. PCR performed on cDNA (+) from cultured mouse keratinocytes. PCR amplifications using water (H_2O) or total RNA without prior reverse transcription (−) served as controls. Note multiple bands for PC7, which likely represent splice variants.

expression reverses malignancy in these cells [20]. The results therefore suggested convertase inhibitors as good candidates for therapeutical agents. However, the effects of α1-PDX on certain convertase substrates, which could potentially contribute to tumor progression, like desmosomal cadherins, have not been investigated. Having shown in the current study that these cadherins are processed by furin in the heterologous baculovirus overexpression system, we addressed the effect of α1-PDX on processing of Dsg1 and Dsg3 in addition to E-cadherin in normal keratinocytes.

The mouse keratinocytes used in this study express furin, PACE4, PC5/6 and PC7 (Fig. 3), which corresponds to the expression pattern in the epidermis [35]. It is noteworthy that we observed multiple amplification products for PC7 (Fig. 3). Sequence analyses suggested different splice variants (Posthaus et al., unpublished observation). To inhibit convertase functions in the mouse keratinocytes, we infected them with adenovirus-expressing α1-PDX in presence of the transcriptional transactivator tTa. Transfection efficiency was approximately 80% (data not shown).

In infected keratinocytes, much of the membrane-incorporated cadherin pool was likely to be synthesized before efficient expression of α1-PDX. To circumvent this problem, cell surface-expressed cadherins were digested with trypsin to stimulate de novo synthesis upon incubation in medium containing 1.2 mM calcium. At different time-points during calcium-induced differentiation, cells were lysed and fractionated into membrane-bound Triton X-100-soluble (Fig. 4A) and Triton X-100-insoluble proteins (Fig. 4B) as described [36]. The Triton X-100-soluble fraction contains most of the adherens junction components and desmosomal proteins not yet assembled into desmosomes, while the Triton X-100-insoluble fraction mainly harbors fully assembled desmosomes [36].

Western blot analyses revealed that trypsinization resulted in nearly completely digested cell surface-exposed E-cadherin and Dsg3. Dsg1 was not detected at this time-point because this cadherin is known to be expressed only after incubation in high calcium [36] (Fig. 4). Accordingly, in high calcium medium, keratinocytes synthesized all three cadherin molecules and expression levels rose in a time-dependent manner. Compared to mock-infected or non-infected controls, α1-PDX expression reduced the amount of mature E-cadherin at the expense of the unprocessed form (Fig. 4A). Nonetheless, proE-cadherin processing in mouse keratinocytes was not completely abrogated by α1-PDX. One explanation for this observation is that not all keratinocytes in the cell culture flasks were sufficiently infected with recombinant adenovirus and therefore did not express sufficient amounts of α1-PDX to completely inhibit the cadherin-competent convertases. This is also suggested by our immunofluorescence study where we observed low expressors (as judged by the GFP intensity) next to high expressors (Fig. 5).

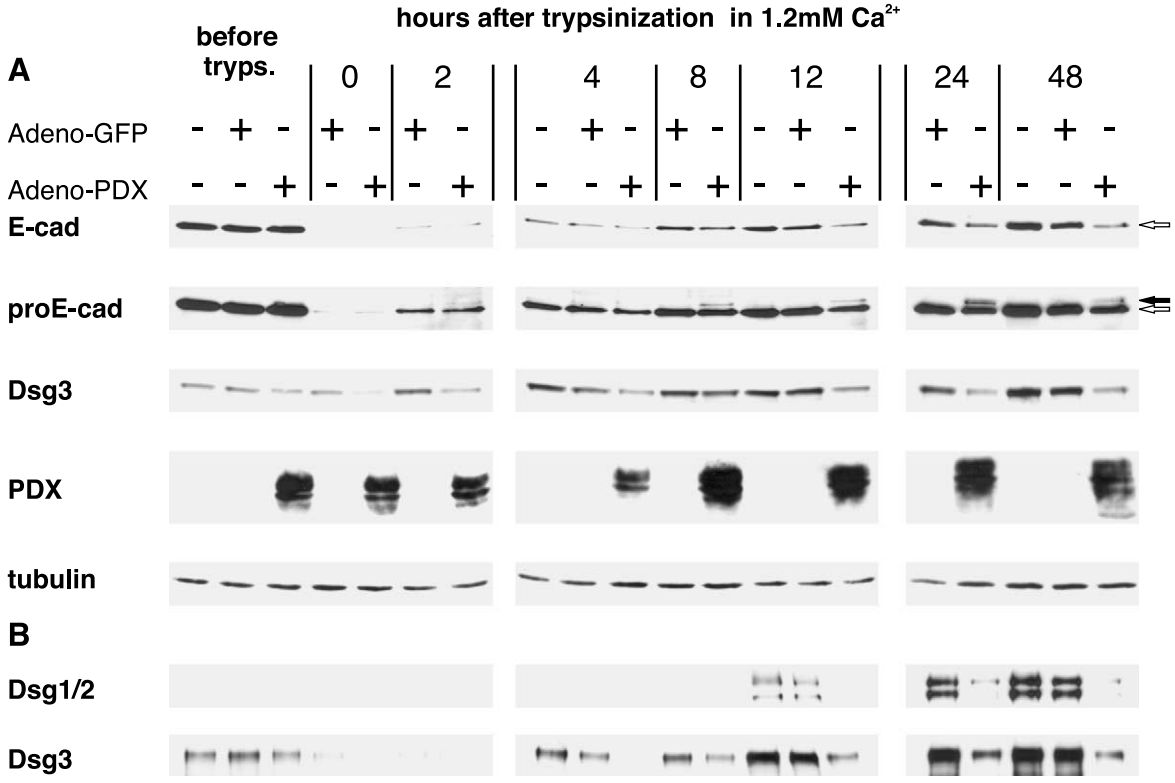

Fig. 4. Inhibition of convertases by α1-PDX in cultured mouse keratinocytes resulted in reduced processing of proE-cadherin and an overall reduction of E-cadherin, Dsg1 and Dsg3 protein levels in Triton X-100-soluble (A) and -insoluble (B) proteins from cultured keratinocytes. Cells were either infected with Adtr5PDX (adeno-PDX) or AdGFP (adeno-GFP) or left uninfected. To induce de novo synthesis of cadherins, cell surface-exposed molecules (before trypsinization) were proteolytically digested with trypsin and subsequently incubated in medium containing 1.2 mM calcium. Only in the α1-PDX-expressing cells, the proE-cadherin form (black arrow) became visible after prolonged exposure of the immunoblots probed with anti-E-cadherin antibodies. Overall expression levels of E-cadherin, Dsg3 and Dsg1 were markedly reduced in α1-PDX-transfected cells as compared to control cells. Equal amounts of protein were separated on 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) for A and 5% SDS–PAGE for B and blotted onto nitrocellulose membranes. Blots were incubated with the indicated antibodies and developed under identical conditions. The anti-Dsg1/2 recognizes two protein bands, of which the upper (150 kDa) corresponds to mature Dsg1 and Dsg2.

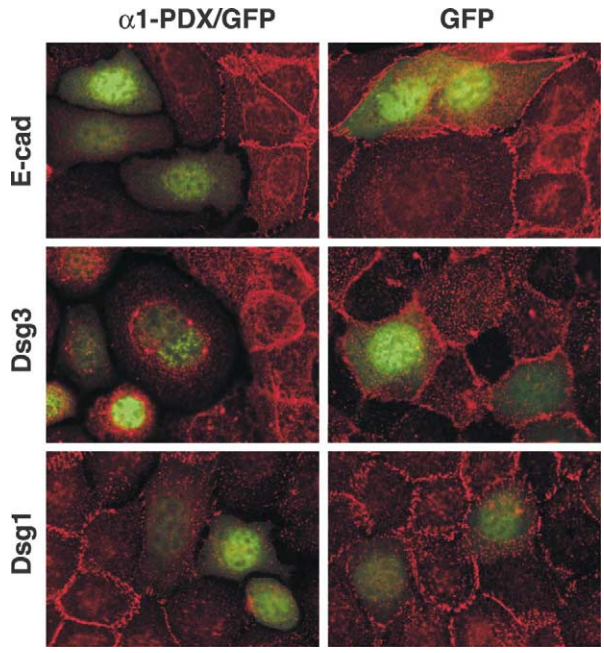

Fig. 5. α1-PDX expression results in lack of surface expression of E-cadherin, Dsg3 and Dsg1. Immunofluorescent staining of cultured keratinocytes infected with adeno-PDX (left) or adeno-GFP (right) after cell surface depletion and subsequent stimulation of de novo synthesis of cadherins for 48 h. Adeno-PDX-infected cells could be identified due to the autofluorescence of the polycistronically expressed GFP. Note that the amount of virus was reduced compared to Western blot analyses to allow non-infected cells in the same well to serve as control.

However, α1-PDX is known to potently inhibit furin and PC6 and to a lesser extent PACE4, whereas it does not effectively inhibit PC7/8 [18,22,37]. Hence, our results are consistent with recent findings indicating partial inhibition of proE-cadherin cleavage in α1-PDX-transfected Lovo cells [18]. The authors of this study suggested that a proportion of procadherin processing relies on cleavage by the α1-PDX-sensitive convertases furin, PACE4 and/or PC5/6. However an α1-PDX-insensitive convertase, possibly PC7/8, could also be involved in procadherin processing or could substitute for other convertases inhibited by α1-PDX. Most interestingly and not been described before, we observed that the overall level of E-cadherin (processed and unprocessed forms) dropped as compared to mock-infected cells. Similarly, α1-PDX-transfected cells expressed markedly less Dsg1 and Dsg3. For Dsg3 this effect was already visible to some extent before calcium-induced de novo synthesis and was accelerated during subsequent differentiation. Furthermore, reduced expression of Dsg1 and Dsg3 resulted in a decrease of these molecules in the Triton-insoluble fraction (Fig. 4B), indicating that fewer desmosomal cadherins were assembled into desmosomes. Unfortunately, the small size of the proregions (approximately 3 kDa) of both Dsg1 and Dsg3 [30,31] made it impossible to detect a potential accumulation of the respective unprocessed proproteins when the full-length mammalian proteins were investigated. Immunofluorescence studies confirmed that α1-PDX-expressing keratinocytes had markedly reduced or even no membrane staining for either of the three cadherin molecules concomitantly with weaker overall fluorescence intensity (Fig. 5). These cells also exhibited reduced adhesion to neighboring keratinocytes highlighted by prominent intercellular spaces. In contrast, in GFP mock-transfected keratinocytes membrane staining for E-cadherin, Dsg3 and Dsg1 did not differ from that of non-infected keratinocytes.

The reduction in E-cadherin, Dsg1 and Dsg3 expression we observed, was not due to an overall reduction in protein synthesis, as other molecules like tubulin were not affected (Fig. 4A). Because adhesion molecules are expressed in a differentiation-dependent manner in keratinocytes [2], another possible explanation of this phenomenon would be a negative effect of convertase inhibition on keratinocyte differentiation. This is conceivable as Notch-1, a cell surface receptor involved in keratinocyte differentiation and cornification [38], was demonstrated to be processed and activated by furin [39]. To investigate whether α1-PDX-expressing keratinocytes were impaired in their overall capability to differentiate, we assessed the expression of the keratinocyte differentiation markers, involucrin and loricrin, at 24 and 48 h after calcium-induced differentiation (Fig. 6). Unlike the results for cadherins, levels of involucrin and loricrin were similar in non-transfected, mock-transfected and α1-PDX-transfected cells. This suggested that the reduced levels of E-cadherin, Dsg1 and Dsg3 cannot be explained by an overall impairment of keratinocyte differentiation. However these results do not exclude the possibility that convertase inhibition leads to reduced processing and activation of other molecules involved in the regulation of cadherin expression.

To further address whether the regulation of cadherin expression was affected by α1-PDX expression we analyzed mRNA steady-state levels of Dsg1 by real-time PCR after induction of its de novo synthesis. In contrast to non-infected and GFP mock-infected keratinocytes Dsg1 mRNA levels did not rise after switching cells to medium containing 1.2 mM calcium (Fig. 7). This finding indicated that α1-PDX expression has an inhibitory effect on the calcium-induced transcription of Dsg1. A possible explanation for this is that accumu-

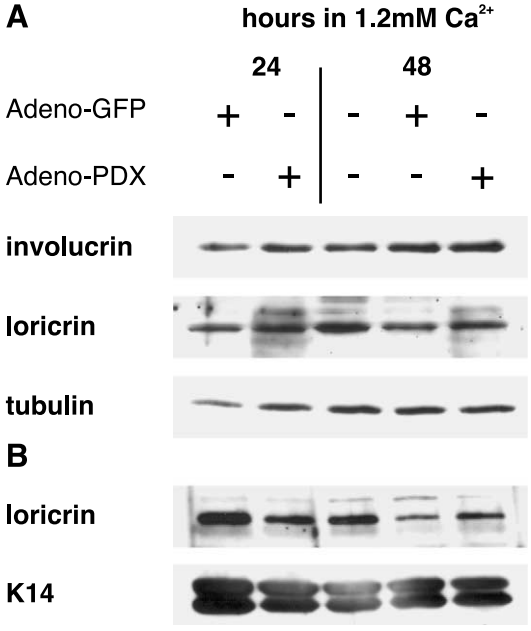

Fig. 6. Convertase inhibition by α1-PDX did not affect expression of the differentiation markers involucrin and loricrin. Western blot analyses of the same lysates as in Fig. 4 using indicated antibodies. A: Triton X-100-soluble, B: Triton X-100-insoluble fractions. Tubulin and K14 served as loading controls.

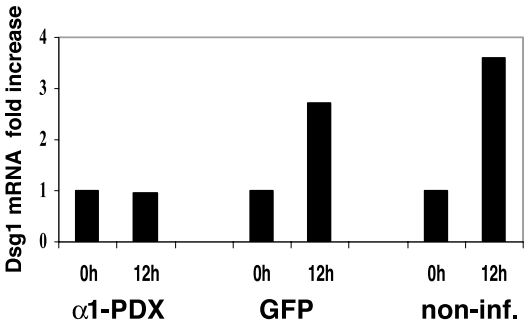

Fig. 7. Quantitative reverse transcription (RT)-PCR revealed reduced transcription of Dsg1 in α1-PDX-infected cells. Representative result of multiple experiments.

lation of the unprocessed procadherin exerts a negative feedback on its transcription. On the other hand, convertase inhibition might lead to inactivation of other factors (e.g. growth factors) that are essential for cadherin expression in keratinocytes.

Despite the fact that the exact mechanism leading to this observation remains unknown, reduced cadherin expression and assembly of intercellular adhesion junctions results in decreased intercellular adhesive strength. This situation is observed for example in many tumors [3]. It is therefore possible that inhibition of one or more convertases negatively affects the overall integrity of tissues which rely on strong intercellular adhesion, such as the epidermis. This observation should be taken into account when considering PC inhibitors as therapeutic tools for a variety of diseases including cancer. Therefore more detailed knowledge of the substrates specific for or favored by each particular convertase is needed. Using such knowledge, the development of more selective convertase inhibitors could potentially reduce side effects, such as the herein reported downregulation of cell–cell adhesion molecules.

In summary, our study demonstrates that the desmosomal molecules Dsg1 and Dsg3 are efficiently and specifically cleaved by the mammalian convertase furin in a baculovirus overexpression system. Furthermore α1-PDX transfection of mouse keratinocytes results in reduced calcium-induced transcription of Dsg1 and in reduced steady-state protein levels of E-cadherin and two desmosomal cadherins which in turn lead to weakened intercellular adhesion. Together these data indicate that the use of PC inhibitors as possible therapeutic tool could have significant consequences for epidermal integrity, which so far have not been recognized.

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